MISSISSIPPI ACADEMY OF SCIENCES

Supplemental Issue

34th Annual Southern Biomedical Engineering Conference

Digest of Papers

March 8-10, 2018

Holiday Inn, Charlotte Airport

Charlotte, NC

Major Sponsor

Mississippi Academy of Science

Journal of the Mississippi Academy of Sciences

Volume 63 April 2018 Supplemental Issue 1

The Journal of the Mississippi Academy of Sciences (ISSN 0076-9436) is published in January (annual meeting abstracts), April, July, and October, by the Mississippi Academy of Sciences. Members of the Academy receive the journal as part of their regular (nonstudent) membership. Inquiries regarding subscriptions, availability of back issues, and address changes should be addressed to The Mississippi Academy of Sciences, Post Office Box 55709, Jackson, MS 39296-5709, telephone 601-977-0627, or email msacademyofscience@comcast.net.

Contents

Contents Continued

265 **EFFECT OF PEDICLE-SCREW FIXATION IN LUMBAR SPINE AT L3-L5 LEVEL: A FINITE ELEMENT STUDY**

Jayanta Kumar Biswas[1], Sreerup Banerjee[2], Santanu Majumder[1], Sandipan Roy[3], Subrata Saha[4,5], Amit RoyChowdhury[1]

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274 **THE EFFECT OF TROGLITAZONE ON C- REACTIVE PROTEIN IN INDIVIDUALS WITH PREDIABETES: DATA FROM THE DIABETES PREVENTION PROGRAM**

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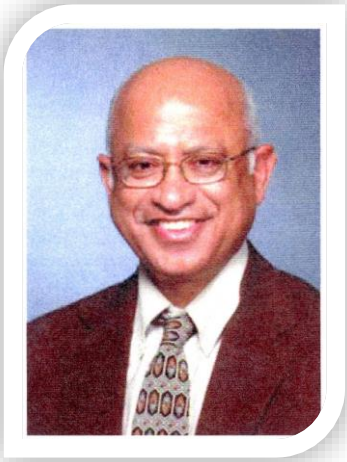
SBEC HISTORY

The Southern Biomedical Engineering Conference (SBEC) series was conceived by bioengineering professionals from academia and industry located primarily in the South of the United States in 1982. The first Southern Biomedical Engineering Conference was held at the LSU Medical Center, Shreveport, Louisiana, in 1982. Since then it has been held annually in different cities, mostly in the southern United States, and has grown to become a global event that regularly attracts attendees from all over the world. Submitted Papers are peer-reviewed, and those papers accepted for presentation and publication appear in the yearly issue of SBEC proceedings.

The SBEC serves a special purpose by emphasizing participation from young professionals and advanced students. Since established investigators present papers in the same sessions with the students, it encourages a high level of professionalism as a standard for young investigators and students. Submission of papers from individuals from around the world is encouraged. However, if their papers are accepted, an author or co-author must attend the conference to present their work and to interact with other attendees. In keeping with the emphasis on student participation, the SBEC presents best paper and presentation awards to undergraduate, graduate, and professional students.

Keynote Speaker – I



"ETHICAL CHALLENGES IN BIOMEDICAL ENGINEERING RESEARCH"

Subrata Saha, Ph.D., FAIMBE, FASME, FBMES, FNYAM

Visiting Research Professor, Department of Biomedical Engineering, Florida International University, Miami,
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University of North Carolina at Charlotte

Dr. Subrata Saha is presently a Research Professor in the Department of Biomedical Engineering at the Florida International University in Miami, Florida and an Affiliated Professor in the Department of Restorative Dentistry and the Department of Oral and Maxillofacial Surgery at the University of Washington, in Seattle, Washington.

He was previously the Director of Musculoskeletal Research and Research Professor in the Department of Orthopaedic Surgery & Rehabilitation Medicine, and the Director of the Biomedical Engineering Program in the School of Graduate Studies at SUNY Downstate Medical Center in Brooklyn, New York. Previously, he was a faculty member at Loma Linda University, Clemson University, Alfred University, and Yale University.

Dr. Saha received a BS in Civil Engineering from Calcutta University in 1963, an MS in Engineering Mechanics in 1969 from Tennessee Technological University, and Engineering and PhD degrees in Applied Mechanics from Stanford University in 1972 and 1974, respectively. He has been a faculty member at Yale University, Louisiana State University Medical Center, Loma Linda University, Clemson University, and Alfred University. Dr. Saha has received many awards from professional societies, including Orthopedic Implant Award, Dr. C. P. Sharma Award, Researcher of the Year Award, C. William Hall Research Award in Biomedical Engineering, Award for Faculty Excellence, Research Career Development Award from NIH, and Engineering Achievement Award. He is a Fellow of The Biomedical Engineering Society (BMES), The American Society of Mechanical Engineers (ASME), and the American Institute for Medical and Biological Engineering (AIMBE).

He has received numerous research grants from federal agencies (NIH and NSF), foundations, and industry. Dr. Saha is the founder of the Southern Biomedical Engineering Conference Series. He also started the International Conference on Ethical Issues in Biomedical Engineering. Dr. Saha has published over 90 papers in journals, 35 book chapters and edited volumes, 347 papers in conference proceedings, and 84 abstracts. His research interests are bone mechanics, biomaterials, orthopedic and dental implants, drug delivery systems, rehabilitation engineering, and bioethics.

Dr. Saha is presently the Editor-in-Chief of the Journal of Long-Term Effects of Medical Implants and Associate Editor of the International Journal of Medical Implants & Devices and was an Associate Editor of the Annals of Biomedical Engineering and Trends in Biomaterials and Artificial Organs. He has been a Member of the Editorial Boards of many journals, including Journal of Biomedical Materials Research; Medical Engineering and Physics; Journal of Applied Biomaterials; Medical Design and Material; Biomaterials, Artificial Cells, and Immobilization Biotechnology; Biomaterials, Medical Device and Artificial Organs; Journal of Bioengineering, Biotelemetry and Patient Monitoring; Journal of Basic & Applied Biomedicine and TM Journal.

Keynote Speaker – II

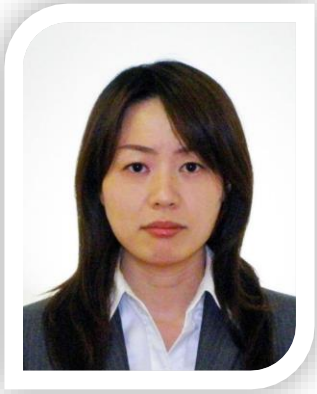
" THE EFFECTS OF SUSTAINED DELIVERY OF NPY RECEPTOR ANTAGONIST ON CELLS TYPES WITHIN THE INTERVERTEBRAL DISC"

Michelle A. Tucci, PhD., FAIMBE

Professor, Department of Anesthesiology
Univeristy of Mississippi Medical Center

Dr. Michelle Tucci, Professor of Anesthesiology at the University of Mississippi Medical Center. Dr. Tucci has been involved in a leadership role for various state, national and international organizations. After completing her undergraduate training at Seton Hill University, in Pennsylvania she completed a Master's degree in Biology at the University of Dayton in Ohio. Following her move to Mississippi, she completed her PhD in pharmacology and Toxicology in 2000. Aside from her work supervising and overseeing resident's basic science research in orthopedic surgery for several years, she has also mentored and supervised a number of undergraduate and graduate students from diverse disciplines. She has served on over 60 doctoral dissertation committees, has published over 300 full journal publications (several in prestigious journals such as J. of Investigative Surgery, J. of Clin Investigation, Analytical Biochemistry, J. of Immunology, Infection & Immunity, Cancer Investigation, Microsurgery, Alcohol, Critical Reviews in Biomed Eng, J. of Gerontology, Pediatric Research, Annals of Pharmacotherapy, J. of Spinal Disorders and Techniques, J. Oral Pathol Med, to name afew), and published over 400 abstracts at state, regional, national and international meetings (Italy, France, Spain, Canada, Poland, and China). Her leadership role in various societies includes Director and program chair at the Rocky Mountain Biomedical Engineering Society; Program Chair at the Academy of Surgical Research, Program and conference organizer at the Southern Biomedical Engineering meetings, Chair of Pathology Implant SIG at the Society for Biomaterials, to name a few. She served/serving in editorial boards in several journals as well as member of various NIH special review panels. She is serving as Chief Editor of the *Biomed Science Instrumentation* and Chief Editor for *Journal of the Mississippi Academy of Sciences*. Previously, she has been recognized for her work and service by the Academy of Surgical Research, the Mississippi Academy of Sciences Outstanding Contribution to Science, Peeler Dudley Outstanding Service Award, Douglas Walker Award and recently was inducted as fellow in American Institute of the Biomedical and Biological Engineering.

Keynote Speaker – III



" A NEW CONCEPT FOR HYDROXYAPATITE IN BONE REMODELING-THE NANOSTRUCTURE AND RESPONSE TO MECHANICAL STRAIN"

Miho Nakamura, Ph.D.
Associate Professor,
Tokyo Medical and Dental University

Miho Nakamura earned a Ph.D. in Biomaterials in 2007 and a Master's Degree in Medical Science in 2003 from Tokyo Medical and Dental University. Dr. Nakamura has received several awards, such as: The International Society for Ceramics in Medicine Excellence Award in 2016, Award for Young Researcher in The Ceramic Society of Japan in 2014, Award for Young Researcher in Japanese Society for Biomaterials in 2013, Award for Encouragement of International Exchange in Japanese Society for Biomaterials in 2012, Award for Young Researcher in Japanese Association of Inorganic Phosphorus Chemistry in 2010, and Award for Encouragement of Research in Materials Science in 2008.Dr. Nakamura is presently the Associate Professor with the Institute of Biomaterials and Bioengineering at Tokyo Medical and Dental University in Tokyo, Japan. She is also a Visiting Professor with the Department of Anatomy and Cell Biology at the University of Oulu in Oulu, Finland.

Keynote Speaker IV

" A BIOACTIVE CERAMIC FOR TREATMENT OF STRESS URINARY INCONTINENCE: ANALYSIS OF THE MECHANISM OF ACTION"

Ahmed El-Ghannam, PhD.
President, International Society for Ceramics in Medicine
Associate Editor, Journal of Biomedical Materials Research
Associate Professor of Tissue Engineering and Biomaterials,
Department of Mechanical Engineering and Engineering Science,
University of North Carolina at Charlotte

Dr. Ahmed El-Ghannam holds a BSc in Chemistry, MSc in Glass Science and Technology, and an MS and Ph.D. in Bioengineering from the University of Pennsylvania. He has over 30 years of experience in material science and bioceramics engineering. He has six US patents, many world renowned collaborators, and has been invited as a keynote and plenary speaker to various national and international meetings. He is the Associate. Editor for the Journal of Biomedical Materials and a leader in various prestigious societies. Dr. El-Ghannam's lab focuses on the development of bioceramics for multifaceted applications in drug delivery to treat cancer and infection, augment soft tissue and reconstruct bone. Dr. El-Ghannam's team includes clinicians, molecular biologists, and scientists who are widely published.

Keynote Speaker V

"Ceramic Delivery Systems and Future Impact on Health and Disease"

Hamed Benghuzzi, MS, PhD, FBSE, FAIMBE
Professor, Health Sciences
University of Mississippi Medical Center

Dr. Benghuzzi is a Professor at the University of Mississippi Medical Center. He is known nationally and internationally as a pioneer in Ceramic Drug Delivery Systems. He has over 250 PubMed indexed articles and over 700 abstracts detailing the release characteristics of various biologicals from the bioceramic carriers. He has trained more than 35 PhD students who are actively involved in academic careers. He has mentored students at all levels (from high school, undergrad, grad, post doc and faculty). He has served as a mentor for residents and faculty on more than 10 funded grants. He has been in research leadership roles in many organizations such President of the Academy of Surgical Research, Vice President of the Rocky Mountain Bioengineering Society, President of MAS, Academy's Executive Director, and also organized and chaired several regional, national and international society programs. He has also served on numerous NIH special emphasis panels including R-25, K01, KO8, T-35, and the P-60 center grants. In addition, he has received numerous awards from various organizations during his career. A few of his awards included: (1) The Presidential Award from the RMBS, (2) Presidential Award from SEM International, (3) the Endocrine's Society Outstanding Investigator Award, (4) MAS Contribution to Science Award, (5) The MAS Dudley Peeler Award, and (6) HEADWAE Award, (7) C. Hall Award, Outstanding Contribution to Biomedical Engineering (32nd SBEC), and (8) ISCM Excellence Award fron the International Society for Ceramics in Medicine. He was invited as a keynote/plenary to speak at state, national and international levels including recent invitations in France, Italy, Spain, Greece, China, Poland, Dubai and Canada. He is a fellow of the American Institute for Medical and Biological Engineering (AIMBE) as well as an International Fellow of Biomaterials Science and Engineering (FBSE).

3D HETEROGENEOUS BREAST TISSUE MICROENVIRONMENT USING POLYLACTIDE BEADS

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ABSTRACT

The microenvironment composition and density critically affect the breast epithelial cells behavior and can promote cancer development and progression. Those parameters are more suitably investigated in three dimensional (3D) *in vitro* culture systems. However, current 3D breast tissue systems poorly account for the heterogeneous density and composition of the extracellular matrix (ECM) observed within breast tissue. Here we investigated whether 3D matrices embedded with polylactide beads more closely mimicked the heterogeneous microenvironment of breast tissue. Briefly, breast epithelial cells were grown in 3D collagen / Matrigel® matrices embedded with polylactide beads and the development of complex structures i.e., acinus- and duct-like structures was monitored over time. Results indicate that polylactide beads coated with either media (117±4 um) or Collagen I (124±2um) had significantly smaller diameters on average than control beads in PBS (133±3um). The cells formed complex structures surrounding cluster of beads with cell strands migrating outward. The cell strands included both acinus- and duct-like structures. The length and the complexity of the cell strands formed in 3D matrix embedded with polylactide beads differed based on the beads' coating. Thus, embedding polylactide beads in an *in vitro* 3D test system may model the density heterogeneity of normal breast tissue.

Keywords: 3D, breast, polylactide beads, collagen, heterogeneous

INTRODUCTION

Breast cancer remains the most common cancer type in women and the second leading cause of death in the US [1]. Normal breast tissue is rather heterogeneous as are the breast cancer types mainly originating from the ducts [2]. Additionally, the microenvironment plays a critical role in the cancer progression through both the chemical and cell-cell interactions [3, 4]. Indeed, while two-dimensional (2D) cultures have provided key information, 3D cultures more closely recapitulate the breast tissue and the associated cancer progression [5, 6].

3D models of breast tissues and of breast cancer progression should allow the tuning of both breast structure and functions mimicking the natural physiology of the breast. Should three dimensional models would be invaluable in the understanding of breast cancer development, progression and in the evaluation of potential treatment regimens. Specifically, such 3D models should deepen our understanding of the complex cell-cell and cell-matrix interactions that are involved in the development of breast tissue as well as cancer initiation and progress. Three dimensional models of breast tissues also can serve as monitoring system for cellular processes that lead to tumor growth and invasion and as an alternative method for investigation new drugs or drug regimen. Importantly, 3D model may also be used as potential in vivo implants personalized to each patient's needs.

The challenges in the generation of 3D breast tissues remain to control specific features of normal breast tissue, the generation of functional acinus- and duct-like structures and the modulation of the complex cell-cell and cell-ECM interactions. In particular, current 3D models of breast tissue do not account for heterogeneous density observed within breast tissue. In prior investigations [7], we demonstrated the key importance of the composition and density of the extracellular matrix (ECM) in the generation of functional acini and ducts.

Here, we further our inquiry of the role of the density on the matrix using embedded poly-lactide beads to mimic the breast tissue heterogeneity. Our results indicate that the nature of the beads and more significantly of their coating affected the size/complexity of the 3D breast tissue model generated.

METHODS

Breast cells. MCF10A were purchased from ATCC (Manassas, VA) and used within passages 4-12. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM)/F12 medium (Cellgro, Manassas, VA, USA) supplemented with 5% horse serum (Lonza, Allendale, NJ, USA), insulin (10 mg/ml; Sigma-Aldrich, St. Louis, MO, USA), epidermal growth factor (20 ng/ml; Sigma-Aldrich), hydrocortisone (0.5 mg/ml; Sigma), amphotericin B (Cellgro), and gentamycin (Cellgro). Cell viability and counts were assessed using trypan blue dye (Cellgro). Cells were expended in 2D cultures conditions

in the media described above, detached using trypsin, washed, counted and used in the 3D model.

Polylactide Beads. Polylactide beads were a gift from Dr. Burg lab (Clemson University). Polylactide beads were prepared and stored under vacuum until use as described elsewhere [8].

3D breast heterogeneity models. After sterilization, beads were immersed in either PBS, F12/DMEM media or collagen I solutions overnight and rinsed in sterile PBS prior to use in 3D models. Bead diameters were determined post-coating. Diameter of the beads were derived from microphotographs obtained using a IX70 microscope displaying a calibrated scale using Image J software.

After layering and gelling a mix collagen I/Matrigel® (Biosciences, Bedford, MA, USA; 1:1 mixture) [7, 9], MCF10A cells alone or admixed with coated beads (17ug) were added to each well and the growth of breast like structures assessed overtime (up to 14 days) through microscopy (IX70 Olympus with camera DP70). For monitoring purposes, MCF10A cells were stained with the nuclear vital dye Hoechst 33342 (Promega, Madison, WI, USA; excitation 350 nm, emission 461 nm). Multiple overlapping microphotographs were stitched together to capture the entire structures. 3D model parameters measured using Image J software (NIH) included the total area of the structure generated and the length of the branching.

Extracellular protein and collagen remodeling. After a 14-day incubations, 3D structures were assessed for collagen and non-collagenous protein deposit following stain with Sirius Red and Fast Green (Chondrex, Redmond, WA), respectively with the MCF10A cells labeled with nuclear vital dye.

Statistical Analyses. All experiments were independently repeated (n=2-3) and data are presented as mean ± SEM. One-way and two-way ANOVAs along with post-hoc tests were used to assess differences in coated bead sizes, 3D area, branching overtime and matrix reorganization. *A priori*, $p<0.05$ was considered significant.

RESULTS

Coating poly-lactide beads with media or collagen I solution affected the diameter of the poly-lactide beads (133±3µm, 117±4µm vs 124±2um for PBS, Media and Collagen I coating, respectively, $p<0.05$).

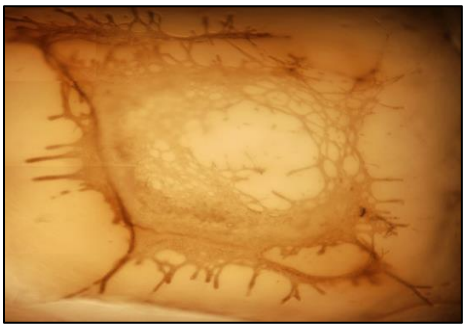
In both the presence and absence of polylactide beads regardless of the coating, MCF10A formed breast like structures including duct-like structures (Fig 1A). When cultured in the presence of beads, the area and the length of the duct-like formation differed (Fig 1A,B).

When compared the areas generated in the presence of media-coated or collagen I coated beads was significantly higher than the area generated without bead or in the presence of PBS coated beads (2-way ANOVA, overtime, $p<0.001$, Fig 2).

The number and length of the extensions outward was highly variable and tended to decrease especially in the presence of beads coated with media or collagens compared to no beads controls (n.s.)

To better ascertain the matrix changes associated with the presence of the coated beads, the presence of collagen I was determined. While overall collagen concentrations were not significantly different, the location of non-collagenous proteins overlapped the location of cells, whereas in highly rich collagen I areas, no MCF10A cells were present (Fig 3).

A **B**



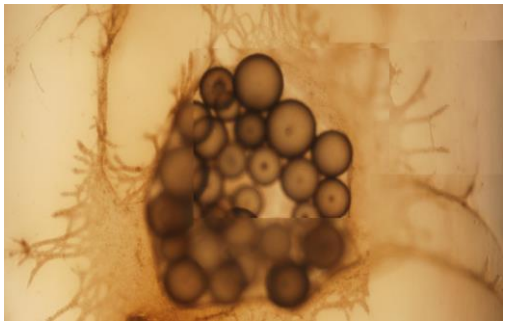


Fig 1. ***3D structures generated by MCF10A in the absence (A) or presence (B) of coated polylactide beads.*** Microphotographs taken on day 11 and stitched together highlighting the presence of 3D structures including duct-like and the complexity of the network formed.

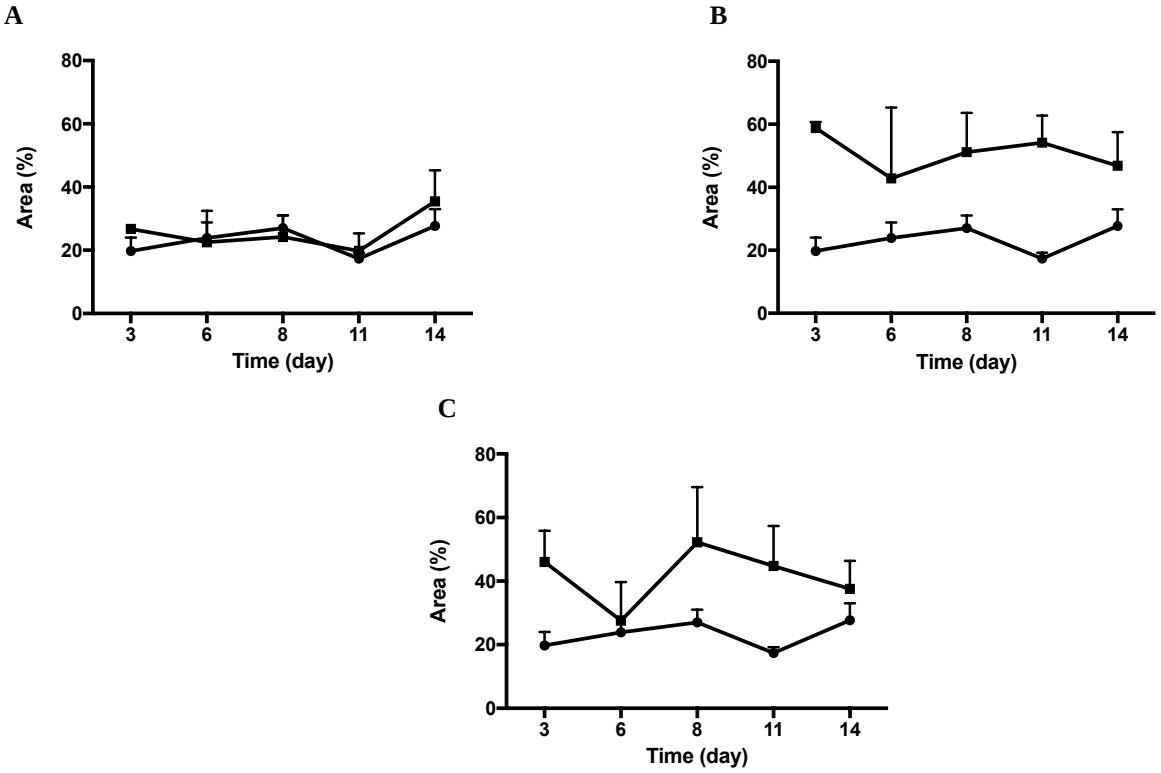


Fig 2. ***Media (B) or collagen I (C) coated beads significantly (ANOVA, p<0.001) increased the areas of the structures generated by MCF10A in contrast with PBS coated poly-lactide beads (A) or the absence of beads.*** On microphotographs taken overtime and stitched together, the total area was determined and compared to 3D MCF10A cultures without beads. Areas are expressed in % of the entire well.

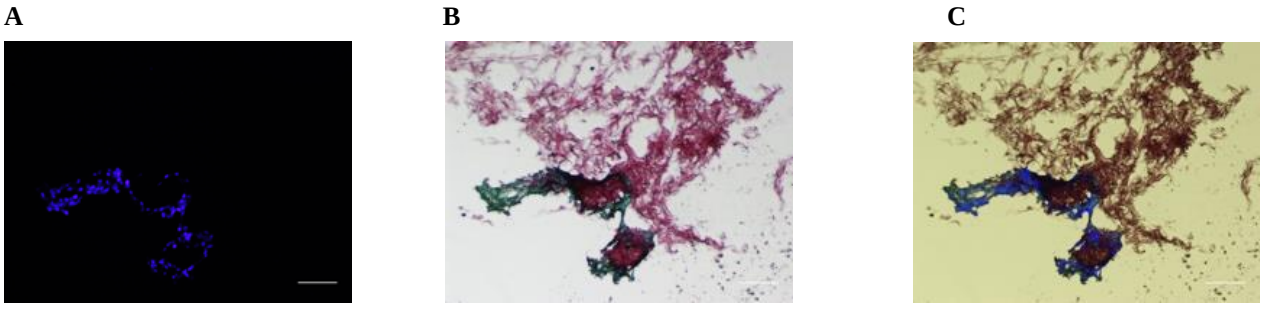


Fig 3. ***In 3D cultures, cells clustered in area rich in non-collageous proteins rather than in area rich in collagen.*** Representative microphotographs of MCF10A cells in 3D cultures with media-coated beads stained on day 14 with Hoechst (nuclear dye, **A**), non-collagenous proteins (green, **B**) and collagen I (red, **B**). The overlapping microphotograph (**C**) further highlights the co-localization of non-collagenous proteins and MCF10A cells.

DISCUSSION

In the present study, we assessed the potential of poly-lactide beads to mimic the heterogeneous breast tissue environment. Indeed, the density of the microenvironment, in particular of the ECM, defines, to a large extent, both the normal breast tissue and the breast

progression [3, 9]. The results indicate that in the presence of beads coated with media or collagen I, the area of the structure generated by MCF10A cells alone is significantly increased. Moreover, the differentiation of outward tubule-like structures appeared to be limited while the complexity of the branching tended to increase.

These results confirmed the observations made using various concentrations of collagen/Matrigel® leading to increased matrix density associated with drastic changes in the formation and function of the generated 3D tissue [7, 9]. Others have successfully developed matrices and cell combinations that closely mimic breast tissue [5, 6, 10]. However, the heterogeneity inherent to breast tissue remains a key challenge in developing 3D culture test system mimicking breast and breast cancer progression. Our data represent a first step in the generation of such models.

Key to the optimization of 3D modeling of breast tissue are the generation of standard tools including software and assays to confirm the structure and functionality of the generated 3D breast tissues. Recent works will facilitate those analyses including the development of new software allowing the estimation of volumes of 3D cell-based structures [11].

Moreover, the results presented here focus on a mono-culture (i.e., one cell type), current and future 3D models aim to mimic not only the heterogeneity of the matrix, of the chemical milieu but also of cell types present [3, 4, 9]. Clinically, the generation of 3D in vitro systems derived from cells isolated from individual patients may be used to further personalize the data gathered on the cancer type, progression, prognostic and especially best treatment approach [12, 13].

As the categorization of breast cancers and cancers in general become more refined and thus more amendable to specific targeted therapy, such 3D breast tissue and/or breast cancer progression models hold great promises both as predictive diagnostic tools and as approach to assess multiple therapeutic options and select the most beneficial.

CONCLUSIONS

Long-term, the data presented here will participate to the definition of reliable tools for the evaluation of breast cancer progression and the testing of specific treatments. Many steps remain before breast tissue 3D models can reliably be used as diagnostic and drug predictive tools in the clinical environment. Nevertheless, 3D models are closer to fulfill the potential of bioengineered tissues as viable options to further our understanding of breast cancer progression and as personalized test tissue systems to assess drug combinations relevant to the treatment of breast cancer.

ACKNOWLEDGMENTS

The authors would like to thanks Dr. Karen Burg (Georgia State University, Athens, GA) and her students at Clemson University for the preparation of the poly-lactide beads. Dr. Dréau also would like to acknowledge the participation of numerous undergraduate and graduate students that spent time in his laboratory collecting the data. This research was supported in part through NSF funding (EFRI program).

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PULSED LASER DEPOSITION OF BIOACTIVE COATING FROM WHITE PORTLAND CEMENT

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ABSTRACT

Objective. We report the study of feasibility to produce the thing bioactive coating from experimental dental cement using pulsed laser deposition (PLD) technique. ***Methods.*** The targets for PLD system (disks 30 mm in diameter × 5 mm thick) were sintered from micronized powder of set Alborg White Portland cement (WPC). The parameters for sintering process were chosen based thermo-gravimetric analysis and differential scanning calorimetry (TGA/DSC). The coatings were deposited by PLD on silicon substrates. The effect of laser power on coating crystallinity and morphology was evaluated by scanning electron microscope (SEM) and X-ray diffraction (XRD). The material transfer from target to substrate were evaluated by X-ray fluorescence (XRF) and X-ray energy dispersive spectroscopy (EDS). The bioactivity of deposited films was evaluated by ability produce the hydroxyapatite (HA) layer on a surface of specimen immersed in a simulated body fluid (Dulbecco's Phosphate-Buffered Saline (DPBS). The formation of hydroxyapatite was confirmed by SEM, X-ray energy dispersive spectroscopy (EDS), XRD and micro-Raman spectroscopy. The formation of HA was evaluated after 1, 3, 7, 14, and 21 days of immersion. ***Results.*** This study demonstrated that White Portland cement can be used as a target material for manufacturing of bio-functional coatings. The films deposited on Si substrates have mainly amorphous structure; the crystallinity of the film can be achieved by increasing the laser power. The biological performance of deposited films was tested by HA forming ability in simulated body fluid. The HA layer was formed on a coated surface after first day of immersion.

Keywords: dental cements, ceramic; hydroxyapatite, thin films, bio-functional coatings.

INTRODUCTION

MTA materials that derived from a Portland cements have been reported to be biocompatible. Their biocompatible nature defined by their ability to form bone-like hydroxyapatite (HA) when exposed to physiologic solutions [1-3]. Portland cements and their properties are well-studied and described in cement chemistry and industrial engineering, where the major focus are on settling/polymerization kinetics, bulk mechanical and corrosion properties of large structures [4-7]. Recently, the White Portland cement (WPC) draws a lot of attention as promising dental material due it property to form hydroxyapatite layer with immersion in PBS solution [3, 8-11]. Most Portland cements studies were focused on influence of composition and setting process parameters on mechanical and corrosion properties of cements. The typical analytical method used for composition analysis and phases determinations are: chemical analysis [12]; XRF and/or EDS analysis; optical methods [5]; X-ray diffractometry; thermogravimetry; IR and Raman spectroscopy [13-15]. Cements are complex multicomponent mixtures of crystalline and amorphous oxides with several levels of hydration, therefore combination of two-three methods commonly used to determine the phase composition. The evaluation of phase transitions in cements is became even more challenging task. Most of published research on cements phase transition focused on a hydration process and used pure components (mixing oxides to prepare cement with known quantities) for samples preparation and analysis [15, 16]. The group from Aalborg White Research and Development center [6] developed a model for predicting the metastable or stable phase composition of hydrated Portland cement systems from the chemical composition of the cement and the water/cement ratio. Besides composition of initial/raw cement, the other parameters of setting process effect on phase equilibria and mechanical properties of hydrated/set cement. It was reported that increasing the temperature and applying pressure during setting process speeds up the curing process and improves the mechanical properties of settled cement [17]. Using the mechanical mixing and ultrasonic agitation techniques also benefit the compressive strength of MTA-like materials [18].

We would like to emphasize that Portland cements were studied and analyzed as a construction materials with the focus on bulk materials properties, therefore in this research the cement is evaluated as a bio- and nano-materials. One of the biggest challenges of this research was to merge the knowledge of construction and medical fields. And so far, no reports were found about applying WPC as a bio-functional coating in a form of a thin film. The purpose of reported study is to evaluate the feasibility deposit the thing bioactive coating from WPC using PLD technique and confirm the bioactivity of deposited films.

METHODS

Materials Preparation and Characterization.

The cement used in this study was Type I WOPC (ASTM C150) Aalborg White from Aalborg, Denmark.

The crystallographic phase content was evaluated by X-ray diffractometry. The X-ray diffraction analysis was performed using Bruker D8 Advance diffractometer (Bruker Corp., Billerica, MA, USA) used CuKα radiation at 40 mA and 40 kV. The data collected in the 2θ range of 10–80°, at step of 0.02° and a step time of 0.5-1 second were used. Phase identification was accomplished using a search-match "EVA" software utilizing JCP database.

The component analysis was conducted using Micro-Raman scattering technique. The measurements were performed at room temperature using a Horiba LabRam Raman microscope equipped with a Leica microscope, a CCD camera, and laser at 532 nm with 10 mW laser power. Typical spectra from 80 to 4000 cm^{-1} were recorded with a resolution of 4 cm^{-1}. The time acquisition was 5 seconds and 10-20 scans were recorded to improve the signal-to-noise ratio. Correct calibration of the instrument was verified by the position of the Si band at 520.6 cm^{-1}. The peaks of cements were assigned in accordance to references [14, 15, 19-21].

The high-resolution scanning electron microscope (HR-SEM; Hitachi SU8000) with the attached energy dispersive X-ray analyzer (EDS) was used for samples imaging and evaluation of elemental compositions.

The thermogravimetric analysis (TGA) was performed using a SDT Q600 system (TA Instruments, USA) under argon atmosphere (50 sccm), at heating rate 20 °C/min, starting from room temperature up to 1200 °C. The average sample weight was 15 mg and an equivalent weight of α-Al_2O_3 was used as a reference.

PLD Deposition and characterization.

Neocera Pulsed Laser Deposition System was utilized for the deposition of films from sintered targets. The system equipped with a pulsed KrF laser (wavelength of 248 nm) operating at a pulse repetition rate of 1-10 Hz, the laser energy was varied from 200 to 500 mJ/pulse. The PLD method is proven to be an excellent method to transfer the stoichiometry of complex target material to substrate [22].

Bioactivity evaluation.

The bioactivity of bone-bonding material is commonly evaluated in vitro by immersion of the test material into simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma, and examining the formation of hydroxyapatite (HA) on its surface [23, 24]. Set of films, deposited in the same conditions, were immersed in 2 ml of SBF (Dulbecco PBS 1x) at 37°C for 1, 3, 7, 14 and 21 days. After immersion, the surface morphology of the samples and theirs composition were investigated by SEM, XRD, and EDS techniques. Based on reported studies [23, 25-27] we expected the formation of HA after 3–7 days of immersion, the crystallized fraction HA commonly reported after 14–21 days. The typical sign of apatite formation is flower-like HA crystals observed by SEM. In addition to imaging the XRD and Raman analysis employed to determine the type of apatite forming. The major characteristic of appetites is Ca/P ratio that was determined by EDS analysis, the ratio 1.67 indicates the bone-like apatite formation [28].

RESULTS

Effect of the sintering parameters on composition of cement.

The temperature dependent stability and reactivity of cement's phases and components were determined by the thermogravimetric analysis. The thermogravimetric (TGA) and differential scanning calorimetry (DSC) curves for the raw, hydrated (set) and sintered WPC are presented on figure 1. The weight loss peak around 100 °C indicates the hygroscopic water i.e. physically absorbed water. The zone between 100 and approximately 200 °C is attributed to dehydration of C–S–H gel. The step at 425–550 °C is due primarily to decomposition of portlandite (CH). Carbonates show distinctive decomposition peaks at the temperature range of 625–875 °C. The loss below the portlandite (550-600 °C) step is due to decomposition of C–S–H and the hydrated aluminate phases, but in this zone cement pastes show only slight indications of steps. The absence of steps can be attributed to a combination of low crystallinity, and/or the presence of other phases and compositions in mixture or solid solution [16, 29, 30].

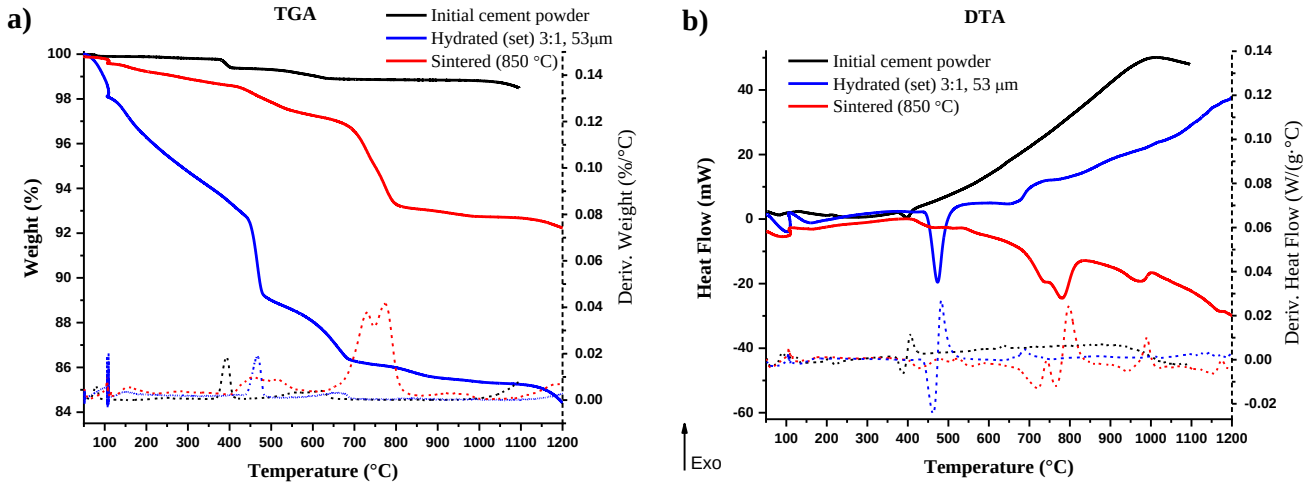

Figure 1. TGA (a) and DSC (b) curves for the raw, hydrated (set) and sintered WPC.

Based on thermogravimetric analysis, the two temperatures 850 °C and 1000 °C were used to sinter the targets for PLD process. The targets preparation process includes following steps: micronizing hydrated cement powder by ball-milling with zirconia balls; pressing green compacts (31 mm die diameter, 75 MPa applied pressure, no binder) and then sintering for 8 hours in Thermolyne™ furnace. The XRD and Raman analysis of sintered targets shown that increasing the sintering temperature changes the balance between alite (C3S) and belite (C2S) phases and decreasing content of portlandite (CH), calcium silicate (CSH) and ettringite (Aft) phases (figure 2). It should be noted that the shelf-life of the targets sintered at high temperatures (more than 900C) is very limited due high content of anhydrous phases that absorb moisture from air.

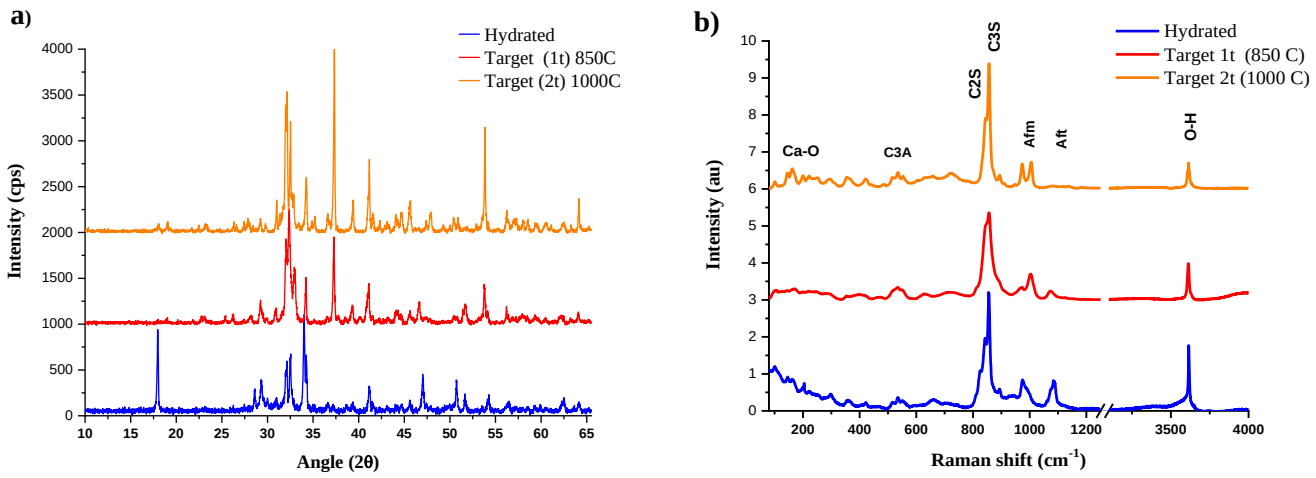

Figure 2. X-Ray patterns and Raman spectra of sintered and initial hydrated WPC.

Films deposition and characterization.

The films were deposited in a vacuum chamber (Neocera PLD system) by utilizing a pulsed KrF laser (Excimer) operating at a repetition rate of 10 Hz with a wavelength of 248 nm. The laser beam was focused on a rotating target at an angle of 45°, the laser energy was varied from 200 to 500 mJ/pulse. The focused laser beam was scanned across the rotating and rastering target to avoid deep crater formation on a target surface. Substrates were fixed at a distance of 4.5 cm from the target. The sample list with deposition parameters are presented in table 1.

SEM was used to give a visualization of the films (figure 3). It could be seen that the films deposited from both targets are amorphous with the particulates of crystalline chunks nucleated on the film surface or embedded in. It should be noted that with higher the laser energy increases the number of crystalline particulates in film (figure 3), what was also supported by XRD analysis. The X-ray diffraction pattern shown that the deposited films have amorphous structure in general (figure 4 a-b). The EDS analysis confirmed that there are no significant changes in elemental (chemical) composition of WPC during the film manufacturing process (figure 4c).

Bioactivity evaluation.

The SEM images shown formation of HA-like crystals after 1 day immersion on films deposited on Si (figure 5). The formation of HA after first day of immersion was also confirmed by Raman spectroscopy and XRD. The Raman spectra and X-ray patterns were compared to the reference calcium phosphates: HA (Sigma-Aldridge), mono-, di- and tri- calcium phosphates (MP Biomedicals).

Table 1. Deposition parameters and film thicknesses.

Sample #	Deposition Pressure (mbar)	Laser energy (mJ/pulse)	# of pulses	Film Thickness (nm)
Film 1	8.6 e-6	200	20,000	378 ± 8
Film 2	2.3 e-6	200	20,000	344 ± 6
Film 3	1.9 e-6	300	20,000	510 ± 12
Film 4	4.6 e-6	400	20,000	489 ± 12

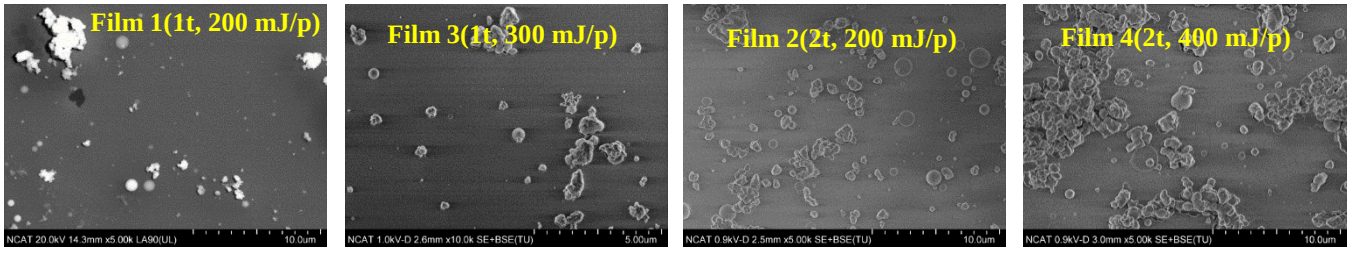

Figure 3. SEM images of films deposited on Si substrate.

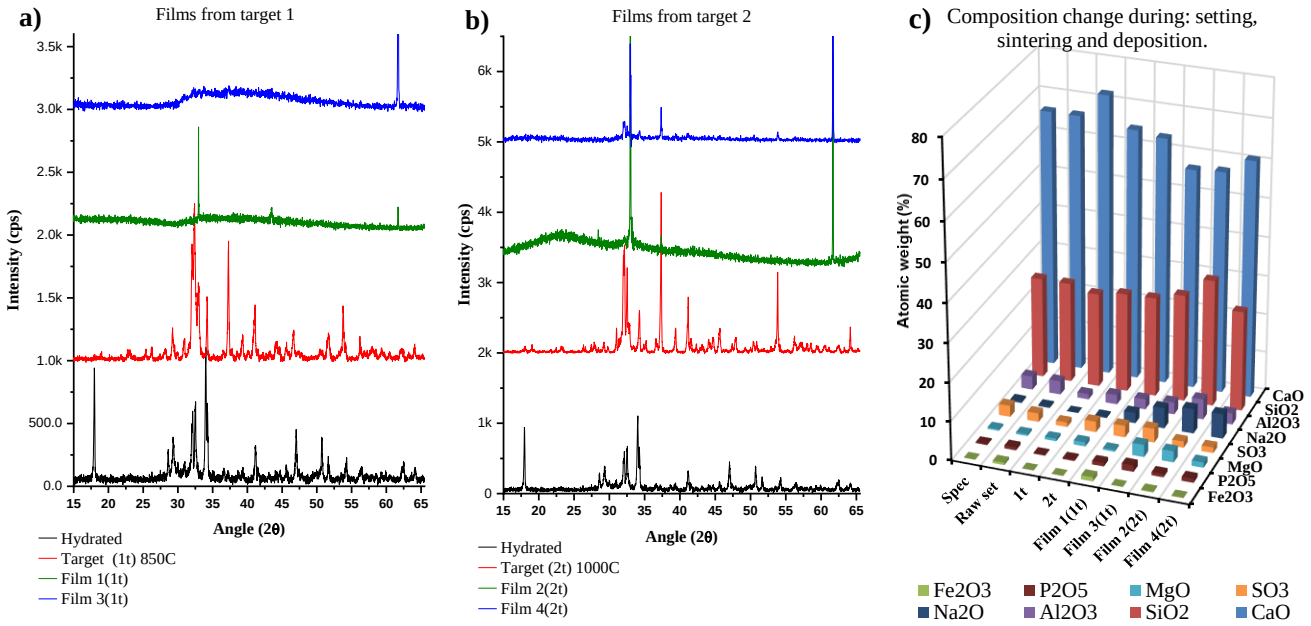

Figure 4. XRD patterns (a-b) and EDS analysis (c) of initial cement, sintered targets and films deposited from targets.

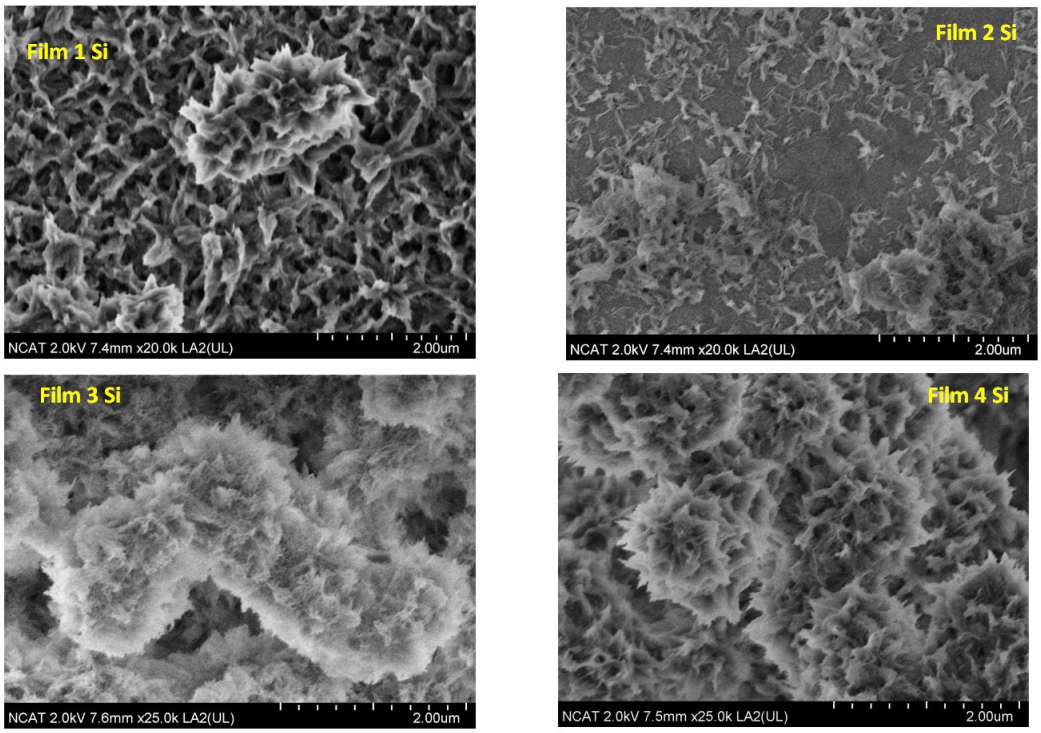

Figure 5. Formation of HA crystals on a surface of films after 1 day of immersion in SBF.

Raman spectra of films after 1 day immersion show the presence of HA peak (950 cm^{-1}) and the other HA characteristic peaks (420 and 570 cm^{-1}) that are overlapped with film spectra (figure 6). The width and shape of the peak shown that there is a mixture of di- and tri-basic calcium phosphates. Figure 6 presents the kinetic of HA formation on a surface of films studied by Raman spectroscopy. We could observe the changes in a shape and width of peak at 950 cm^{-1} from wide (sum of characteristics peaks of HA and dibasic calcium phosphate) to sharp peak of pure HA. Those changes are noticeable after 14 days of immersion for the film #4 (with the highest crystallinity) and after 21 days for films #1 and #3. The similar tendency of increasing HA content and crystallinity was detected by XRD (figure 7).

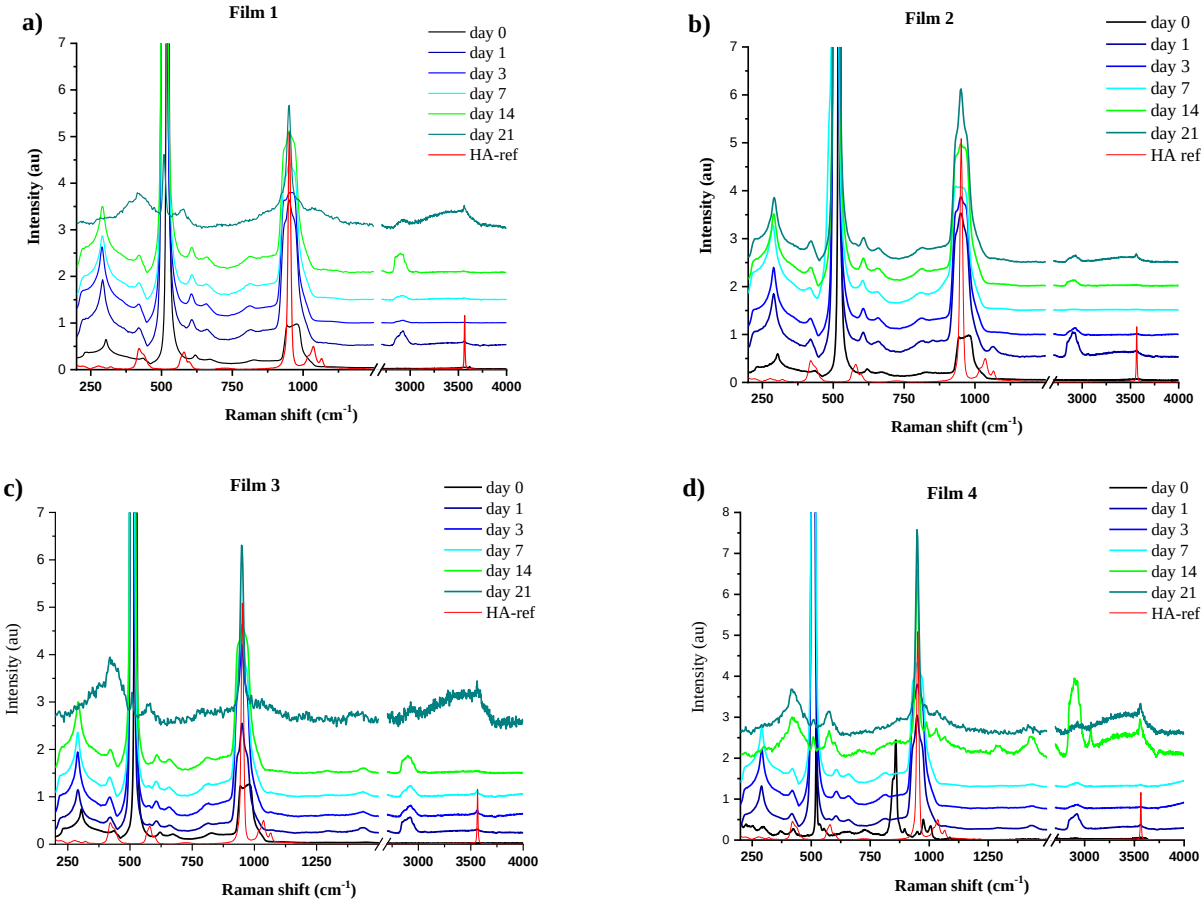

Figure 6. Raman spectra of films after immersion in SBF.

The XRD pattern of film 4 (figure 8) clearly indicates the formation of HA crystals that support the assumption that crystalline film has higher HA-forming ability. It should be noted, that crystalline films also has higher roughness and surface area which are beneficial for HA growth[24]. The EDS analysis revealed a Ca/P ratio of precipitated phosphates after 21 day of immersion values from 1.64 to 1.72.

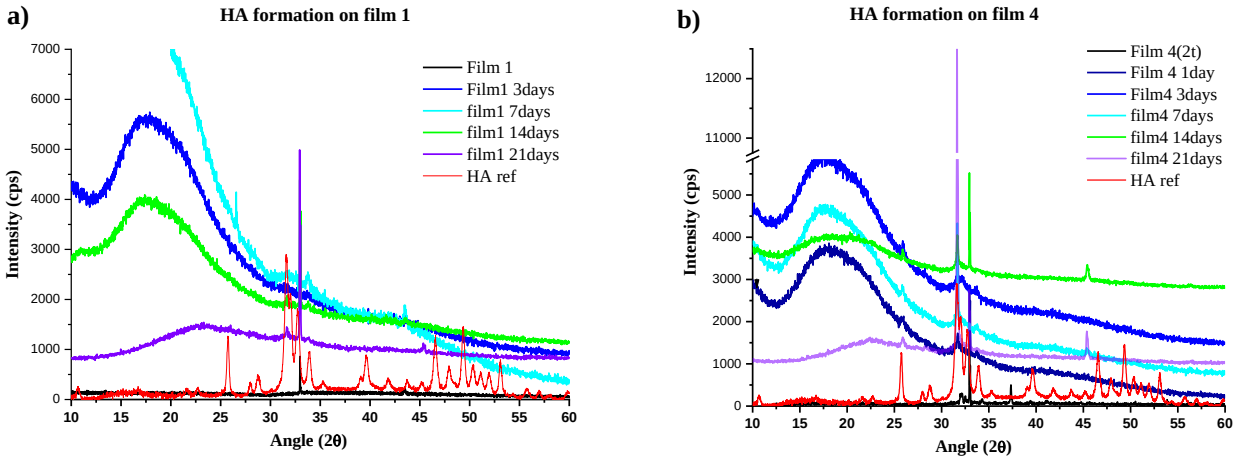

Figure 7. XRD patterns of amorphous (a) and semi-crystalline (b) films after immersion in SBF.

DISCUSSION

One of strategies to improve the osteointegration of implantable devices is to modify the surface of implant with the materials that are similar to the human hard tissues. The pure HA and other calcium phosphates are the most well-known and studied biocompatible materials that are used as implantable ceramic due to its chemical and structural similarity to the bone tissue [31-35]. However the simple mimicking the chemical composition of a bone is not enough to promote the osteointegration process at an adequate rate. It was suggested that Si might play an essential role in connective tissue metabolism [31]. The calcium-silicates and calcium-silicates-phosphates systems demonstrated improved bioactivity compared to pure HA [31, 36, 37]. The White Portland Cement used in our study belongs to calcium-silicates chemical group and shown bioactivity similar to Bioglass 45S5 system [23, 37].

Besides the chemical composition of implant surface the osteointegration process is highly depends on a structural properties of a surface such as roughness, degree of crystallinity, hydrophility, etc. The PLD process used to develop the thin films of WPC allows us to control the degree of crystallinity and thickness of films by varying the laser energy and number of pulses.

CONCLUSIONS

This study demonstrated that White Portland cement can be used as a target material for manufacturing of bio-functional coatings. The set of films was deposited from a sintered targets on Si substrates. The films have mainly amorphous structure; the crystallinity of the film can be improved by increasing the laser power.

The biological performance of deposited films was tested by HA forming ability in SBF. The results are very promising: all samples (films and sintered targets) have shown the formation of HA after 1 day of immersion.

ACKNOWLEDGMENTS

The authors acknowledge the financial support of the Engineering Research Center for Revolutionizing Metallic Biomaterials (NSF EEC 0812348) for their financial support and the Center for Advanced Materials and Smart Structures at North Carolina A&T State University for access to the necessary laboratory facilities and equipment.

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Data Analytics for Improved Decision Making at a Veterans Affairs Medical Center

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ABSTRACT

This paper reports on a study using data analytics for decision making at a Veterans Affairs (VA) Medical Center in Marion, IL, to improve patient outcomes. The SAIL (Strategic Analytics for Improvement and Learning) reports, released each quarter, are used to extract the outcome metrics, and a case is made to extract similar data for each division internally, a practice not currently used. At the overall VA level, the SAIL data is used for the following: a) color-coded visual charts that immediately indicate the problem areas, as compared to the national benchmarks, b) a color-coded chart that compares selected metrics to other VAs, to learn from them, c) a regression analysis that points to which metric to change for maximal impact, d) a mathematical model that provides results on potential changes to other metrics. A case study using more than four years of data is used to demonstrate the effectiveness of the methodology. It is possible that after seeing the data and studying the trend at other VAs, a decision is made to reduce the SMR30 by 5%. After running correlation algorithms, IHC (In Hospital Complications) is shown to be the most correlated with SMR30. A regression model is then developed that highlights that IHC would have to be decreased by 44% to attain the desired result. Past data shows that this is certainly feasible, and then a regression analysis is done to create models between IHC and the other metrics to see the consequence of the change. The model then predicts that MRSA (Methicillin-Resistant Staphylococcus Aureus) infection rate may decrease by 17%, but CAUTI (Catheter Associated Urinary Tract Infection) and PSI (Patient Safety Indicator) may increase slightly. This does not mean that this will come true, but one should monitor all of these metrics to ensure that there are minimal unintended consequences. The objective is to lay the groundwork for a healthy discussion between the executives, staff and clinicians on the path forward, resources required, and more importantly a progress dashboard that reflects weekly progress (data available internally from the IT department) rather than waiting three months for the SAIL report to show up.

Keywords: Veteran Affairs, patient outcomes, SAIL report, data analytics, executive decision

INTRODUCTION

There are over 5,000 registered hospitals in the US with approximately 900,000 beds [1]. Hospital care expenditure in the US is over $970B, up from about $9B in 1960 [2]. Healthcare is going through a major transformation, and will see significant efforts to lower costs, improve patient outcomes, and in general go towards increased efficiencies. Using data analytics for improved decision making is no longer a luxury but a necessity, and yet most major hospitals do not use the full potential of the data that they have to make decisions. The federal VA program is approximately 4% of the total healthcare industry, and provides care to the nation's veterans through 152 centrally administered hospitals. These hospitals do the best they can under trying conditions, severe budget issues and limited direction from the center other than to increase efficiency and provide improved care/outcomes. All of these problems stem from not having standardized tools for better decision making, so most decisions are made ad-hoc, typically responding to immediate concerns (e.g. reduce the 30-day mortality rate at the VA in Marion, IL). Managing this large enterprise is challenging, and using data analytics to improve patient outcomes and reduce healthcare costs has become a huge priority [3,4]. *Strategic Analytics for Improvement and Learning Value Model* or *SAIL* [5], is a system for summarizing hospital system performance within the VAs. The VA developed the SAIL model to measure, evaluate and benchmark quality and efficiency at all its medical centers [6]. The SAIL report is an excellent tool, and provides benchmarked data for all 152 VA centers. *It must be noted here that SAIL reports do not provide division level data, and a case is made in this paper for divisions, such as*

Cardiology, to request similar data from their IT departments on a weekly basis, if they are to have any chance of making lasting changes in their divisions.

Each quarter when the SAIL reports come out, there is usually a meeting called by the VA Chief of Staff, and is attended by numerous division heads, clinicians and staff. The SAIL report, which is a single Excel worksheet is usually put up on the screen. For the Marion VA, the immediate focus has been the 30-Day mortality rate (SMR30), as they are shown to be in the lowest 10% so there is intense discussion on how to improve it. The hospital has some of the finest clinicians in the country, so the executives and the staff struggle to find a viable roadmap to make changes as they do not know if this is a resource issue or a system inefficiency. There are very few visual tools to help the staff make sense of the data, and there are certainly no tools to see past trends, model the system, play what-if scenarios, etc. Hence, typically, committees are put together to consider the matter, make suggestions, and usually there is little appreciable difference year after year. That is certainly not due to the staff not making a concerted effort, but it is more to do with not having the tools to make informed data-driven decisions.

There is a critical need for new ways to use the SAIL data, identify inefficiencies, and execute/monitor the changes made. This paper provides a methodology to do exactly that, so that individual divisions can compare themselves to other more efficient counterpart divisions around the country, and make deliberate changes, and monitor the progress weekly, rather than quarterly.

Data Analytics: Visualization leading to Prioritization

The SAIL reports are an excellent system to concisely provide information on 32 metrics to all 152 VAs along with benchmark data, but the VAs do not have an easy-to-understand visualization tool, and most discussions are still based on just pointing to the numbers on the SAIL report (see Figure 1 for a typical SAIL report). Our approach is to take the numbers from the SAIL report and provide a visual method for honing in to what are the most critical areas to talk about. The SAIL report, as mentioned before, has 32 metrics. Each metric row is followed by 5 items in the 5 columns:

Col 1: how the metric was scored

Col 2: preferred direction for metric (should be low or high)

Col 3: metric score for that VA

Col 4: benchmark for all 152 VAs

Col 5: 10^{th}-50^{th}-90^{th} percentiles

Trying to make decisions by studying the SAIL report is not easy, and often leads to more confusion, especially since for some metrics, the preferred direction is going up, while for others it may be going down.

This paper extracts the data from the SAIL report and provides an easy-to-understand and the intuitive first step is to provide a visualization tool that gives a **"Green"** color for those in the top 50%, **"Yellow"** for those between 50 – 10%, and **"Red"** for those in the lowest 10% in the country, and therefore calling for immediate action (see Figure 2). Just a simple glance at the plot shows that there are five metrics that need immediate attention as they are in the lowest 10^{th} percentile, and it turns out that one of the most important metrics, SMR30, is one of these (the second bar in Figure 2).

Now that we have identified that SMR30 is the outcome metric that we need to bring down, we consider five variables given below, as the primary variables influencing SMR30. All of the data is available in the SAIL reports, but at the hospital level, but each division within the hospital can request division level data from their IT department.

1. In Hospital Complications → IHC
2. Health care Associated Infections (HAI)
    a. Catheter Associated Urinary Tract Infection: → CAUTI
    b. Central Line Associated Bloodstream Infection: → CLABI
    c. Ventilator Associated Pneumonia: → VAP (Not used due to lack of data)
    d. Methicillin-Resistant Staphylococcus Aureus Infection: → MRSA
3. Patient Safety Indicator → PSI

Strategic Analytics for Improvement and Learning (SAIL)

NOTE: EFFICIENCY FOR FY2013-2014 IS BASED ON FY2013 DATA; INPATIENT SHEP AND PCMH SURVEY FOR FY2014Q4-FY2015Q1 IS BASED ON FY2014Q4 DATA.
SAIL IS REFRESHED ON A QUARTERLY BASIS. MEASURE VALUES MAY CHANGE IN ACCORDANCE WITH CHANGES IN THE SOURCE DATA.

Marion IL Scorecard for FY2015Q1

Measure	Measure Unit	Preferred	Marion IL	Benchmark	10th-50th-90th ptile
Acute care mortality					
1. Acute care Standardized Mortality Ratio (SMR)	O/E	↓	0.951	0.483	0.483 - 0.877 - 1.178
2. Acute care 30-day Standardized Mortality Ratio (SMR30)	O/E	↓	1.455	0.731	0.731 - 0.955 - 1.192
Avoidable adverse events					
1. In-hospital complications	O/E	↓	1.868	0.339	0.339 - 1.052 - 1.523
2. Health care associated infections (HAI)					
a. Catheter associated urinary tract infection	inf/1k device days	↓	0.731	0.000	0.000 - 1.013 - 3.013
b. Central line associated bloodstream infection	inf/1k device days	↓	0.000	0.000	0.000 - 0.478 - 1.471
c. Ventilator associated Pneumonia	inf/1k device days	↓	0.000	0.000	0.000 - 0.000 - 3.442
d. Methicillin-resistant Staphylococcus aureus (MRSA) infection	inf/1k bed days	↓	0.137	0.000	0.000 - 0.082 - 0.284
3. Patient safety indicator (PSI)	O/E	↓	0.000	0.000	0.000 - 0.759 - 1.184
CMS 30-day Risk Standardized Mortality Rate (RSMR)					
1. AMI RSMR	%	↓			
2. CHF RSMR	%	↓	7.248	6.469	6.469 - 7.508 - 8.850
3. Pneumonia RSMR	%	↓	9.069	7.474	7.474 - 9.152 - 11.290
CMS 30-day Risk Standardized Readmission Rate (RSRR)					
1. AMI RSRR	%	↓		16.336	16.336 - 16.386 - 16.451
2. CHF RSRR	%	↓	18.304	17.892	17.892 - 19.422 - 21.678
3. Pneumonia RSRR	%	↓	14.583	13.507	13.507 - 14.956 - 16.540
Adjusted length of stay	days	↓	4.540	3.674	3.674 - 4.500 - 5.395
Performance measures					
1. Inpatient performance measures (ORYX)	%	↑	92.584	99.492	95.407 - 97.734 - 99.492
2. Outpatient performance measures (HEDIS like)	wct %	↑	91.190	91.473	87.271 - 89.457 - 91.473
Customer satisfaction					
1. Patient satisfaction	score (0-300)	↑	265.670	267.586	238.804 - 256.250 - 267.586
2. Best places to work	score (1-100)	↑	58.254	64.734	49.650 - 58.185 - 64.734
a. Overall job satisfaction	score (1-5)	↑	3.631	3.737	3.448 - 3.609 - 3.737
b. Satisfaction with organization	score (1-5)	↑	3.461	3.605	3.150 - 3.410 - 3.605
c. Recommend my organization as a good place to work	score (1-5)	↑	3.751	3.872	3.430 - 3.659 - 3.872
3. Registered nurse turnover rate	%	↓	3.814	3.491	3.491 - 6.242 - 11.213
Ambulatory Care Sensitive Condition hospitalizations	hosp/1000 pts	↓	33.579	20.257	20.257 - 26.169 - 32.975
Access					
1. Primary care wait time					
a. New primary care appointments completed within 30 days from preferred date	%	↑	98.857	99.740	83.536 - 97.367 - 99.740
b. PCMH Access composite	casemix adjusted %	↑	40.996	53.800	32.078 - 42.025 - 53.800
i. Get an urgent care appointment as soon as needed	casemix adjusted %	↑	43.785	59.919	30.553 - 45.549 - 59.919
ii. Get a routine care appointment as soon as needed	casemix adjusted %	↑	60.330	66.284	41.417 - 54.364 - 66.284
2. Specialty care wait time					
a. New specialty care appointments completed within 30 days from preferred date	%	↑	93.936	98.551	89.604 - 95.595 - 98.551
3. Mental health wait time					
a. New mental health appointments completed within 30 days from preferred date	%	↑	99.508	99.885	96.667 - 99.196 - 99.885
4. Call responsiveness					
a. Call center speed in responding to calls in seconds	seconds	↓	114.648	19.052	19.052 - 58.461 - 195.731
b. Call center abandonment rate	%	↓	24.943	3.359	3.359 - 8.918 - 22.063
Mental Health	Standardized score	↑	-0.204	1.130	-1.351 - -0.094 - 1.130
1. Population coverage	Standardized score	↑	-0.407	1.147	-1.194 - 0.009 - 1.147
2. Continuity of care	Standardized score	↑	-0.579	0.994	-1.167 - -0.083 - 0.994
3. Experience of care	Standardized score	↑	0.586	1.023	-1.153 - -0.046 - 1.023
Efficiency (1/SFA)	score (0-100)	↑	91.466	96.093	91.008 - 94.386 - 96.093

Figure 1. SAIL Report

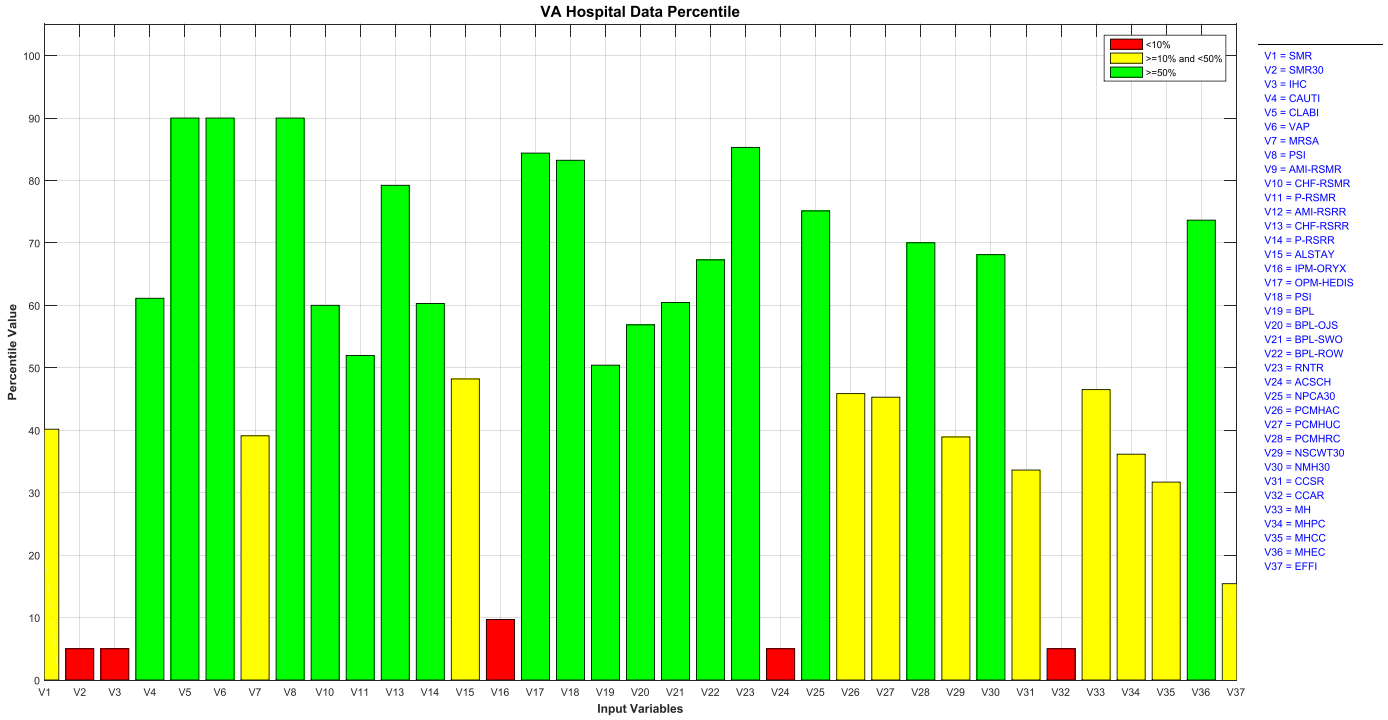

Figure 8. New visualization tool

Data Analytics: Comparison with other VAs

Given in Figure 3 is a color-coded chart that compares SMR30 and the five of the six metrics, that have data available, for various other VAs around the country (Erie, PA; Hampton, VA; St. Louis, MO; Wichita, KS). This chart helps in visualizing how important the metrics are in relation to the SMR30. For example, better IHC and CAUTI numbers lead to a better SMR30 for Erie, PA. For Marion, IL, to improve their SMR30, they must improve their IHC and CAUTI numbers.

The question then is which one to target first: IHC or CAUTI? We have at our disposal data from 17 quarters for all VAs, and we would like to use that to see if we can find these correlations. For the purposes of this study, we will look at the 17 quarters worth of SAIL data for Marion VA, Illinois. It must be noted here, that the objective of this study is to develop a methodology to use data analytics to find insight from the data, and once we can reach some actionable conclusions, we can easily scale the study to any VA in the country. It must also be noted that some VAs do not have data for some metrics in one or more quarters, so any approach must consider missing data (as we did in this section by not considering VAP).

	Erie	Hampton	Marion	St.Louis	Wichita
SMR30	63.028%	90.683%	5%	35%	46.719%
IHC	100%	73.511%	20.952%	21.275%	28.528%
CAUTI	90%	90%	46.49%	49.58%	90%
CLABI	No Data	17.74%	90%	5%	90%
MRSA	90%	34.182%	37.339%	30.727%	10%
PSI	10%	5%	90%	65.827%	49.097%

Figure 9. Color-coded visualization tool

Data Analytics: Identifying a roadmap for change

This paper reports on using regression analysis to find the correlations between SMR30 and the other metrics, with the correlation coefficient being the number that is to be calculated. The correlation coefficient is a measure that determines the degree to which two variables are associated. The range of values for the correlation coefficient is -1.0 to 1.0 and it can't be greater than 1.0 or less than -1.0. A correlation of -1.0 indicates a perfect negative correlation, while a correlation of 1.0 indicates a perfect positive correlation. The most common calculation is known as the Pearson product-moment correlation. It is determined using the below equation,

$$r_{xy} = \frac{\sum_{i=1}^{n}(x_i-\bar{x})(y_i-\bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i-\bar{x})^2 \sum_{i=1}^{n}(y_i-\bar{y})^2}} \quad \textbf{(eq.1)}$$

$\sum$ is sigma, the symbol of 'sum up'

$(x_i - \bar{x})$ is the difference between each x value and the mean of *x*

$(y_i - \bar{y})$ is the difference between each x value and the mean of *y*

The correlation values between SMR30 and the selected metrics are shown in Table 1 As can be seen above, SMR30 is the most correlated with IHC (correlation coefficient of 0.4725). This is a very reliable outcome as we had data for all 17 quarters.

Table 1. Correlation Values

	IHC	HAI - CAUTI	HAI - CLABI	HAI - MRSA	PSI
Marion	**0.4725**	-0.1424	Missing Data	0.1682	-0.0573

Data Analytics: Modeling to study the impact of changes

This, by far is the most significant contribution of this paper, i.e. the ability to model the impact of changing any metric on other metrics. A stepwise regression analysis was performed on the data to come up with the following linear models with a confidence level of 93%. In the regression analysis, response variable was taken as SMR30 and the continuous predictors were IHC, CAUTI, MRSA and PSI. The regression equation ended up with just IHC and excluded other predictors demonstrating that SMR30 is highly related to IHC. This statistical model that relates SMR30 to IHC is used to target a change to be made in IHC based on a desired change in SMR30:

SMR30 = 1.028 + 0.1934 IHC

The models which predict the consequences of making the above change in IHC on other critical metrics such as MRSA, CAUTI and PSI are obtained by simple linear regression and are given by:

MRSA = 0.1075 + 0.0600 IHC

CAUTI = 1.889 - 0.488 IHC

PSI = 0.761 - 0.102 IHC

Figure 4 is the plot of the four models. The x-axis is values for IHC ranging from 0.0 to 2.0. The solid black line is the plot for SMR30, and desired direction for it is shown (high to low). This is the driver for all changes to be made.

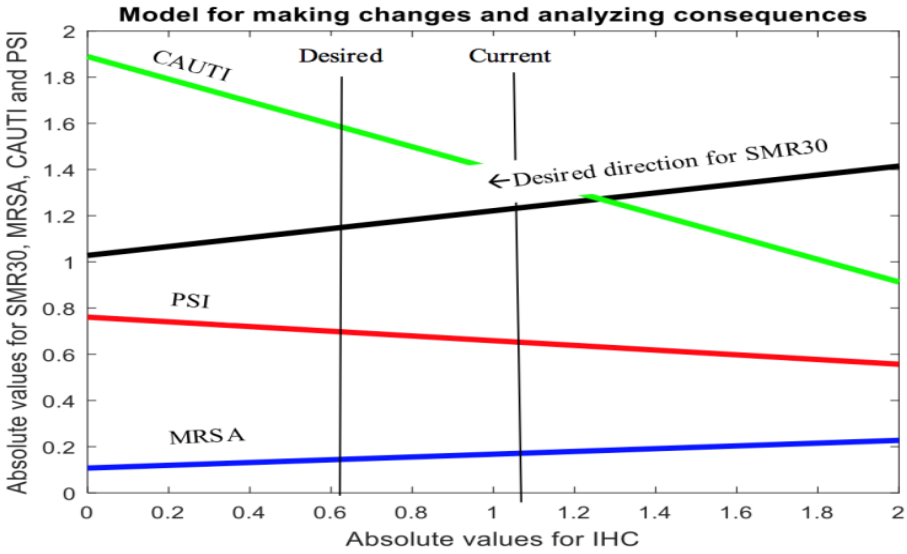

Figure 4. Modelling Results

Data Analytics: Case Study

Given below are the results from the model (also shown visually in Figure 4). Let us try and see what happens when we want to reduce the SMR30 value by 5%.

Current SMR30 value: 1.2070

Corresponding IHC value: 1.1030 (vertical line on plot labeled current)

New desired SMR30 value: 1.2070*0.95 = 1.1466

Targeted IHC value from model: 0.6135 (decrease of 44% from 1.1030)

Consequence on MRSA: 0.1443 (decrease of 16.9% from 0.1737)

Consequence on CAUTI: 1.5896 (increase of 17.7% from 1.3507)

Consequence on PSI: 0.6984 (increase of 7.7% from 0.6485)

As can be seen above, a desired 5% change in SMR30 would require a targeted 44% decrease in IHC. If this change was accomplished, then this would come with good consequences on MRSA, but potentially unintended consequences on CAUTI and PSI. It is this knowledge that allows healthcare executives to make informed decisions, and monitor the progress to make sure that the unintended consequences are minimized.

CONCLUSION

This reports on a study using data analytics for decision making at the Veterans Affairs (VA) Medical Center in Marion, IL, to improve patient outcomes, specifically the SMR30 (30-day Standardized Mortality Ratio). At the overall VA level, the SAIL data is used for visualizing the data so that critical problem areas can be quickly identified and then compared to other VAs around the country. A regression analysis is then conducted to see which metric to target so as to have the maximum impact on SMR30, and finally a statistical

model is developed to have some idea on intended and unintended consequences of making any changes. A case study using more than four years of data is used to demonstrate the power of the methodology. It must be noted here, that the output of the model is based on data analytics, and may not necessarily be clinically accurate, but it does provide a framework for making changes, and monitoring the consequences. This paper has shown a data-driven methodology for using the SAIL data to make decisions at the overall VA level, but more importantly this approach can be used at the division level, where the actual changes need to be made. The division level data is not part of the SAIL report, but may be requested by the division heads internally, and then used exactly via the methodology presented in this paper to improve their division level patient outcomes.

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THE EFFECTIVENESS OF DRY NEEDLING ON THE REDUCTION OF PROXIMAL UPPER QUARANT PAIN USING COHEN'S d: A SYSTEMATIC REVIEW

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ABSTRACT

Upper quadrant pain secondary to active myofascial trigger points (MTrPs) is a common cause of referral for physical therapy intervention. Dry needling (DN) is the use of needles inserted directly into the muscle at an MTrP. The literature contains conflicting reports to whether DN of MTrPs or trigger points (TrPs) in the upper quadrant is efficacious in reducing pain. The purpose was to determine if DN treatment to the trapezius muscle in patients with proximal upper quadrant pain reduced pain intensity in comparison to controls and other interventions using outcome measures known as Cohen's d. Eleven randomized clinical trials were assessed in this systematic review. The mean PEDro score of the studies was 6.5 with a range of 4 – 8. Using Cohen's d to measure the efficacy of DN treatment, DN had a large effect and more superior for pain control in five studies compared to the controls/interventions (ES range of 0.81 to 17.46; CI of 0.08 – 1.50 and 15.19 – 19.50 respectively); moderate effect in one study which was not significant (ES of 0.52; CI: -0.15 – 1.8) and trivial/small effect, also not significant, in two studies (ES of 0.07 to 0.23; CI: -0.86 – 1.00 and -0.18 – 0.63). In three studies, DN appeared to be less effective but insignificant, when compared to the control or other interventions (ES range of -0.32 to -0.14; CI of -0.18 – 0.63 and -0.89 – 0.60 respectively). Besides having a large effect size in favor of DN in study 2, DN appeared less effective (but not significant) versus STT post-intervention and OMT at both post-intervention and follow up (ES range of -0.61 to -0.23; CI of -1.41 – 0.23 and -1.03 – 0.58.). However, p-value and Cohen's d as measures of outcomes do not totally not see results or outcomes at the same lenses. Cohen's d may probably be better in looking for clinical significance and the magnitude of such significance. Chronic pain, such as pain in the upper quadrant/trapezius that may not respond to traditional physical therapy, may be relieved with DN treatment. When considering treatment options for pain reduction, it is suggested that DN be taken into consideration. More research is necessary to document the effectiveness and to elucidate the mechanism of DN.

Keywords: Dry needling, pain, myofascial trigger points, acupuncture, mechanism.

INTRODUCTON

The management of Pain continues to be a mayor focus in physical therapy clinics and varying therapeutic interventions are employed. There are conflicting reports in the literature that DN of myofascial trigger points (MTrPs) or trigger points (TrPs) is efficacious in reducing spinal pain. The mechanism by which DN reduces pain is also not fully elucidated. The application of DN (thin filiform needles) employs different techniques, including but not limited to winding, several stabbings (pistonings) of the needle into the site, inserting the needle into the site for a certain amount of time and electrical dry needling (EDN). Irrespective of the technique of application, pain reduction of varying degrees may be observed as demonstrated in the literature. Also, Acupuncture and DN may have different philosophies but procedure of inserting the needle into the body is essentially the same and both are targeting pain reduction essentially. How effective DN is may be dependent on the statistical analysis used, p-value or effect size. There is argument in the literature that p-value that measures statistical significance may not be enough when measuring clinical effectiveness of an intervention. In differentiating between effect size and p-value, Sullivan and Feinn [37] stated that "a *P* value can inform the reader whether an effect exists, the *P* value will not reveal the size of the effect". In arguing the need for effect size in reporting quantitative study, Cohens [38] stated that "The primary product of a research inquiry is one or more measures of effect size, not p values". Effect size is defined as the *magnitude of the difference between groups*, whereas the *absolute effect size* is the difference between the average, or mean, outcomes in two different intervention groups [37]. If DN has a clinical effect, using p-value to measure the effect may not be enough.

The development of MTrPs and subsequent pain formation is complex. It is speculated that MTrP is developed due to increased release of acetylcholine (ACh) from motor endplates and $Ca2^{+}$ resulting in continuous state of localized shortening and contractures of sarcomeres (muscle contraction) [8]. There is localized hypertonicity which then begin to cause ischemia and hypoxia [9], and subsequently, chemicals (such as bradykinin, prostaglandins, serotonin, calcitonin gene-related peptide (CGRP) and substance P) responsible for the propagation of pain and inflammation are released. In addition, several inflammatory cytokines, such as tumor necrosis factor-α (TNF- α), interleukin-1ß (IL-1ß), interleukin-6 (IL-6), and interleukin-8 (IL-8) are released [9]. High level of H^{+} ions and adenosine triphosphate (ATP) [10], are released resulting in low pH which further propagates the action of ACh (inhibition of acetylcholinesterase). The continuous presence of factors of pain and inflammation results in sensitization of the sensory afferent nerve fibers of the muscle and this may explain the point tenderness of MTrPs [10, 11, 12]. Eventually, this may lead to central sensitization of the dorsal horn neurons (which is an increase of the excitability of neurons within the central nervous system) causing pain hypersensitivity. The question is, why is DN able to mediate pain reduction in MTrPs and other tissues?

If DN has effects on pain control, what is the best way the effects can be measured to determine clinical efficacy? The use of p-value is being challenged as it may not give a clear clinical effects magnitude. Therefore, the purpose of this review is to evaluate the effect of DN treatment on proximal quadrant pain and using Cohen's d as an outcome.

METHODS

Databases, including Pubmed, Embase and PEDro databases were accessed October – November 2016 for articles published not more than 10 years at the time of the search . The randomized control trial articles were selected with inclusion criteria including intervention of DN into the trapezius muscles, clinical trials, musculoskeletal pain in proximal upper quadrant (neck, cervical, shoulder, scapular, upper back, and upper thorax), and studies that compared the effects of DN to alternative treatments pain reduction, sham DN, or pure control. Study quality was evaluated using PEDro criteria. The PEDro is a 10-point scale for assessing internal validity (higher scores indicating higher quality). PEDro risk of bias assessment was used. Information taken from the articles includes population, intervention group, comparison group(s), and post-intervention and follow up pain outcomes (VAS). Clinical trial quality was evaluated for each article and data was extracted for comparison. Outcome measures used for comparison is Cohen's d which measures the effect size of the study. Effect size was measured by taking the difference between two means, or mean (group 1) – mean (group 2) / standard deviation. Cohen classified effect sizes as small ($d = 0.2$), medium ($d = 0.5$), and large ($d \geq 0.8$).

RESULTS

Eleven randomized clinical trials were assessed in this systematic review (Table 1). The mean PEDro score of the studies was 6.5 with a range of 4 – 8. Using Cohen's d to measure the efficacy of DN treatment, DN had a large effect and more superior for pain control in five studies compared to the controls/interventions (ES range of 0.81 to 17.46; CI of 0.08 – 1.50 and 15.19 – 19.50 respectively) [31,32,6,7,36]; moderate effect in one study which was not significant (ES of 0.52; CI: -0.15 – 1.8) [33]; and trivial/small effect which was also not significant, in two studies (ES of 0.07 to 0.23; CI: -0.86 – 1.00 and -0.18 – 0.63) [5,35]. In three studies, DN appeared to be less effective but insignificant, when compared to the control.or other interventions (ES range of -0.32 to -0.14; CI of -0.18 – 0.63 and -0.89 – 0.60 respectively). Besides having a large effect size in favor of DN in study 2, DN appeared less effective (but not significant) versus STT post-intervention and OMT at both post-intervention and follow up (ES range of -0.61 to -0.23; CI of -1.41 – 0.23 and -1.03 – 0.58.) [31] (Figure 1).

DISCUSSION

This review determines the effects of DN on upper quadrant pain and using Cohen's to measure outcomes unlike the popular p-value that has been used to measure statistical significance. Statistical significance may not give the effects or magnitude of effects of an intervention [37]. This study presented the results of the study in categories of large, moderate or small effects. It is important to compare some of the results of the studies using the p-value and Cohen's d. Some of the studies presented confusing results using p-value or Cohen's d. For example, the study of Llamos-Ramos, R., et al [5], presented statistical significance of p-value of $p < 0.01$ to 0.001 and using Cohen's d and the same data, the results favor DN to Trp MT but at a trivial level that is not significant. On the other hand, Mejuto-Vazquez, M. [6], study presented p-value of statistical significance of $p < 0.001$, and using Cohen's d, DN resulted in large effect compared to the control and this large effect size is

significant. In this study, we can see where p-value and Cohen's d for measurement of outcomes tend to agree in some of the studies. This is true of the study of Pecos-Martin, D [7] that presented a $p < 0.001$ and using Cohen's d, a large effect with significance is also reported. There was no main difference between DN of TrPs of upper trapezius muscles and DN of TrPs plus paraspinal muscles ($p<0.05$) but using Cohen's d, DN of TrPs of upper trapezius plus paraspinal muscle produced small effect (not significant) but in favor of the DN TrPs plus paraspinal muscles. Using Cohen's d to measure the efficacy of DN treatment, DN had a large effect and more superior for pain control in five studies compared to the controls/interventions (ES range of 0.81 to 17.46; CI of 0.08 – 1.50 and 15.19 – 19.50 respectively) [31,32,6,7,36]. Moderate effect in one study which was not significant (ES of 0.52; CI: - 0.15 – 1.8) [33]. Of the eleven articles reviewed we have trivial/small effect, also not significant, in two studies (ES of 0.07 to 0.23; CI: -0.86 – 1.00 and -0.18 – 0.63) [5,35]. Three of the eleven studies favored control and other interventions over DN but the differences were not significant. The application of DN for upper quadrant pain is seen in these reviews to be positive for pain control. Using Cohen's d for magnitude of effects of intervention may be more useful clinically as there might be a difference between statistical significance and outcome measures like Cohen's d that gives a magnitude of the difference and whether that difference is significant or not looking at the CI. What is not clear is the mechanism with which DN controls pain, and this is not covered in this study. The application of DN (thin filiform needles) employs different techniques, including but not limited to winding, several stabbings of the needle into the site (sometimes referred to as "pistoning"), inserting the needle into the site for a certain amount of time and electrical dry needling (EDN) in some cases.

Irrespective of the technique of application, pain reduction of varying degrees may be observed as demonstrated by this current review. Also, Acupuncture and DN may have different philosophies but the procedure of inserting the needle into the body is essentially the same and both are targeting pain reduction essentially.

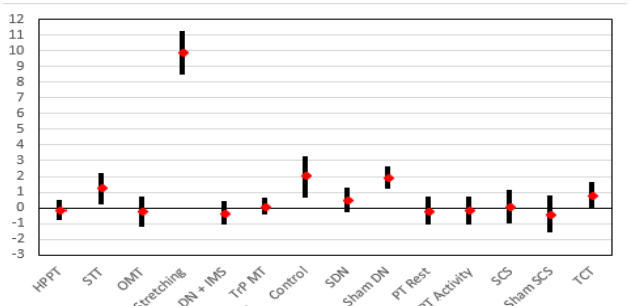

Fig.1 • Effect Size | Confidence Interval (Lower & Upper)

Study Summary Table 1.

Article	Population	Intervention Group(s) (Includes DN)	Comparison (Control or w/o DN)	Outcome (Pain)	Post	Follow Up	Effect Size Post	Effect Size Follow Up	Conclusion
1. Aridici, R. 2016 8/10	36 patients (29 females) age 18-73 with chronic mechanical neck pain involving the upper trapezius	DN: n = 30	HPPT: n = 31	Visual Analog Scale (VAS)	1 week DN: 4.58 (1.85) HPPT: 4.20 (2.05)	4 weeks HTTP: 5.93 (1.98) DN: 6.25 (1.99)	ES = -0.19 CI lower = -0.70 CI upper = 0.31	ES = -0.16 CI lower = -0.66 CI upper = 0.34	Post/Follow up: HPPT > DN Trivial (NS)
2. Campa-Moran, I. 2015 7/10	36 patients (29 females) age 18-73 with chronic mechanical neck pain involving the upper trapezius	DN-S: n = 12	STT: n = 12 OMT: n = 12	Visual Analog Scale (VAS) (0-100 mm)	Post treatment 2 DN-S: 36.0 (24.0 to 48.0) STT: 30.1 (18.0 to 42.1) OMT: 23.0 (10.8 to 35.0)	DN-S: 13.3 (3.9 to 22.7) STT: 34.3 (24.9 to 43.7) OMT: 9.4 (0.03 to 18.8)	ES (DN-S/STT) = -0.28 CI lower = -1.07 CI upper = 0.54 ES (DN-S/OMT) = -0.61 CI lower = -1.41 CI upper = 0.23	ES (DN-S/STT) = 1.27 CI lower = 0.35 CI upper = 2.09 ES (DN-S/OMT) = -0.23 CI lower = -1.03 CI upper = 0.58	Post: STT > DN-S Small (NS) OMT > DN-S Moderate (NS) Follow up: DN-S > STT Large (DN*) OMT > DN-S Small (NS)
3. Cerezo-Tellez, E. 2016 6/10	130 patients (no gender specified) with non specific neck pain in the cervical region (trapezius, cervical multifidi, splenius cervicus, and levator scapulae)	DN-S: n = 64	Stretching: n = 64	Visual Analog Scale (VAS)	DN-S: -4.81 (0.2) Stretching: -1.57 (0.17)	6 months DN-S: -4.08 (0.25) Stretching: -1.60 (0.25)	ES = 17.46 CI lower = 15.19 CI upper = 19.50	ES = 9.92 CI lower = 8.60 CI upper = 11.11	Post/Follow up: DN-S > Stretching Large (DN*)
4. Ga, H. 2007 6/10 4. Ga, H. 2007 6/10	40 patients (36 females) age 63-90 with myofascial pain syndrome of the upper trapezius	DN: n = 18	DN + IMS: n = 22	Visual Analog Scale (VAS)	DN: 3.82 (2.47) DN + IMS: 3.11 (2.01)	N/A	ES = -0.32 CI lower = -0.94 CI upper = 0.31	N/A	DN + IMS > DN Small (NS)
5. Llamas-Ramos, R. 2014 8/10	94 patients (62 females) (mean 31 yrs age) with chronic idiopathic mechanical neck pain	DN: n = 47	TrP MT: n = 47	11 point numeric pain rating scale (0=no pain,10=max pain)	DN: 1.9 (1.4) TrP MT: 2.2 (1.8)	1 week DN: 1.3 (1.1) TrP MT: 1.6 (1.5) 2 weeks DN: 0.9 (0.8) TrP MT: 1.0 (1.1)	ES = 0.19 CI lower = -0.22 CI upper = 0.59	1 week ES = 0.23 CI lower = -0.18 CI upper = 0.63 2 week ES = 0.10 CI lower = -0.31 CI upper = 0.51	Post: DN > Trp MT Trivial (NS) Follow up: DN > TrP MT Small/Trivial (NS)
6. Mejuto-Vazquez, M. 2014 8/10	17 patients (9 females) (mean 25 yrs age) with acute(<7 days) mechanical, idiopathic, unilateral neck pain	DN: n = 9	Control: n = 8	11 point numeric pain rating scale (0=no pain,10=max pain)	DN: 3.8 (1.9) Control: 5.5 (2.1)	2 weeks DN: 2.0 (1.7) Control: 4.6 (2.1)	ES = 0.85 CI lower = -0.18 CI upper = 1.8	ES = 2.09 CI lower = 0.82 CI upper = 3.15	Post: DN > Control Large (NS) Follow up: DN > Control Large (DN*)
7. Myburgh, C. 2012 7/10	77 female patients age 25-46 with and without neck/shoulder pain were observed with respect to self-reported pain	DN: n = 17	SDN: n = 20	Self-reported pain (NRS-101 eleven point pain rating scale)	48 hours DN: 3.41 (2.31 to 4.53) SDN: 4.60 (3.62 to 5.58)	N/A	ES = 0.52 CI lower = -0.15 CI upper = 1.17	N/A	DN > SDN Moderate (NS)
8. Pecos-Martin, D. 2015 8/10	72 patients (58 females) age 18-42 with unilateral neck pain for ≥3 months	DN: n = 36	Sham DN: n = 36	Visual Analog Scale (VAS)	1 week DN: 2.6 (1.8) Sham DN: 5.3 (1.6)	1 month DN: 2.1 (1.6) Sham DN: 5.1 (1.5)	ES = 1.57 CI lower = 1.04 CI upper = 2.01	ES = 1.93 CI lower = 1.36 CI upper = 2.47	Post/Follow up: DN > Sham DN Large (DN*)
9. Rayegani, S. M. 2014	28 patients (no gender specified) age 20-46 with the diagnosis of	DN: n = 14	PT: Heat, Ultrasoun	Visual Analog Scale	1 week DN Rest: 1.8	N/A	Rest ES = -0.20 CI lower = -0.94	N/A	Rest: PT > DN

4/10	myofascial pain syndrome of upper trapezius for at least 2 months		d, Tens, Stretching : n = 14	(VAS)	(1.5) DN Activity: 2.8 (2.2)		CI upper = 0.54 Activity ES = -0.14 CI lower = -0.89 CI upper = 0.60		Small (NS) Activity: PT > DN Trivial (NS)
10. Segura-Orti, E. 2016 6/10	34 patients (25 females) (mean 33 yrs age) with active trigger points in the upper trapezius	DN + 8 reps shoulder shrugs/ abduction + stretching: n = 10	SCS: n = 8 Sham SCS: n = 6	Visual Analog Scale (VAS) (0-100 mm	3 weeks DN: 17.7 (14.7) SCS: 18.6 (10.3) Sham SCS: 12.3 (9.3)	N/A	ES (DN/SCS) = 0.07 CI lower = -0.86 CI upper = 1.00 ES (DN/Sham SCS) = -0.41 CI lower = -1.41 CI upper = 0.63	N/A	DN > SCS Trivial (NS) Sham SCS > DN Small (NS)
11. Ziaeifar, M. 2014 4/10	33 patients (no gender specified) age 20-48 with active trigger points in the upper trapezius	DN: n = 16	TCT: n = 17	Visual Analog Scale (VAS)	1 week DN: 1.34 (1.93) TCT: 3.05 (2.27)	N/A	ES = 0.81 CI Lower = 0.08 CI Upper = 1.50	N/A	DN > TCT Large (DN*)

DN: Dry Needling; ES: Effect Size; CI: Confidence Interval; DN*: Dry Needling is Significant; NS: Not Significant; HPPT: High Powered Pain Threshold Ultrasound; DN-S: Dry Needling plus Stretching; STT: Soft Tissue Technique; OMT: Orthopedic Manual Therapy; IMS: Intramuscular Stimulation; TrP

DISCUSSION

This review determines the effects of DN on upper quadrant pain and using Cohen's to measure outcomes unlike the popular p-value that has been used to measure statistical significance. Statistical significance may not give the effects or magnitude of effects of an intervention [37]. This study presented the results of the study in categories of large, moderate or small effects. It is important to compare some of the results of the studies using the p-value and Cohen's d. Some of the studies presented confusing results using p-value or Cohen's d. For example, the study of Llamos-Ramos, R., et al [5], presented statistical significance of p-value of $p < 0.01$ to 0.001 and using Cohen's d and the same data, the results favor DN to Trp MT but at a trivial level that is not significant. On the other hand, Mejuto-Vazquez, M. [6], study presented p-value of statistical significance of $p < 0.001$, and using Cohen's d, DN resulted in large effect compared to the control and this large effect size is significant. In this study, we can see where p-value and Cohen's d for measurement of outcomes tend to agree in some of the studies. This is true of the study of Pecos-Martin, D [7] that presented a $p < 0.001$ and using Cohen's d, a large effect with significance is also reported. There was no main difference between DN of TrPs of upper trapezius muscles and DN of TrPs plus paraspinal muscles ($p<0.05$) but using Cohen's d, DN of TrPs of upper trapezius plus paraspinal muscle produced small effect (not significant) but in favor of the DN TrPs plus paraspinal muscles. Using Cohen's d to measure the efficacy of DN treatment, DN had a large effect and more superior for pain control in five studies compared to the controls/interventions (ES range of 0.81 to 17.46; CI of 0.08 – 1.50 and 15.19 – 19.50 respectively) [31,32,6,7,36]. Moderate effect in one study which was not significant (ES of 0.52; CI: - 0.15 – 1.8) [33]. Of the eleven articles reviewed we have trivial/small effect, also not significant, in two studies (ES of 0.07 to 0.23; CI: -0.86 – 1.00 and -0.18 – 0.63) [5,35]. Three of the eleven studies favored control and other interventions over DN but the differences were not significant. The application of DN for upper quadrant pain is seen in these reviews to be positive for pain control. Using Cohen's d for magnitude of effects of intervention may be more useful clinically as there might be a difference between statistical significance and outcome measures like Cohen's d that gives a magnitude of the difference and whether that difference is significant or not looking at the CI. What is not clear is the mechanism with which DN controls pain, and this is not covered in this study. The application of DN (thin filiform needles) employs different techniques, including but not limited to winding, several stabbings of the needle into the site (sometimes referred to as "pistoning"), inserting the needle into the site for a certain amount of time and electrical dry needling (EDN) in some cases.

Irrespective of the technique of application, pain reduction of varying degrees may be observed as demonstrated by this current review. Also, Acupuncture and DN may have different philosophies but the procedure of inserting the needle into the body is essentially the same and both are targeting pain reduction essentially.

CONCLUSION

Using Cohen's d to measure the efficacy of DN treatment, of the eleven studies reviewed, DN had a large effect and more superior for pain control in five studies compared to the controls/interventions, moderate effect in one study which was not significant and trivial/small effect, not significant was observed in two studies. However, p-value and Cohen's d as measures of outcomes do not totally not see results or outcomes at the same lenses. Cohen's d may probably be better in looking for clinical significance and the magnitude of such significance.

With the positive effect of DN treatment on pain reduction, one can deduce that patients with pain treated by DN can have an increase in function and ultimately improved quality of life. DN as a modality is new in physical therapy. Chronic pain, such as upper quadrant pain or trapezius pain that may not respond to the traditional physical therapy (heat, exercise, and massage) may be *relieved with DN treatment and therapists need to consider this new modality when treating chronic pain.*

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LASER PROBE WITH INTEGRATED CONTACT COOLING FOR SUBSURFACE TISSUE THERMAL REMODELING

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ABSTRACT

Over 6.5 million women in the United States suffer from female stress urinary incontinence (SUI). Only ~200,000 women choose surgery. There may be a role for a non-surgical, minimally invasive procedure that provides thermal shrinkage/remodeling of submucosal collagen in the endopelvic fascia. This study describes design, characterization, and preliminary testing of a novel probe with integrated contact cooling for potential use in transvaginal laser treatment of SUI. Laser energy at a deeply penetrating, near-infrared wavelength of 1075 nm was delivered through a 600-□m-core fiber optic patchcord into a 90° side-firing probe head (19 x 22 mm) with integrated flow cell and sapphire window cooled to -2°C by circulating an alcohol-based solution. An inflatable balloon attached to the probe insured contact with vaginal wall. A force sensor and thermocouples monitored pressure and temperature. Thermal lesions were created in vaginal tissue of three cadavers (power = 4.6-6.4 W; spot diameter = 5.2 mm; time = 30 s). Thermal lesion areas measured 3.1-4.6 mm^2, while preserving the vaginal wall to a depth of 0.8-1.1 mm. Consistent tissue contact and cooling was maintained using the force sensors. Preliminary cadaver studies demonstrated subsurface treatment of endopelvic fascia with partial preservation of the vaginal wall. Future studies will optimize parameters for thermal remodeling with further tissue surface preservation.

Keywords: cadaver, laser, minimally invasive, nonsurgical, remodeling, stress urinary incontinence, thermal

INTRODUCTION

Over 6.5 million women in the U.S. suffer from stress urinary incontinence (SUI), while only ~200,000 women choose surgery [1,2]. The need for general anesthesia, long recovery time, incisions, potential treatment failures with future pregnancy, and procedural morbidity are multiple reasons for the hesitation of patients to have surgery. There is a role for a non-surgical method which improves patient quality of life if it can be rapidly performed with minimal morbidity and short recovery time.

Radiofrequency (RF) energy has been used for transurethral thermal shrinkage and remodeling of submucosal collagen in bladder neck and proximal urethra with success rates up to 80% [3,4]. However, RF therapy requires multiple needles inserted into endopelvic fascia and is more invasive than desired.

Our previous laboratory studies demonstrated subsurface thermal denaturation of tissues using near-infrared (IR) laser energy with applied cooling, to preserve 1-2 mm of the tissue surface [5-9]. For SUI, the goal is to thermally remodel the endopelvic fascia without damaging adjacent tissue. The vaginal wall, endopelvic fascia, and urethral wall measure about 2.7, 4.3, and 2.4 mm in thickness [10,11]. The vaginal wall may potentially be preserved while submucosal tissue is thermally remodeled, using a transvaginal laser probe with integrated cooling. Previous computer simulations of light transport, heat transfer, and tissue damage showed that a transvaginal approach is more feasible than a transurethral approach [12].

In this preliminary study, we use a cadaver model as an intermediate step between our previous *ex vivo* tissue studies and future preclinical and clinical studies, *in vivo*, to test a novel transvaginal laser probe with integrated contact cooling for subsurface thermal coagulation of the endopelvic fascia.

METHODS

Thermal lesions were created in vaginal tissue using an Ytterbium fiber laser operating at a near-IR wavelength of 1075 nm with incident power of 4.6 - 6.4 W, incident spot diameter of 5.2 mm on the tissue surface, and total laser irradiation time of 30 s. Laser energy was delivered through a custom-built transvaginal probe head (19 x 22 mm) with integrated pressure and temperature sensors, and flow cell circulating an alcohol-based solution for cooling the sapphire window (Figure 1).

A 6.25 mm right angle prism was used to reflect laser radiation at 90 degrees from the side-firing laser probe (with only ~ 4% of laser power lost due to aperture mismatch). The outer surface of the sapphire window on

the laser probe was cooled to -2 °C, to protect the vaginal wall from potential thermal damage. To confirm consistent contact with the vaginal tissue, a force sensor was added to the laser probe and attached to a custom built circuit, with indicators that would light up at various forces (e.g. three red LEDs at 0.3, 0.6, and 0.9 N, two green LEDs at 1.2, and 1.8 N, and two blue LEDs at 2.5 and 2.9 N) (Figure 2). The LED and force sensor was programmable via USB interface (Atmel Studio software). This force sensor was intended to assist the surgeon in applying consistent force to the tissue for reproducible procedures. Two micro-thermocouples (Type T, 125-µm-OD, Omega Engineering, Stamford, CT) were also attached with thermal epoxy near the outer rim of the sapphire window on the laser probe to provide temperature monitoring and feedback at the interface between the probe and vaginal wall.

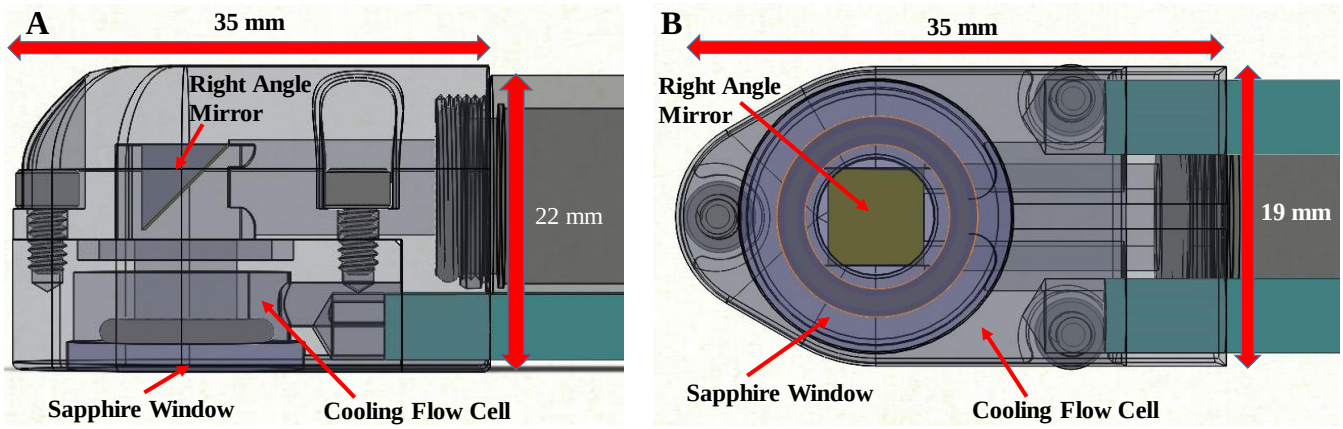

Figure 1. (A) Side view of probe design. (B) Bottom view of probe design.

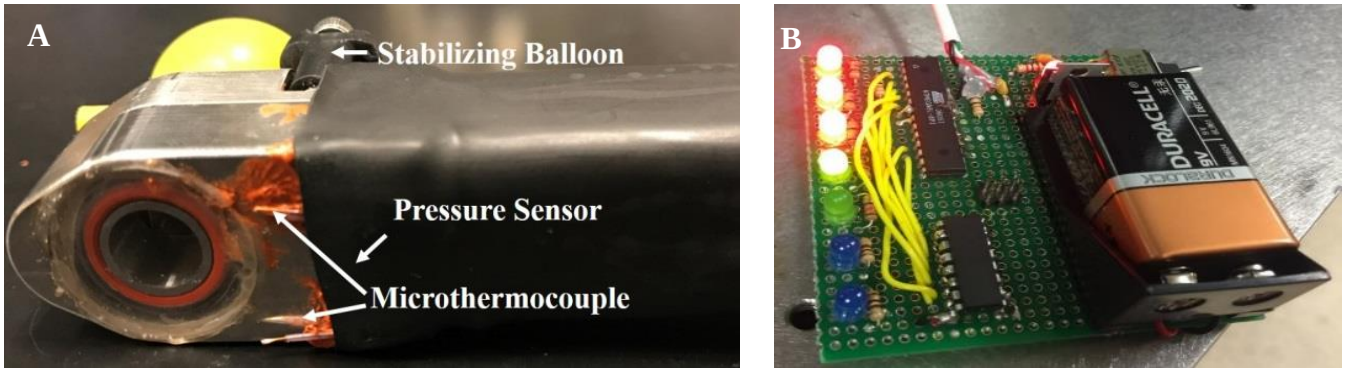

Figure 2. (A) Photograph of probe. (B) Circuit board with LED display for pressure sensor.

RESULTS

Since human cadaver tissue was non-uniform from distal to proximal end, a power escalation study was conducted with 30 s irradiation time and 5 min between studies. Subsurface thermal lesions were made with both 4.6 and 6.4 W settings. The 4.6 W setting preserved a deeper surface tissue layer than the 6.4 W setting (1.1 vs 0.8 mm) while also creating a larger lesion area (4.6 vs 3.1 mm^2) (Table 1 and Figure 3). This result may be attributed to small differences in the initial tissue temperatures as well as the non-uniformity of the tissue from distal to proximal end.

Table 1. Lesion characteristics (λ = 1075 nm).

Parameters		
Laser Incident Power (W):	4.6	6.4
Irradiation Time (s):	30	30
Initial Tissue Temp. (°C):	20	17
Probe Temp. (°C):	-2	-2
Lesion Characteristics		
Preserved Tissue (mm):	1.1 ± 0.2	0.8 ± 0.1
Lesion Area (mm^2):	4.6 ± 0.6	3.1 ± 0.3
Lesion Shape:	Circular Elliptical	

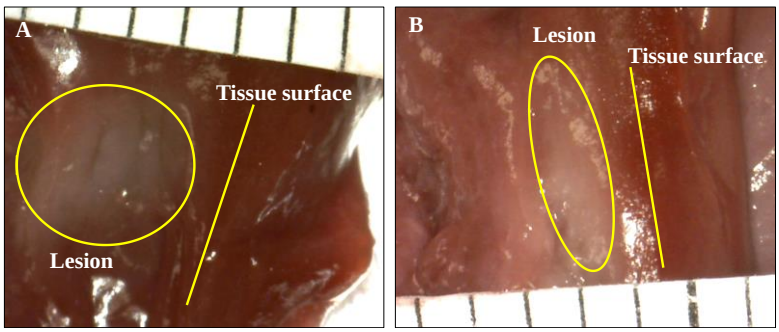

Figure 3. Gross images of thermal lesions produced in human vaginal tissue, ex vivo, using 1075 nm laser wavelength. A) 4.6 W for 30 s preserved 1.1 ± 0.2 mm B) 6.4 W for 30 s preserved 0.78 ± 0.1 mm.

DISCUSSION

Radiofrequency (RF) energy has previously been used as nonsurgical SUI treatment with improvement in patient quality of life. RF heating decays rapidly with depth as $1/r^4$, resulting in a superficial penetration depth and direct heating of only 1-2 mm. RF probes for SUI therapy therefore typically require needles inserted through the urethral wall and into the submucosal tissue for collagen heating, denaturation, and shrinkage, with saline irrigation of the mucosa to prevent overheating. RF therapy is thus more invasive than desired by many patients who are seeking a minimally invasive, nonsurgical treatment for SUI.

Carbon dioxide (= 10.6 m) and Erbium:YAG (= 2.94 m) lasers have also recently been used for sub-ablative resurfacing of atrophic vaginal tissue in post-menopausal women, and as a side benefit, for treatment of SUI. Short-term studies appear promising for vaginal rejuvenation and also show moderate improvements for SUI. However, one limitation is that these lasers (adapted from the cosmetic skin resurfacing field in dermatology) produce superficial optical penetration depths (OPD) of only tens of micrometers, resulting in a thermal treatment zone of less than about 500 m.

Preliminary laboratory studies in our laboratory demonstrated subsurface thermal denaturation of a variety of different soft tissues using a deeply penetrating near-IR laser wavelength (= 1064 or 1075 nm) in combination with applied surface cooling, preserving 1-2 mm of the tissue surface. Thus, laser denaturation and shrinkage of endopelvic fascia may potentially produce improved tissue remodeling results similar to RF approach, but in a less invasive manner with preservation of vaginal mucosa. Computer simulations utilizing optical, thermal, and damage parameters for vaginal wall, endopelvic fascia, and urethral wall, were also previously conducted for a transvaginal endoscopic approach for minimally invasive laser treatment of female SUI.

In this study, we explored an intermediate cadaver model to test a miniaturized transvaginal laser probe with integrated cooling, an anchoring balloon, and temperature and force sensors, for producing subsurface thermal lesions in the endopelvic fascia with preservation of the vaginal wall.

CONCLUSIONS

Optical studies in cadavers demonstrated subsurface targeting of endopelvic fascia with partial preservation of vaginal wall, using near-IR laser and contact cooling. Further studies are planned to optimize laser parameters for thermal remodeling with greater tissue surface preservation prior to preclinical and clinical studies, *in vivo*.

ACKNOWLEDGMENTS

This research was supported by the National Institutes of Health, Grant #1R15DK099774. The authors thank Michael Peters, Dina Bastawros, Erinn Myers, and Michael Kennelly for their help with the cadaver studies.

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THE EFFECTS OF MIRROR THERAPY ON UPPER EXTREMITY FUNCTION POST-STROKE: A SYSTEMATIC REVIEW

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ABSTRACT

Background and Significance: Impairment in upper extremity (UE) function post stroke is detrimental to independence in activities of daily living (ADL's). During mirror therapy (MT), an individual watches the movement of the unaffected hand reflected in a mirror giving the illusion of correctly moving the paretic hand. The purpose of this study was to determine if mirror therapy improved upper extremity function in patients post-stroke to increase functional independence.
Methods: PubMed Database was searched through November 15, 2017, to include randomized control trials comparing conventional treatment and/or sham treatment to MT for improving UE weakness/paresis and independence in ADL's. Seven articles met the inclusion criteria. Study quality was evaluated using the PEDro scale, a 10-point scale developed to assess the internal validity of clinical trials in physical therapy. Studies were also scored using the 2011 Centre of Evidence Based Medicine (CEBM) scale, a 5-level scale in which lower numbers indicate higher levels of evidence.
Results: Evidence in five of the seven articles demonstrated that MT led to statistically significant improvements in function ($p < 0.05$) as shown on the Functional Independence Measure (FIM) when compared to a control. The mean PEDro score was 6.7 with a range of 5 to 8. The CEBM levels of evidence included five level II studies and two level III studies.
Conclusion: This systematic review provides evidence to support the use of MT as a therapeutic intervention in individuals with chronic or subacute stroke to increase UE motor control and functional skills.

Keywords: Cerebrovascular Accident, Upper Extremity, Mirror Therapy.

INTRODUCTION

Stroke is caused by damage to the brain and can lead to a variety of impairments including motor, sensory, perception, and language disorders [1]. Hemiplegia is a consequence in 85% of patients who survive a stroke. Upper extremity (UE) function is impaired in approximately 69% of these individuals. Upper extremity impairment after stroke can be due to paresis and/or spasticity and can be detrimental to independence in activities of daily living [2]. Basic activities of daily living include but are not limited to: personal hygiene, dressing, eating, transferring from sitting to standing, getting in and out of bed, and walking. If unable to perform the basic activities of daily living, stroke survivors may not be able to live independently. Most evidence-based rehabilitation treatments for the hemiplegic UE require direct interaction with a therapist for many weeks. Mirror therapy is inexpensive, easy to construct, and is directed by the patient. During MT, the individual watches the movement of the unaffected hand in a mirror that is placed in the mid-sagittal plane between the patient's two upper extremities. The reflecting side of the mirror blocks the patient's view of the affected extremity while the patient watches the mirror image of the unaffected extremity. This gives the patient the illusion of correctly moving the paretic hand [3]. Mirror therapy effects "motor image and motor execution overlap particularly in the premotor and parietal areas, basal ganglia and the cerebellum." [4 p. 576] The theory is that MT promotes neuroplasticity of the brain while having effects in the motor and somatosensory cortices [4]. Functional magnetic resonance imaging has given credence to this theory [5].

METHODS

An electronic search of the PubMed Database was conducted through November 15, 2017, which resulted in 116 articles related to this topic. Specific search terms included those related to cerebral vascular accident and upper extremity and mirror therapy. These articles were screened according to title, which eliminated 101 articles and left 15 for continued screen. Following the abstract screen, nine articles remained for consideration. Two of these were eliminated according to the inclusion/exclusion criteria. Inclusion criteria were; randomized control trials comparing conventional treatment and/or sham treatment to MT, (UE) weakness/paresis, and the use of the Functional Independence Measure (FIM) as an outcome measure.

Exclusion criteria consisted of the use of Mirror Image Movement Enabler, or robotics, and the use of MT as the control group.

The seven articles included in the review were scored using the PEDro scale, a 10-point scale developed to assess internal validity and the quality of methodology of clinical trials in physical therapy research. Higher PEDro scores suggest higher quality clinical trials. PEDro scores for this review ranged from 5 to 8 with a mean of 6.7. The level of evidence was also assessed using the 2011 Centre of Evidence Based Medicine (CEBM) scale which is a 5-point scale with a lower score indicating a higher level of evidence. CEBM is based primarily on study design. The CEBM levels of evidence included five level II studies and two level III studies.

RESULTS

In a recent study by Kim, et al., [1] 25 adults with hemiplegia post-stroke were randomly assigned to one of two groups. The intervention group received four weeks of MT for 30 minutes per day five days a week. The control group received four weeks of conventional therapy for 30 min per day five days a week. Upper extremity function and activities of daily living were measured using the Action Research Arm Test (ARAT), Fugl-Meyer Assessment (FMA), Box and Block Test (BBT), and the Functional Independence Measure (FIM) total. The within group comparisons demonstrated that both the intervention and control groups showed statistically significant improvements ($p < 0.05$) after therapy in the ARAT, FMA, BBT, and FIMtotal. The MT group showed greater improvements than the control group. The post intervention mean score improvements were as follows: ARAT- MT group increased by 5.7 while the control increased by 2.8; FMA- MT increased by 5.9 while the control increased by 3.4; BBT-MT increased by 4.2 while the control increased by 2.8; FIMtotal- MT increased by 6.8 while the control increased by 3.7. The between group comparison demonstrated that the intervention group (MT) showed statistically significant improvements ($p < 0.05$) in mean scores when compared to the control group in all outcome measures.

In a study by Park, *et al*. [2], 30 adult participants with hemiplegia post-stroke were randomly assigned to one of two groups. The intervention group participated in four weeks of MT five times each week for 30 minutes in addition to conventional therapy. The control group participated in four weeks of sham therapy using a nonreflecting mirror five times each week for 30 minutes in addition to conventional therapy. Outcome measures included the FIMtotal, FIMself-care, FMA, and the BBT. Results of the between group comparison revealed a significant difference in mean scores in favor of the intervention group (MT) for UE function in FMA ($p = 0.000$), coordination in BBT ($p = 0.002$), self-care in FIMself-care ($p = 0.001$), and activities of daily living in FIM total ($p = 0.008$) (see Figure 1).

In a 2015 study by Park, et al., [3] 30 adults with hemiplegia post-stroke were randomly assigned to one of two groups. The intervention group participated in six weeks of MT incorporating eight specific functional tasks five times per week. The control group participated in six weeks of sham therapy utilizing the same functional tasks five times per week but utilizing the nonreflecting side of the mirror. Outcome measures included the Manual Function Test (MFT) and the FIM self-care. Within group comparisons revealed that both the intervention and the control group showed significant improvements in the mean scores on MFT and FIMself-care ($p < 0.05$).The between group comparisons revealed that the intervention group had statistically significant improvements in the mean scores in MFT and FIMself-care ($p < 0.05$) (see Figure 1).

In a study comparing the outcomes of 30 adult subjects with hemiplegia post-stroke accompanied by complex regional pain syndrome type 1, Vural, et al. [4], randomly assigned patients into two groups. The intervention group received four weeks of conventional stroke rehabilitation five times per week for two to four hours a day, plus MT five times a week for 30 minutes. The control group received four weeks of conventional stroke rehabilitation five times a week for two to four hours per day. Outcome measures included were the FIMmotor, Brunnstrom Recovery Stages (BRS) hand, BRSarm, Modified Ashworth Scale (MAS), FMAwrist, and FMAhand. Results of the within group comparisons revealed that the intervention group (MT) showed significant improvement in median scores in FIMmotor ($p = 0.01$), BRShand ($p = 0.01$), BRSarm ($p = 0.02$), FMAwrist ($p < 0.001$), and FMAhand ($p < 0.001$).The control group showed significant improvement only in the median scores of FIMmotor ($p = 0.01$). Between group comparisons upon study completion revealed that the intervention group (MT) showed significant improvement over the control group in the median scores in FMAwrist ($p < 0.001$) and FMAhand ($p < 0.001$).

Thirty-one adult hemiplegic patients post-stroke were randomly assigned to two groups in a study by

Gurbuz, et al. [5]. The intervention group participated in four weeks of conventional stroke rehabilitation consisting of 1-2 hours of neurodevelopmental treatment (NDT) and 20 minutes of MT. The control group participated in four weeks of the same interventions but used the non-reflecting side of the mirror. Outcome measures used were the BRS, FMA upper extremity score, and the FIMself-care score. Within group comparisons revealed that both groups showed statistically significant improvement ($p < 0.05$) in BRS, FMA upper extremity score, and FIMself-care scores. Between group comparisons showed greater improvement with MT in the BRS and the FIMself-care, however, the improvements were not statistically significant. The FMA upper extremity score was significantly higher ($p < 0.05$) in the MT group (see Figure 1).

In an earlier study, by Invernizzi, et al. [6], 26 adult subjects with hemiplegia post-stroke were randomly assigned to two groups. The intervention group received four weeks of conventional stroke rehabilitation five times per week for 60 minutes, including 30 minutes of MT for the first two weeks and 60 minutes of MT for the last two weeks. The control group received four weeks of conventional stroke rehabilitation five times per week for 60 minutes. The outcome measures performed included the Action Research Arm Test (ARAT), Motricity Index (MI), and the FIMtotal. Results of the within group comparisons revealed that both the intervention and control group showed significant improvements in mean scores of the ARAT, MI, and FIM total ($p < 0.05$). Between group comparisons indicated that the intervention group had statistically significant improvements in the mean scores of ARAT, MI, and FIM total ($p < 0.001$) as compared to the control group.

In the final article in this review, Yavuzer , et al. [7] studied 40 adult subjects with hemiplegia post-stroke. Participants were randomly assigned to one of two groups. The intervention group participated in 4 weeks of conventional stroke rehabilitation five times per week for two to five hours per day plus 30 minutes of MT. The control group participated in four weeks of conventional stroke rehabilitation five times per week for two to five hours per day. The outcome measures utilized were the BRShand, BRSUE, MAS, FIMself-care. Following the intervention, the between group comparison showed significant improvements in mean scores in favor of the intervention group (MT) in motor recovery in BRShand ($p = 0.001$) and BRSUE ($p = 0.001$) and functioning in FIMself-care ($p = 0.001$) at both post-treatment and 6-month follow up (see Figure 1).

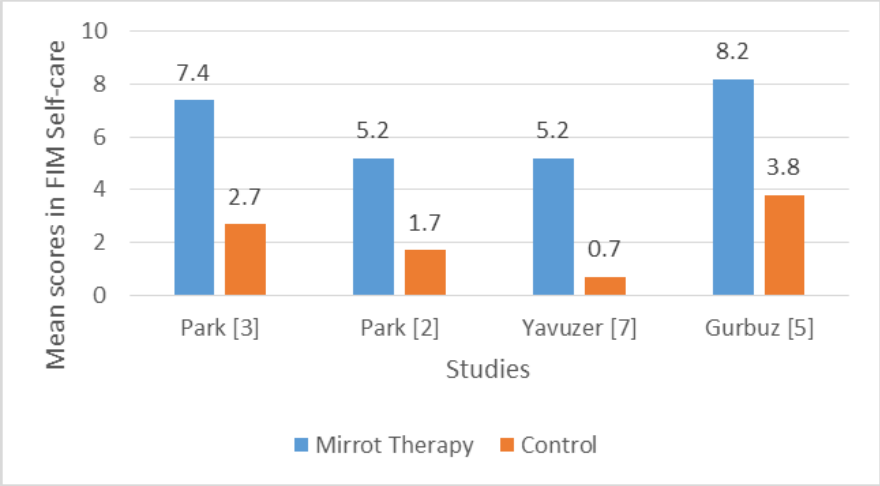

Figure 1. Comparison of Improved UE Function: Differences in Mean FIM Self-Care Scores from Pre-Treatment to Post-Treatment

Vural, Invernizzi and Kim are not depicted in Figure 1 because the authors did not separate self-care from motor or total FIM scores. Self-care scores are directly related to UE function; whereas, motor and total FIM scores include lower extremity function.

DISCUSSION

The inclusion of MT as a therapeutic intervention for patients post-stroke has been shown to improve UE function. Evidence in five out of the seven articles demonstrated that MT led to statistically significant improvements in self-care, as demonstrated by increased FIM scores when compared to conventional therapy and/or sham therapy. Longer follow-up studies might further improve these results due to the fact that continued muscle activity is necessary to facilitate neuroplasticity [5]. Further studies that include functional imaging are needed to determine the time frame necessary to produce a change in cortico-motor reorganization. While motor function was evaluated using different clinical outcome measures, the studies reviewed demonstrated good evidence that MT increased motor function in patients post-stroke.

CONCLUSIONS

This systematic review provides evidence to support the use of MT as a therapeutic intervention in patients with chronic or subacute stroke. A mirror therapy box can be constructed for less than ten dollars out of a cardboard box, making this an easily accessible and inexpensive treatment option for this client population. Another attractive aspect of MT is that it can be done by the patient, independent of therapist interaction. It can be easily added to conventional therapy and incorporated into home exercise programs. Depending upon the individual client's motivation to consistently participate in a MT program, this intervention could lead to improved motor control of the affected UE and allow increased independence in basic activities of daily living. Further studies are needed to determine the optimal MT protocol.

ACKNOWLEDGMENTS

The authors would like to acknowledge Emily Bright, Allison Dean, Samantha Klein, Kala Ross, Mary Clark Strange, and Carolyn Wellborn for their assistance in gathering the information for this review.

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HIGH-RATE MECHANICAL INSULT CONTRIBUTES TO ALTERATIONS IN BRAIN CELL SIGNALING AND REACTIVITY

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ABSTRACT

Traumatic brain injury (TBI) is complex pathology with numerous long-term debilitating symptoms associated with damage to the brain parenchyma. It is necessary to better understand TBI at the cellular and molecular level to mitigate organ-level dysfunction. In particular, there is a lack in understanding the interplay of injury mechanics and mechanobiological responses in brain cells. This study aimed to analyze such effects from high-rate mechanical injuries, through an *in vitro* injury model of primary mixed brain cell cultures. Neurons and astrocytes interact in the proper functioning of neural networks and are critical components in the injury response of the central nervous system. The goal of this study was to analyze expression of abundant structural and adhesion molecules expressed by neurons and astrocytes to understand how intra- and intercellular signaling may be compromised as a result of high-rate mechanical insult. Target expression was measured for β-actin, vinculin, β-tubulin, ezrin, connexin-43, and glial fibrillary acidic protein. Transient alterations in both neuron and astrocyte-specific molecules occurred over the time course of 48 hours after insult. This has implications for compromised cell-cell communication and provides potential molecular targets for mechanobiological mechanisms associated with neuron and astrocyte dysfunction following high-rate mechanical insult.

Keywords: injury, shock wave, astrocyte, neuron, signaling, reactivity, connexin-43

INTRODUCTION

Traumatic brain injury (TBI) is defined broadly as the complex cellular and molecular sequelae caused by external physical force enacting damage on the brain tissue. TBI can present clinically as long-term impaired cognition, mood changes, and increased anxiety [1]. Importantly, no therapeutic strategies have yet been successful in completely mitigating the symptoms of TBI. Many approaches are aimed at general hallmarks of cellular injury, yet there is still a need to understand the scope of mechanobiological and molecular contributors in brain cell injury to properly target them. *In vitro* TBI models have shown differential cellular outcomes associated with a range of injury mechanics, including injury severity and rate, and evidence shows that brain cells are differentially susceptible to strain rate, in particular, depending on cell type and mechanics [2-4]. In this study, high-rate mechanical insult was the focus for exploring cellular dysfunction in neuronal-glial cultures. High-rate mechanical insult was defined as one millisecond or less exposure to a transient overpressure compression wave. The specific injury model was designed to simulate military-relevant conditions that result from exposure to explosive devices. Blast neurotrauma affects a significant number of Veterans and presents clinically in conjunction with post-traumatic stress disorder [5, 6]. It is debated how the shock wave from an explosive event interacts with the skull and brain to create pressure gradients inside the tissue leading to cognitive dysfunction [7].

Well-characterized cellular hallmarks of blast neurotrauma include oxidative stress, neuroinflammation, glial reactivity, and neuronal damage [8, 9]. However, little is known about the mechanisms which may initiate and/or prolong diffuse cellular dysfunction and phenotypic shifts in cells after blast injury. Glial cells, especially astrocytes, are both biochemically and structurally coupled with neuronal networks, and thus, the feedback mechanisms between these cell types have numerous implications for the prolonging secondary injury mechanisms like neuroinflammation and oxidative stress. Moreover, Sword et al showed that there is a complex relationship between biochemical alterations and structural disruption of neuron-astrocyte coupling after trauma [10]. This may initiate or influence glial contributions to neurodegeneration and cell death after injury [11]. It is therefore critical to identify mechanisms of glial cell reactivity after injury in order to harness their potential to repair rather than instigate further damage to the brain. Previous work has established a mechanical basis for astrocyte reactivity following simulate blast events [12]. The objective of this study was to expand

understanding of mechanobiological mechanisms in astrocyte reactivity to include intercellular signaling with neurons following high-rate insult exposure. It is hypothesized that the presence of other cells may significantly influence astrocytic signaling potentiation in the secondary sequelae associated with blast injury.

METHODS

Primary cell cultures. Two types of primary cell cultures were prepared for these studies: (1) mixed brain cells and (2) astrocyte-only. Primary rat mixed brain cell preparations were obtained as a gift from Dr. Beverly Rzigalinski (Edward Via College of Osteopathic Medicine, Blacksburg VA). Culture preparation and maintenance is described by Ahmed et al [13]. Briefly, cortices were extracted from one-two day old rat pups and seeded in equal volume amounts (~$1x10^6$ cells) per well. Previous characterization showed that glial cells adhere to the culture plates with neurons adhering on top. Astrocyte-only cultures were also extracted from two day old rat pup cortices and mechanically purified from other cells by gentle shaking for 48 hours. Cells were maintained up to 14 days after extraction before use and were seeded at $1x10^4/cm^2$ in standard six-well plates for 6-7 days prior to testing. Samples were routinely stained for glial fibrillary acidic protein (GFAP) to assess cell population composition. For testing, samples were prepared in DMEM and sealed with parafilm (ensuring no air bubbles) before being placed in the overpressure generator, described below. Sham groups were treated equally apart from overpressure exposure.

High-rate insult device. Figure 1 shows the custom water-filled, temperature-controlled chamber used for *in vitro* exposure to high-rate compression wave which mimics a shock wave profile. This device was fabricated as a means to isolate and study pressure mechanisms associated with high-rate injury. It works by an exploding bridge wire mechanism, in which a thin wire is vaporized at the smaller end of the chamber using high electrical current. The vaporization creates an underwater explosion which propagates down the conical section of the tube exposing cells that were located adjacent to the pressure wave. Pressure measurements were captured using Meggitt 8350C or PCB 113B21 sensor at a location directly above and adjacent to the cell cultures.

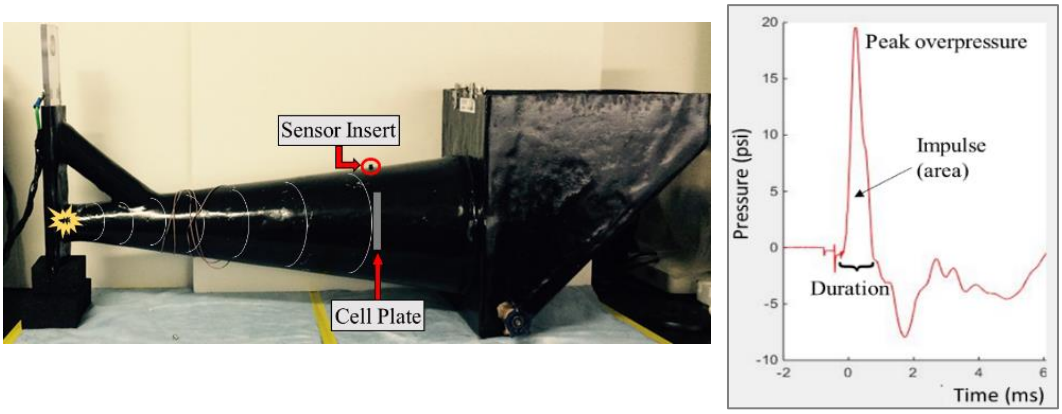

Figure 1. The overpressure generator was used for *in vitro* high-rate overpressure testing. Upon wire vaporization, the wave front travels down the test section over cultures denoted by "Cell Plate." The underwater pressure wave as represented in the pressure trace is meant to mimic a Friedlander waveform.

Gene expression. At either 24 or 48 hours post exposure, TRIzol reagent (Ambion 15596018) was added to the cell samples for RNA and protein extraction. Manufacturer's protocols were followed for the RNA extraction followed by DNase digestion (Promega M6101) to remove potential contaminants. RNA was quantified using a spectrophotometer, and a 260/280 of 1.8-2 was considered suitable for further analysis. Gene expression analysis was conducted by reverse transcription real-time polymerase chain reaction. Complementary DNA was prepared using random hexamers with one microgram of RNA. Gene target primers were obtained from PrimerExpress (Thermo-Fisher), and optimized in a reaction with SYBR green (Qiagen 330523) detection. A reference gene, glyceraldehyde triphosphate dehydrogenase (GAPDH) was used for computing normalized gene expression by a delta-C_t method.

Protein expression. Protein lysates were prepared by two methods. First, proteins were precipitated from the phenol phase after RNA was isolated from TRIzol using propanol. Pellets were washed in guanidine-HCl in 95% ethanol. Resuspension buffer for proteins was a 1:1 ratio

of 1% sodium dodecyl sulfate to 8M urea in 1M Tris-HCl (pH=8) with protease inhibitor cocktail (Sigma P8340). To facilitate better resuspension, samples were sonicated and heated for 10 minutes at 55 ºC three times. Otherwise, protein samples were prepared by incubation/shaking with lysis buffer containing 40 mM Tris-HCl (pH=7.5), 150 mM NaCl, 2.5 mM EDTA, 1% Triton-X, and protease inhibitor. Relative quantification of protein targets was completed using an automatic Simple Western apparatus, Wes (Protein Simple). Manufacturer's protocol was followed for sample preparation. Antibodies used for this analysis were anti-GFAP (Abcam 7260), anti-connexin-43 (CX43, Novus NB100-91717), anti-superoxide dismutase 2 (SOD2, Novus NB100-1992), β-actin (Sigma A5441) and anti-GAPDH (Novus NB600-502).

Statistics. Statistical comparisons were made using JMP software (Virginia Tech license). ANOVA analysis was used to determine differences between groups. Assumptions for normality were confirmed by Shapiro-Wilk test, and Kruskal-Wallis (KW) test was used for nonparametric data sets. Equal variances were assessed using Brown-Forsythe test. If necessary, data was transformed using natural log. Post-hoc student's t-test with a p-value<0.05 was considered statistically significant for comparisons between groups. Statistical blocks were used in gene expression analysis for replicates in each sample set. For graphical presentation, non-transformed data is shown. It should be noted that Figure 4 is presented as box plots with medians, but statistical comparisons were computed between means by methods described here.

RESULTS

High-rate insult mechanics. Table 1 provides the average pressure wave parameters for this study. Samples were exposed to an average of 18.37 psi (127 kPa) peak overpressure, which corresponds to mild severity injury in preclinical rodent models [14, 15]. Previous studies with underwater transient pressure chambers have shown that 17 psi overpressure causes minimal astrocyte death or detachment immediately following exposure [12]. It should be noted that pressure data was not obtained for one mixed cell plate due to sensor malfunction, but it was exposed to the same conditions as the other samples and thus was considered to be well represented by the average parameters listed here.

Phenotypic alterations. Gene expression for structural proteins was measured in mixed cell samples at 24 and 48 hours post overpressure (Fig. 2). The neuronal marker β-tubulin was significantly increased relative to sham (p-value=0.031) at 24 hours but returned to sham levels by 48 hours. Astrocyte-specific GFAP was also increased relative to sham but was delayed to the 48 hour time point (p-value=0.022). This reactive response was preceded by a significant increase in CX43 gene expression (p-value=0.011) at 24 hours and was coupled with decreased CX43 expression at 48 hours (p-value=0.018). Increased GFAP gene expression at 48 hours also corresponded to a decreased protein levels as compared to sham (p-value=0.037, Fig. 3). The increased gene expression may therefore be a compensatory mechanism in response to disruption of the protein by high-rate overpressure and/or cellular sequelae.

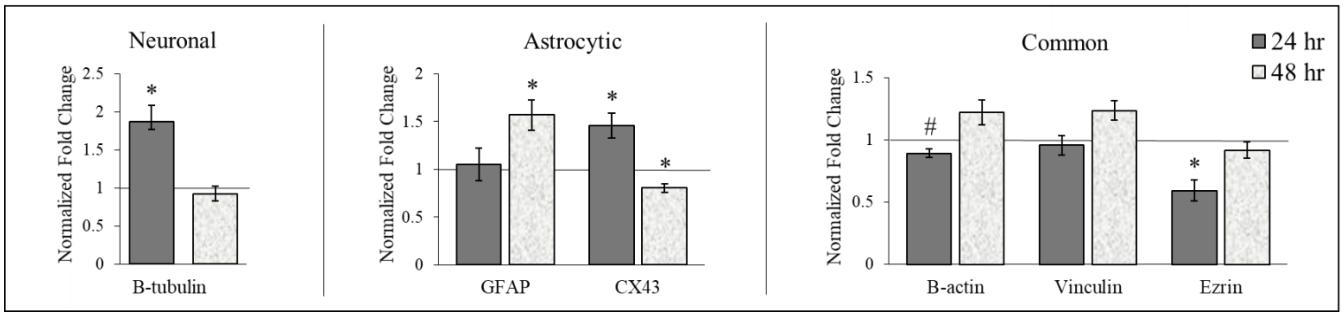

Figure 2. High-rate insult induced gene expression alterations in both neuron and astrocyte-specific molecular targets. All values are mean ± standard error and are normalized to average sham (i.e. sham=1). *p-value<0.05 as compared to respective sham, #p-value=0.055 as compared to respective sham

Table 1 | Summary of overpressure parameters

	Avg ± Std Dev
Peak Overpressure	18.37 ± 3.66 psi
Positive Peak Duration	1.03 ± 0.17 ms
Rise Time	0.48 ± 0.24 ms
Impulse	803.02 ± 107.53 psi*ms

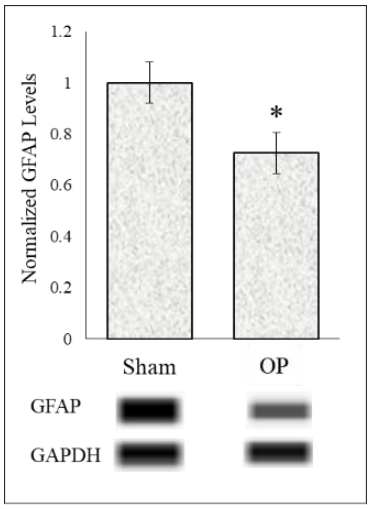

Figure 3. GFAP disruption which was found at 48 hours post-overpressure exposure. OP denotes pressure exposed group. Data are mean ± standard error and are normalized to sham. *p-value<0.05

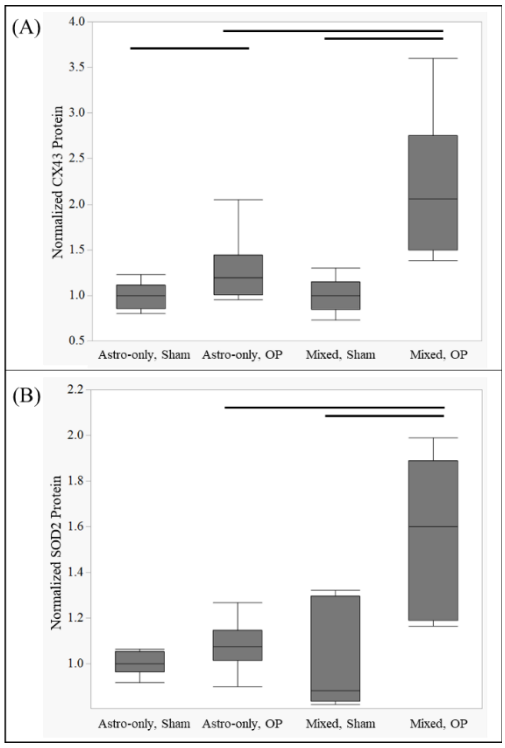

Figure 4. Mixed brain cells displayed increased (A) CX43 and (B) SOD2 protein levels compared to astrocyte-only cultures at 24 hours after overpressure exposure. OP denotes exposed group. Data are normalized to average sham and are shown as box plots (centerline=median) to represent distributions across groups. For significance, means were compared across groups by ANOVA/KW and post-hoc student's t-test. Bar indicates p-value<0.05 as

Metabolic and signaling aberrations. Mixed brain cells displayed increased protein expression of CX43 at 24 hours as compared to sham and astrocyte-only cultures (p-value<0.0001, Fig. 4A). While astrocyte-only cultures had a similar trend in increased CX43 expression compared to sham, mixed brain cells had a more substantial and variable increase. A similar pattern was found with SOD2, a mitochondrial-linked antioxidant molecule. Figure 4B shows no change in SOD2 protein levels in the astrocyte-only cultures with a significant increase in the mixed cells compared to sham and astrocyte-only groups at 24 hours (p-value=0.0094).

DISCUSSION

Shock wave overpressure is a unique injury paradigm with many aspects that remain unclear. This study found that there is a mechanical basis for structural alterations in both neurons and astrocytes following exposure to high-rate insult. Neurons can experience disruption of tubulin proteins as a result of mechanical deformation of axons [16]. Therefore, increased gene expression may indicate initial repair to compensate for damage to neuronal extensions [17]. Although not evaluated, this response may have been coordinated by support mechanisms from surrounding astrocytes. Because astrocytes are central for repair and signaling processes in the brain, they were largely the focus of this study. Astrocyte reactivity is one of the most common features across a variety of central nervous system insults, including most TBI modes. Prior work conducted with astrocyte-only cultures suggested that astrocytes undergo classical reactivity (as measured by increased GFAP expression) in response to high-rate insult [12, 18]. However, here we find that while increased GFAP gene expression occurs, there was decreased protein levels at 48 hours after exposure. Because this response has only been noted in the presence of other cell types, it suggests that disruption or controlled downregulation of GFAP may be influenced by crosstalk between cell types. It is possible that astrocyte stiffness is modulated to facilitate repair, or astrocyte disruption could precede neuronal death as they compensate for neuronal damage via increased CX43 and other signaling processes [11, 19].

Astrocytes displayed dynamic changes in mRNA and protein levels of specialized gap junctional protein CX43. CX43 is specifically expressed within astrocyte networks for small molecule communication and transport. It has been implicated in astrocyte reactivity to multiple injury mechanisms [20, 21]. Although CX43 regulation is still unclear, differential CX43 expression in astrocytes is influenced by the presence of injured neurons [22]. Spatial distribution of enhanced CX43 is highly varied depending on insult and has roles in both detriment and repair of neurons [11, 21, 23]. Earlier studies showed that CX43-mediated astrocyte communication was imperative

to clearance of excess neurotransmitter release and neuroprotection [21, 23], but recent studies have concluded that negative consequences involve increased cell death [11]. Results of this study were consistent with previous findings that neurons influence CX43 regulation, as expression was higher in the mixed cell cultures as compared to astrocytes alone. This may be critical for astrocyte roles in coordinating inflammatory and metabolic processes after injury and suggests that neuronal signaling specifically influences astrocytic network communication [21, 24]. CX43 upregulation also temporally corresponded to increased SOD2 protein in mixed brain cell cultures after overpressure exposure. Astrocytes have a robust antioxidant potential, and thus, may be responsible for coordinating this response to address high susceptibility of neurons to oxidative stresses [25]. No studies have previously implicated CX43 in blast neurotrauma mechanisms, however the results here suggest that future work should focus on CX43 influence in outcomes related to neuronal repair and oxidative stress.

While cell-specific alterations occurred by the 48 hour time point, the insult did not elicit measureable signs of compensatory gene expression of β-actin or vinculin. Taken together, these results indicate that neuronal and astrocytic reactivity to high-rate insult were largely a progressive response to signaling sequelae between cells. There could be a variety of factors that influence these outcomes, from inflammation to cellular adhesion. Moreover, high-rate insult caused increased signaling potential within astrocyte networks both in the presence and absence of neurons. These results may imply that the initiating factors for astrocyte reactivity are mechanically based, while neuronal signaling may influence the extent of astrocyte reactivity. Future work will employ functional manipulations of cell-specific pathways to understand their precise contributions to outcomes observed in this study.

CONCLUSIONS

High-rate insults initiated sub-acute changes in mixed brain cells at 24-48 hours post exposure. Cell-specific proteins were differentially expressed in neurons and astrocytes. Importantly, the presence of neurons influences the potentiation of gap junctional and antioxidant signaling mechanisms in astrocytes. CX43 may be an important mediator of early intercellular signaling after high rate mechanical insult to the brain and is regulated to some extent by non-mechanical cues. This work motivates future studies which will decipher and differentiate the molecular basis for mechanical versus cellular signaling in driving astrocytes ability to assume a reparative phenotype.

ACKNOWLEDGMENTS

The authors would like to acknowledge Samuel Miller for his contributions to sample collection and preparation. We would also like to thank Dr. Beverly Rzigalinski for her generous contribution of mixed cell preparations for use in these studies.

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COMPUTATIONAL FLUID DYNAMIC EVALUTATION OF AN AORTIC BENCH-TOP MODEL

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ABSTRACT

In treating diseases within the aorta, minimally invasive endovascular techniques like transcatheter aortic valve replacement (TAVR) are becoming more prevalent. In order to evaluate and optimize the design of catheter devices a robust and durable aortic bench-top model was created for experimentation and testing. In this study, numerous computational fluid dynamic (CFD) simulations were performed on the aortic bench-top model in order to computationally characterize effective fluid resistance at the aortic branches and descending aorta, in order to influence hardware settings for future testing and experimentation. By introducing and manipulating nozzles at the bench-top model outlets in the CFD simulations, it was found that the model is capable of producing aortic branch fluid resistance values from 1.5 - 2000 mmHg/L/min *in silico*, which is well within the range of physiological relevance. Once determining the dependence of fluid resistance on nozzle geometry, effective fluid resistance values were applied to the branching arteries and descending aorta in the CFD model to perform pulsatile flow simulations with both blood and water glycerol to verify water glycerol as a blood analog for this bench-top. Water glycerol generated flow profiles and volume flow waveforms comparable to blood in the simulations of the aortic bench-top model, thus computationally verifying the mixture created in the lab as an effective blood analog.

Keywords: Computational Fluid Dynamics, Aorta, Blood Flow, Bench-top, Fluid Resistance, Blood Analog

INTRODUCTION

With minimally invasive catheter surgeries becoming prevalent for treating various heart diseases like aortic aneurysms or aortic valve stenosis, there is a need for bench-top models to assess catheter effectiveness and train surgeons for these types of surgical interventions. Aortic bench-top models have been utilized for years to develop an understanding of secondary flows, vortices, and wall-shear stresses (WSS) existing at the walls of the aortic arch that can lead to numerous arterial diseases. One of the first aortic bench-top models was developed by Yearwood and Chandran (1979), and took into account the complex geometry, tapering, and irregular cross-sections in the aorta. They found high WSS existent along the outer wall of the aortic arch with a prevalence of secondary flows developing on the inner wall during ventricular systole. Despite the lack of important geometrical factors like the aortic sinus region and branching arteries, they quantified important flow conditions generated during high flow rates in the aortic arch [1]. A few years later Khalighi *et al.* (1983) developed an acrylic *in situ* casting of the aortic arch including the branching arteries and aortic sinus. This further developed an understanding of flows existing in the regions of interest and how they are influenced by the bifurcating branches at the mid-aortic arch region. This work also allowed for the evaluation of valve prostheses [2]. Since then, Liepsch *et al.* (1992) developed a non-rigid silicone model of the aorta to understand fluid-structure interaction (FSI) and the impact of wall compliance on the flow patterns generated in the aortic arch [3]. This work has subsequently lead to the use of non-rigid, patient-specific aortic models in the evaluation of endovascular techniques and the assessment of various medical devices as in the works by Sulaiman *et al.* (2008) and Biglino *et al.* (2013) [4,5].

Computational fluid dynamics (CFD) is often utilized within the aortic arch region to analyze the complex three-dimensional (3D) flow patterns generated during ventricular systole, and to measure parameters that are difficult to obtain *in vivo*. CFD in the cardiovascular system was first validated in the work by Olufsen *et al.* (2000) where the one-dimensional theory from the Navier-Stokes equation was utilized in a CFD of the entire arterial tree and generated blood flow and pressure measurements in agreement with values obtained from magnetic resonance imaging (MRI) [6]. Since then, numerous works have validated the use of CFD analysis in the aorta, like the work done by Morris (2005). This work developed a CFD simulation of an aortic model including the aortic sinuses and branching arteries, and although making the assumption of rigid walls, still

quantified values relevant to *in vivo* data [7]. CFD simulations have continued to advance to point of being utilized to assess the efficacy of endovascular treatments. The work by Tokuda *et al.* (2008) displayed the effects on aortic flow from arterial cannulas during cardiopulmonary bypass surgery, and the work by Fung (2008) quantified factors of failure for stent-grafts within the aorta from high velocity blood flow [8,9].

This paper provides a computational fluid dynamic (CFD) model that simulates flows in an aortic bench-top apparatus in order to influence hardware settings on the bench through the quantification of fluid resistance at the aortic branches. Also, using the resistance values determined, perform time-variable flow simulations to verify the water glycerol mixture developed in the lab as a physiologically relevant blood analog fluid. The CFD simulation seeks to characterize the complex three-dimensional flows in an aortic arch bench-top model during physiological pulsatile flow. By quantifying the flow patterns generated, parameters affecting a catheter's path can be determined and utilized in the optimization of the device. The acrylic bench-top model must be robust and durable to accommodate for numerous engineering changes during prototyping and the repeated *in vitro* testing of preliminary catheter devices. This is reflected in the CFD model by allowing for the integration of design tables, various bench-top configurations, and patient-specific aortic arch geometry.

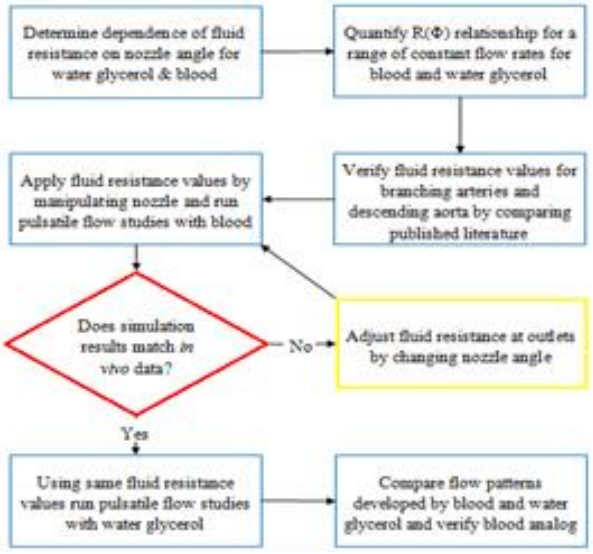

Figure 1. Flow chart outlining chronology of research.

METHODS

Geometry

All three-dimensional models were created using the computer-aided design software SolidWorks (SolidWorks, Concord, MA, USA). The aortic arch was modeled from a set of DICOM files obtained from the Visible Human Project's CT scans of a middle-aged female. An STL model of the aortic arch was developed in image segmentation software called ITK-SNAP (open-source software, www.itk-snap.org). The STL was then imported into SolidWorks where it was sliced into numerous cross-sections orthogonal to the centerline of the aortic arch, and each cross-section was traced and lofted together to create a functional solid-body model as seen in Fig. 2(a).

Due to the non-planar geometry of the aortic arch and branching arteries, visualizing flow through the center of the geometry becomes difficult. In order to illustrate the flow profiles existent in the bench-top model, a series of lofted surfaces were constructed. Using a series of slices of the aortic arch and branching arteries, centroid points were sketched at each cross-section. These were used to construct centerlines for each branch and the arch, and a series of horizontal lines coinciding with the centerline were sketched at each cross-section. All lines were then lofted together generating the non-planar, centerline surfaces as seen in Fig. 4.

Figure 2. Steps of bench-top fabrication (a) 3D SolidWorks model, (b) aortic arch printed in PVA

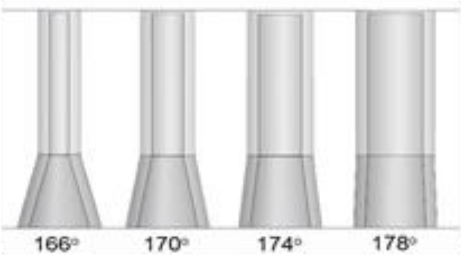

Figure 3. Illustration of nozzle geometry's dependence on nozzle angle

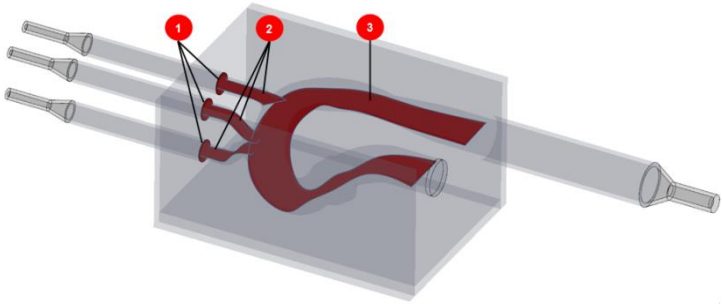

Figure 4. 3-D render of the aortic bench-top model with lofted plot surfaces (red) for (1) aortic branch cross-sections, (2) aortic branches, and (3) aortic arch

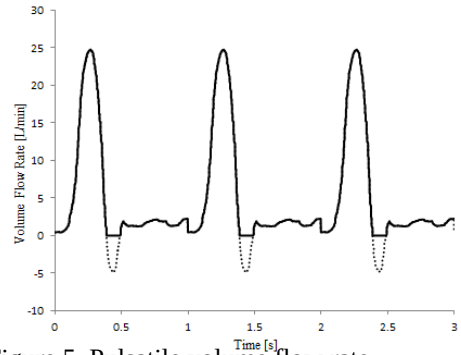

Figure 5. Pulsatile volume flow rate waveform aortic inlet (60 bpm) [10]

Computational Fluid Dynamics

Numerous computational fluid dynamic (CFD) simulations were performed in order to quantify the dependence of fluid resistance on nozzle angle. Once this relationship was determined, appropriate fluid resistance values were applied to each branching artery and descending aorta, and numerous time-variable CFD simulations were performed with both blood and water glycerol in order to verify water glycerol as a blood analog fluid for this bench-top model. "SolidWorks Flow Simulation" was the CFD software utilized in order to characterize the flows existent within the bench-top model.

Numerical Model

Like other CFD software, "SolidWorks Flow Simulation" combines ease of use with high-level functionality. This was desired in order to accommodate for the numerous engineering changes to bench-top configuration and patient-specific aortic arch geometry utilized during prototyping. The CFD code is based on solving 3-D Navier-Stokes equations with a finite volume method (FVM). This method divides the computational domain into smaller volumes around each node in the grid; this ensures continuity of flow between each node. The continuity and momentum equations governing flow for blood and water glycerol can be found in Eq. 1 and 2 [11].

In order to ensure accurate convergence of the CFD simulations, numerous surface goals with flow controlling criteria were introduced into the simulation. Surface goals were placed on probes at areas of interest within the bench-top model – aortic branch cross-sections (Fig. 4) and the bench-top outlets. Each probe had a volumetric flow rate and bulk average total pressure surface goal, and in order for the CFD simulation to converge all surface goals had to be satisfied.

Continuity Equation:

$$\frac{\partial \rho}{\partial t} + \frac{\partial (\rho u_i)}{\partial x_i} = 0 \qquad \text{(eq. 1)}$$

Momentum Equation:

$$\frac{\partial \rho u_i}{\partial t} + \frac{\partial (\rho u_i u_J)}{\partial x_j} = -\frac{\partial p}{\partial x_i} + \frac{\partial}{\partial x_j}\left(\tau_{ij} + \tau_{ij}^R\right) + S_i \qquad \text{(eq. 2)}$$

Mesh

Flow Simulation calculates the minimum gap size using information about the faces where boundary conditions and goals are specified. Therefore, before generating the mesh, all conditions are set to their desired values. Minimum gap size is a certain number of cells per specified gap that can be generated, thus this is the primary parameter driving the computational mesh [11]. Large gap sizes result in inadequate simulation results due to the low fidelity of the automatically generated mesh. In order to generate sufficient simulations results, and stay within the computational resources of the computer, gap size was incrementally decreased until an optimal gap size was determined. For all meshes, the minimum gap size was specified to be 4.2 cm.

Boundary Conditions

Boundary conditions during this study were obtained through the experimental work done by Bazan and Ortiz (2016), which characterized the volume flow rate waveforms at the inlet of the aorta for various heart rates [10]. Inflow conditions are initiated ~50mm upstream to the aortic valve. In order to reduce computational work load, a series of three steady flow rates were applied to

determine the nozzle angle-fluid resistance relationship for both blood and water glycerol. The three steady flow rates simulated onset ventricular systole (5L/min), mid-systole (15L/min), and peak systole (25 L/min) [10]. The inflow waveform employed during the time-variable CFD simulations can be seen in Fig. 5. Due to the small role in high-velocity hemodynamic conditions and the targeted region of the heart cycle for this bench-top model, the small portion of retrograde flow during ventricular diastole has been assumed zero (as displayed by the solid line in Fig. 5). The assumption of a flat or plug velocity profile at the aortic inlet is used and has been verified by various *in vivo* measurements [12,13]. A uniform, static pressure at the outlets of the model were applied for all simulations. Due to the nature of the bench-top model application, the approximation of rigid walls was also applied to the simulations. Previous works have also displayed how the assumption of rigid walls within the aorta generates results comparable to *in vivo* measurements [7].

Flow Simulation is capable of computing laminar flows of inelastic non-Newtonian fluids. Thus, blood is characterized as an incompressible, non-Newtonian liquid with a density of 1005 kg/m^3. The viscous shear stress tensor (τ) governing the fluid properties of blood are defined by Eq. 3 and 4. The dynamic viscosity (μ) of blood was determined using the Power-Law model (Eq. 5), where the values of μ are restricted to maximum and minimum values and K is the consistency coefficient. Water glycerol has been used in various other studies on hemodynamics, and has proven to display characteristics similar to blood [10,14]. The water glycerol mixture used for this bench-top is 60%, by volume, glycerin, and has a constant density of 1153 kg/m^3 and viscosity of 0.00936 Pa-s as seen in Table 1.

$$\tau_{ij} = \mu(\dot{\gamma}) \cdot (\frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i}), \qquad \text{(eq. 3)}$$

where shear rate,

$$\dot{\gamma} = \sqrt{d_{ij}^2 - d_{ii} \cdot d_{jj}}\,, \qquad d_{ij} = \frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i} \qquad \text{(eq. 4)}$$

$$\mu(\dot{\gamma}) = K \cdot (\dot{\gamma})^{n-1} \qquad \text{(eq. 5)}$$

By quantifying the pressure drop from the aortic branches to the nozzle outlet and the volume flow rate at the exit of the model, fluid resistance could be calculated using eq. 6. Once the fluid resistance-nozzle angle relationship was defined, effective fluid resistances were applied at the branching arteries and descending aorta to perform the time-variable simulations of blood and water glycerol. The values applied to the simulation can be found in Table 2 [14].

$$\frac{P_2 - P_1}{\dot{V}} \qquad \text{(eq. 6)}$$

RESULTS

Fluid Resistance

During the fluid resistance analysis, a series of parametric CFD simulations were performed with both blood and water glycerol. During each study, the cone nozzles applied to the outlet of the simulation were incrementally increased at each design point in order to determine fluid resistance dependence on nozzle angle. The results of the various steady flow rate simulations can be found in Fig. 6. Fluid resistance is expected to remain relatively the same regardless of changing flow rate, but at a nozzle angle of 163^o, the fluid resistance increased as the flow rate was increased. However, starting at a nozzle angle of 164^o, all flow rates displayed a similar fluid resistance – 300 mmHg/L/min. The CFD simulations performed with water glycerol (dashed lines) generated fluid resistances significantly higher than blood (solid lines), although this is to be expected due to the shear-thinning nature of blood and the effect of viscous drag on pressure drop. Now, that fluid resistance is quantified with nozzle angles, fluid resistances are applied to each branch of the study in order to run time-variable simulations.

Table 1. Fluid properties applied to CFD simulation.

Properties	Blood	Water Glycerol
Density [kg/m^3]	1003	1153
Maximum Dynamic Viscosity [Pa-s]	0.012171	0.00936
Minimum Dynamic Viscosity [Pa-s]	0.003038	-
Constant Viscosity [Pa-s]	0.0037	-

Table 2. Fluid resistance values applied to time-variable simulation [14]

Vessel	Resistance (mmHg/L/min)
Brachiocephalic	182.7
Left Common Carotid	230.7
Left Subclavian	878.6
Descending Aorta	25.85

Blood v. Water Glycerol

Fluid resistance values, found in Table 2, where applied to the simulation and time-variable CFD simulations were performed with both water glycerol and blood using the flow rate waveform found in Fig. 5. The resulting velocity magnitude contours can be found in Fig. 7. Both contours displayed similar flow patterns in the regions of interest — aortic arch, aortic branches, and descending aorta. There are slight discontinuities near the aortic root region; however, due to the primary interest of flows downstream, this difference can be ignored. During the time-variable simulations, blood and water glycerol generated flow waveforms nearly identical to each other at all areas of interest. This can be seen in Fig. 8, where at the ascending aorta, mid-arch, and descending aorta water glycerol (blue) generates a flow rate slightly higher than blood (red); however these differences are negligible and it can be assumed that water glycerol will generate hemodynamic flows.

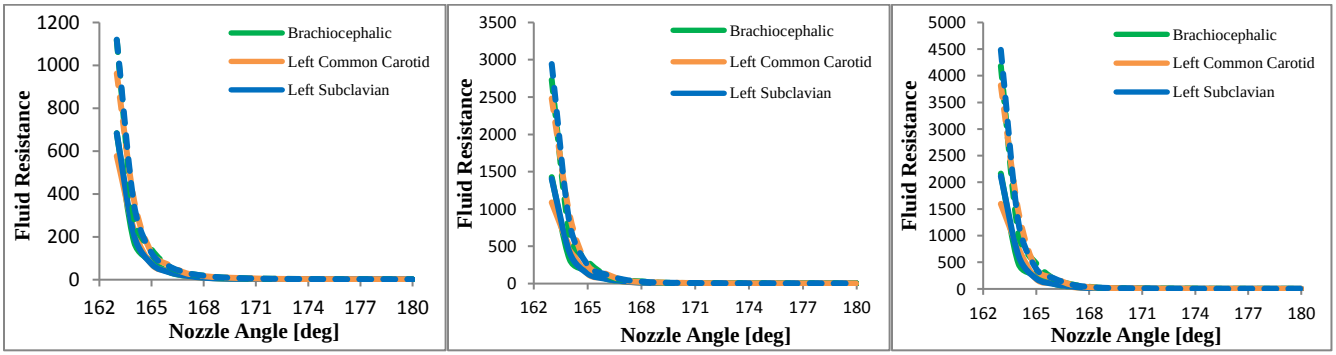

Figure 6. Fluid resistance plots for steady flow rates (a) 5 L/min, (b) 15 L/min, and (c) 25L/min. All fluid resistance values are in mmHg/L/min. Solid lines represent blood and dashed lines represent water glycerol.

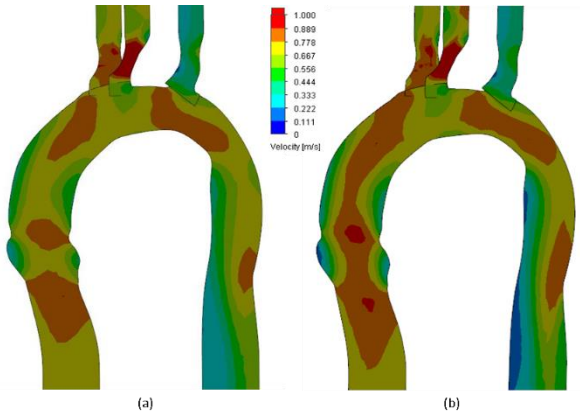

Figure 7. Comparison of resulting velocity magnitude surface contours at 15 L/min between (a) blood and (b) water glycerol.

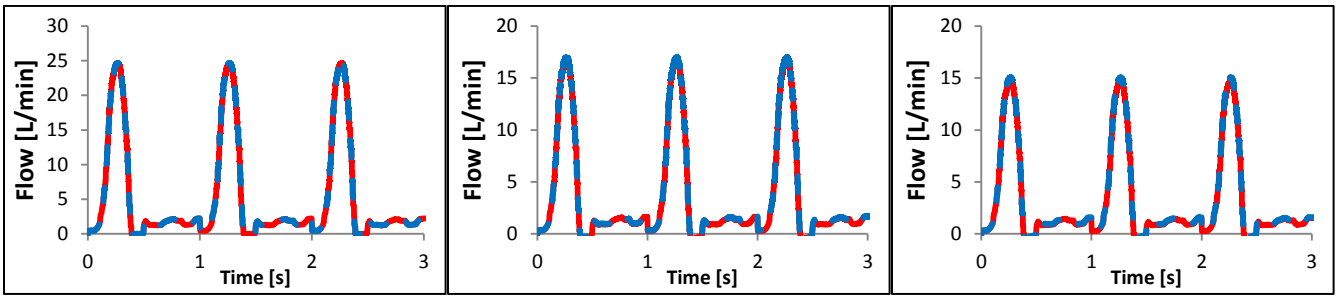

Figure 8. Volume flow rate waveform (60 BPM) for regions of interest – (a) ascending aorta, (b) mid-arch, and (c) descending aorta. Blood is represented in red and water glycerol is represented in blue.

DISCUSSION

Fluid Resistance

This study generated data that is capable of being utilized when sizing fluid resistance hardware settings during future experimentation on the bench. The cone nozzle at the outlets of the model are capable of producing fluid resistances within the range of physiological relevancy [14,15]. There are discontinuities at each of the steady flow rates for fluid resistance in the branching arteries when the nozzle angle is set to 163°. However, the fluid resistances generated at this design point are far too high to apply to a physiologically relevant experiment, so these differences can be ignored. Once the nozzle angle geometry begins to produce relevant fluid resistances (164°), the values are similar across all steady flow rates (5 L/min, 15 L/min, 25 L/min).

Water Glycerol v. Blood

This study computationally verifies water glycerol as an effective blood analog fluid for this bench-top during constant and pulsatile flow conditions. During the pulsatile flow CFD simulations, both blood and water glycerol were evaluated in order to determine the relevance of water glycerol to natural hemodynamics. Water glycerol produced results similar to blood in these CFD simulations, and also to previous published clinical data [15]. The velocity magnitude surface contour comparison (Fig. 7) illustrates how the flow patterns generated at the regions of interest for both blood and water glycerol are comparable to each other. There are slight differences in flow along the wall of the aorta that influence flow downstream in the descending aortic region due to the high viscosity of blood. However, these discontinuities are distinguishable only during the high velocities generated during peak systole, and it can be assumed that the flow patterns generated by water glycerol correspond to blood performance. Flow rate waveforms generated from the CFD simulations in the regions of interest by water glycerol are nearly identical to those generated by blood (Fig. 8); further verifying water glycerol as a blood analog for this bench-top configuration.

Technical Limitations

A high-fidelity CFD simulation was avoided during this study in order to accommodate for the numerous design changes during prototyping and their timely integration into a computational model. Due to the preliminary catheter designs tested on this bench-top, the apparatus had to be robust and durable to accommodate for repeated testing under various conditions. This is partially reflected in the assumption of no wall motion in the aortic arch which reduces the physiological relevancy of the CFD simulation; however, wall motion has been determined to have a small effect on flow patterns generated in the aortic arch [7]. Also, because CFD analysis of the aorta requires numerous complex processes, only one representative case of aortic geometry and pulsatile waveform was simulated during this study. Finally, the boundary conditions at the outlets of the model were assumed to be the same and constant. Although, this is one of the most commonly used assumptions for outflow boundary conditions while simulating blood flow, ideally there should be a comprehensive representation of all downstream effects from vasculature [13,16]. However, this would greatly increase the complexity and solving time of the computational model.

Future Research

Once effective catheter devices have been developed through utilizing the rigid-wall bench-top model, high-

fidelity bench-tops can be employed. This would mean a variety of patient-specific aortic bench-top models that include complaint wall motion and diseased aortic leaflets to further determine the flow characteristics existent within the aorta to optimize catheter devices. This would also be reflected in the computational modeling of the bench-top by implementing more advanced CFD software like CRIMSON or SimVascular, and incorporate aortic leaflet motion simulation model for verification and validation (V&V) of prosthetic aortic heart valves.

CONCLUSIONS

This study attempted to computationally characterize flow characteristics within an aortic bench-top model for the optimization of minimally invasive surgical strategies for endovascular repair. By computationally quantifying fluid resistance values in order to influence bench-top hardware setting, future testing and experimentation can be expedited, and optimal catheter design can be determined for a range of conditions. The CFD simulations of the bench-top model was found capable of producing fluid resistance values within a wide range of physiological relevance. Water glycerol also produced comparable results to hemodynamic performance, thus computationally verifying it as an effective blood analog for this bench-top apparatus.

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OBSERVING TRENDS IN VITAMIN D SUPPLEMENTATION: NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY 2009-2014

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ABSTRACT

Decades of research have proven the efficacy of vitamin D in health and wellness, as well as, disease prevention. Adequate consumption of vitamin D is not commonly obtained by nutrient consumption in food alone. Therefore vitamin D supplementation is commonly recommended or prescribed to obtained adequate intake of vitamin D. This study analyzed trends in the frequency of Vitamin D supplement consumption and the reasons vitamin D were consumed. Supplement information was obtained from National Health and Nutrition Examination Survey (NHANES) responses during cycle years 2009-2010 through 2013-2014. Supplements which contained vitamin D were uniquely included in this study. The results of this study revealed that vitamin D is consumed mostly through multivitamins. The results also revealed an incline of vitamin D supplement consumption and a decline of those individuals who consume calcium supplements containing vitamin D. Overall, there is a 91.74% increase in vitamin D supplement consumption. This may have been attributed to the findings of the 90.05% increase in the reason of consumption being due to physicians' recommendation of consumption.

Keywords: NHANES, Vitamin D Supplementation, Vitamin D

Abbreviations: Cal-D calcium plus vitamin D, CLO cod liver oil, FLO-D fish liver oil plus D, MVMM multivitamin/multi-mineral, NHANES RDA (Recommended Daily Allowance), VitA-D vitamin A plus D , VitD vitamin D supplements, UV (ultraviolet)

INTRODUCTION

Vitamin D is a fat soluble secosteroid and prohormone. Its consumption is essential to preventing vitamin D deficiency and vitamin D deficiency related diseases. Vitamin D is primarily known for its relationship to skeletal formation and other skeletal diseases. One of the main functions of vitamin D is its ability to increase calcium absorption [1]. Maintaining adequate levels of calcium is vital for bone development, growth and remodeling. In the recent decades, it is being realized that vitamin D plays an important role in other diseases. Vitamin D deficiency has been linked to diseases such as cardiovascular diseases [2], cancer [3], autoimmune diseases [4], as well as, other diseases [5].

Vitamin D is endogenously acquired through ultraviolet (UV)-B radiation in the skin. However, through UV-B radiation, vitamin D consumption is not always easily accessible. One of the effects of low sun exposure is vitamin D deficiency. Skin absorption of vitamin D depends on the degree of UV-B radiation. This may include factors such as sunscreen use and darker skin pigment [6]. Other factors to be considered for vitamin D deficiency due to low sun exposure, include those who live in areas not commonly exposed to sunlight. In geographical locations that do not receive sufficient sunlight, the prevalence of vitamin D deficiency is increased in comparison to areas that do receive ample sunlight [7]. Vitamin D deficiency is also indicated in the population during cold seasons when UV-B radiation is insufficient [8].

Another method of vitamin D consumption is through dietary nutrition. Naturally found in many of the oily fish and in some mushrooms which were exposed to sunlight [9], vitamin D is not consumed in large amounts in the human diet. Since the early discoveries of the vitamin D, foods have been irradiated with UV-B [10, 11]. This irradiation fortified foods with vitamin D to increase the chance of vitamin D consumption in foods. Foods which did not naturally contain vitamin D, now do. In addition, foods are currently supplemented with vitamin D. This includes foods such as milk, cheese and cereal. However, the vitamin D fortification of foods still does not provide adequate intakes of vitamin D which will prevent vitamin D deficiency.

In order to maintain an adequate vitamin D status, one must consume the minimum recommended daily allowance (RDA) of vitamin D. The RDA of dietary intake is the daily intake necessary to meet nutritional requirements and is issued by the Food and Nutrition Board of the Institute of Medicine. The recommended daily allowance for children is 400 IU/day, 600 IU/d for adults 70 years or younger, and 800 IU/d for those older

than 70 years [12]. Even with the recommendations of obtaining vitamin D through UV-B absorption and food consumption of vitamin D, it is nearly impossible to prevent vitamin D deficiency. With the realization that the UV-B absorption and food consumption of vitamin D is insufficient to maintain an adequate vitamin D status, the needs to consider and recommend vitamin D supplementation is quite often necessary.

Vitamin D supplementation may be achieved through prescription supplements or via over-the-counter purchase. Due to upper tolerable limits of 4,000 IU [13], high dose vitamin D supplements are usually prescribed by physicians to treat cases of vitamin D deficiency. Vitamin D supplementation is essential to obtaining a sufficient vitamin D status and optimal health. In addition to being consumed as a stand-alone supplement, vitamin D may also be consumed through several different methods. Vitamin D may be consumed as a part of a multivitamin/multi-mineral supplement, in combination with another vitamin or minerals or as an additive ingredient of other supplements that are neither vitamins nor minerals, such as fish oil, natural herbs or manufactured chemicals which are used as supplements. In addition to being an added ingredient, there are supplements that naturally contain vitamin D, such as cod liver oil.

Studies show a rise in vitamin D supplement consumption, including those supplements with higher doses of vitamin D [14]. However, there are not many studies which indicate the reasons for the increase of vitamin D supplementation. This study assessed all supplements containing vitamin D. This study also evaluated the reasons why supplements that contained vitamin D were consumed. Mainly, two roles are identified: the physician's role in vitamin D supplementation and the individual's decision to consume on their own. It is believed that physicians' recommendations to consume vitamin D supplements effected the frequency of vitamin D supplements consumed over the years.

METHODS

This is a quantitative study which analyzes vitamin D supplementation in the U.S. population. Methods for this study included a cross-sectional analysis of all responses obtained from the NHANES data. NHANES is a program of studies designed to assess the health and nutritional status of adults and children in the United States. This previously surveyed data is free to the public, therefore also exempt from human subjects ethics review. Individual dietary supplement information was obtained from the NHANES data in cycle years 2009-2010, 2011-2012 and 2013-2014. All responses were included regardless of age or other demographic information. Missing or unknown data was excluded. Supplements were excluded if there was a lack of evidence of vitamin D as an ingredient. Evidence was determined from known or labeled ingredients and/or the reported consumption of vitamin D (mcg) within the supplement.

Each individual supplement name was reported by the respondent during the survey. To prepare the data for this study, the supplements were coded into supplement categories which contained vitamin D. This included vitamin D supplements (VitD) which contains vitamin D as the primary ingredient, calcium supplements with added vitamin D (Cal-D), vitamin A which also contained vitamin D (VitA-D), cod liver oil (CLO), which naturally contains vitamin D, and fish oil with added vitamin D (FO-D). Multivitamins/multi-minerals (MVMM) contained at least a combination of at least three ingredients of vitamins and minerals and were considered in the analysis when it contained vitamin D. Other supplements which contained vitamin D (Other-D) were also included. These supplements were neither vitamins, minerals or but may consist of natural or chemical properties.

This dietary data was utilized in order to obtain the frequency of supplements which contain vitamin D, as well as, to examine the reasons why vitamin D supplements are consumed. SPSS, Inc. version 24.0 was used to calculate the counts and the frequency of supplements used and to obtain the frequency of supplements used which contained vitamin D by the reason for consumption.

RESULTS

The findings of this study demonstrated that out of 25,786 supplements consumed from the 2009-2010 cycle year to the 2013-2014 cycle year, 11,415 contained a form of vitamin D. Of these, 30.6% derived from multi-vitamins/multi-minerals, 7.6% from vitamin D supplements, 4.6% from calcium supplements which contained vitamin D, 0.3% of vitamin A supplements which contained vitamin D, 0.3% of cod liver oil, 0.2% fish oil supplements which contained vitamin D and 0.8% of other supplements which contained vitamin D. This information is found in table 1 and is illustrated in figure 1.

Only referring to the top three supplements which contain vitamin D from this point on, MVMM, VitD and

Cal-D where the supplements which contained vitamin D that were consumed the most in all three cycle years (figure 2).

In the 2009-2010 cycle year, it was reported that doctors advised 520 out of 2732 MVMM supplements, 198 of 402 Vitamin D supplement and 229 out of 463 Cal-D supplements to be consumed. In the 2011-2012 cycle year, data shows a reported 486 of 2453 MVMM supplements consumed, a 383 of 622 vitD supplements consumed, and 208 of 396 Cal-D supplements consumed. In the 2013-2014 cycle year, it was reported that doctors advised the consumption of 461 of 2,573 MVMM supplements, 465 of 765 VitD supplement and 140 of 289 cal-D supplements. The remainder of each of these supplements were taken for reasons of the respondent's own. Trends in reasons consumed by doctor's advice is illustrated in figure 4.

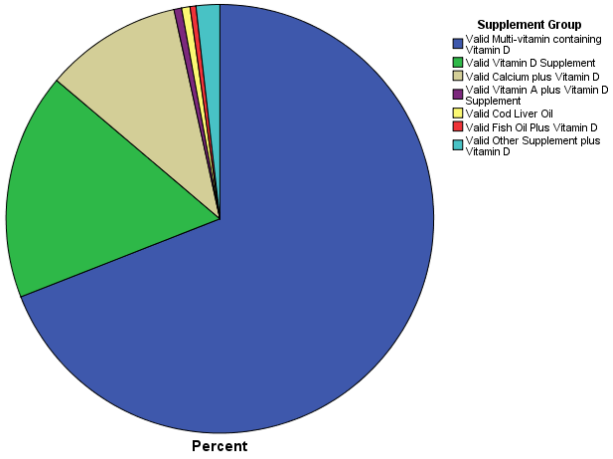

Figure 1. Percent of dietary supplements consumed from 2009 through 2014 that contained vitamin D.

Table 1. Frequency of Dietary Supplements containing Vitamin D Consumed 2009-2014

Frequency of Supplements Consumed by Cycle Year							
Cycle Year	MVMM	VitD	Cal-D	VitA-D	CLO	FO-D	Other
2009-2010	2775	436	475	26	20	2	68
2011-2012	2494	681	403	36	29	13	53
2013-2014	2615	836	306	4	24	36	83

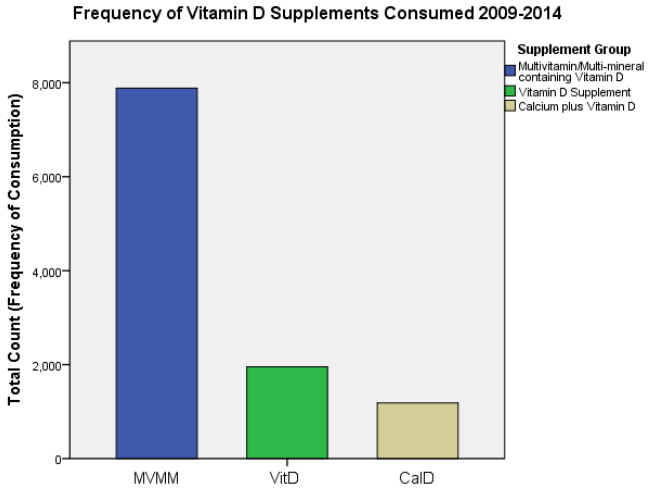

Figure 2. MVMM, VitD and Cal-D were consecutively the most consumed supplements which contained vitamin D from 2009 through 2014.

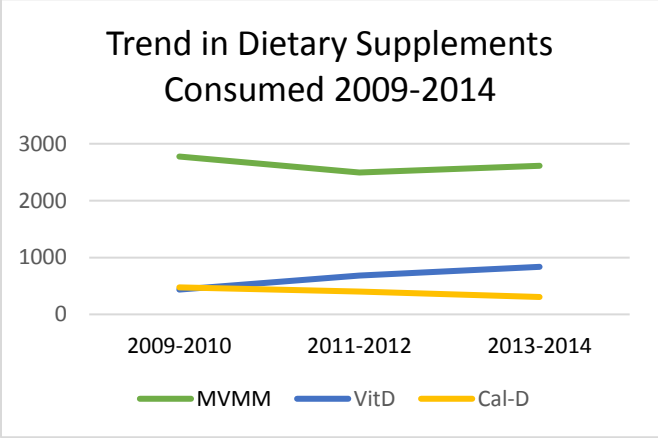

Figure 3. Trends observed in the frequency of the supplement consumption containing vitamin D.

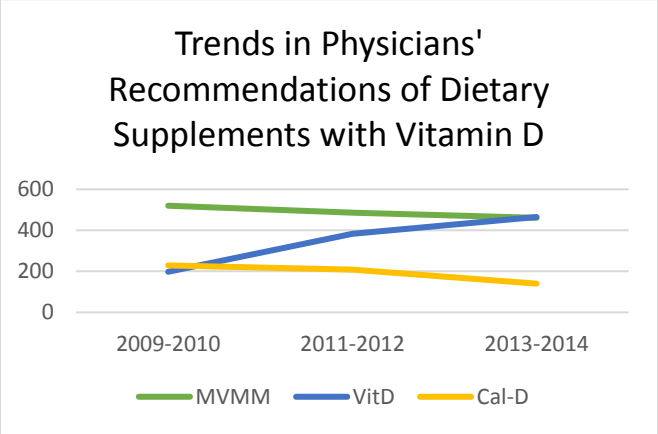

Figure 4. Trends in doctors recommending supplements containing vitamin D

DISCUSSION

Since multivitamins contain more than one - usually more than three vitamins and minerals [15], it would seem practical to consume as many vitamins and minerals as possible to fulfill the recommended allowances for dietary intake. In this study, multivitamins/multi-minerals were the most consumed dietary supplements which contained vitamin D in all cycle years. Past studies [16, 17, 18] have drawn similar conclusions of the most consumed dietary supplements being multivitamins. In Bailey's 2013 [19] study, it was concluded that most supplements were taken in the form of multivitamins, followed by fish oil supplements. Mohebi-Nejad (2014) stated that omega-3 and fish oil supplements were amongst the top prescribed supplements worldwide [20]. These results are slightly differently than ours. Omega-3 and fish oil supplements were not amongst the top three consumed dietary supplements that contained vitamin D. This dissimilarity may be in part to the focus of our study. In this study, only supplements which contained vitamin D were analyzed. Therefore, omega-3 and fish oil supplements were not considered unless it contained vitamin D. In the instances that omega-3 and fish oil supplements did contain vitamin D, fish oil was

categorized in its own group and omega-3 was grouped in the supplement category "other".

Studying consumption trends, multivitamin/multi-mineral consumption declined from 2009-2010 to 2011-2012. A similar decline has been observed by Kantar in 2016 [21]. The second most consumed dietary supplement was the vitamin D supplement. As seen in table 1 and figure 2, there is a positive trend in vitamin D supplement consumption. According to Rooney [22], there has been a trend in the supplement consumption of vitamin D since 1999. Calcium supplements containing vitamin D have been used for the prevention and treatment of bone diseases and disorders. However, our results show a decline in the consumption of calcium supplements with added vitamin D. The reasons for this trend is unknown. According to some studies, hip and other bone fractures are effectively reduced by calcium supplementation when taken with vitamin D [23].

Reasons for trends in the consumption of supplements that contain vitamin D may be easier to determine when the reasons why the supplements were taken are analyzed. When analyzing the reasons for supplement consumption, respondents consumed supplements for reasons of their own, overall. The data analyses, however, reflect a progressive trend in doctors advising their patients to consumed vitamin D supplements and other supplements that contain vitamin D. It must also be noted that there is a similar trend in individuals taking vitamin D supplements own their own. However more doctors advised patients to consume vitamin D which showed a greater influence from physicians. Reasons for the decline of calcium supplements which contain vitamin D may be reflection of the declining advice of physicians to consume it. Over the years, doctors have advised the consumption of vitamin D supplements. This may be reflected in the increase of vitamin D supplement consumption since 2009. In contrast, some observations also included a decline in trend. The analysis from the responses indicated doctors decreased the occurrence of advising their patients to consume multivitamins. The declining advice of physicians to take multivitamins may be due to the uncertainties of safety of multivitamins. Or it may be due to poor evidence of efficacy of multivitamins [23]. While studies have shown there may be consequences to taking single vitamins or minerals [22], other studies show that when consuming multivitamins as instructed, the benefits outweigh the risks [23]. The decline in calcium supplements with vitamin D is also imitated in the decline in physicians advising their patients to consume these supplements. While the physicians' advice has decreased, the respondents' reasons for consuming on their own decreased as well.

EXCLUSIONS AND LIMITATIONS

Exclusions include supplements in which vitamin D could not be indicated as an ingredient. Missing or data that is reported as unknown was also excluded from the analysis.

It should be understood that while most multivitamins/multi-minerals contain vitamin D, their consumption may entail factors which may not represent an association with the need to consume vitamin D. It is also to be noted that the use of supplements, the ingredients within the supplements, and the reasons why the supplements were consumed, were self-reported. These are limitations to the study. There were, however, times that the supplements bottles were presented to the interviewer for confirmation of responses. Another limitation of this study was the absence of prescription information. It is not known whether the reported supplements were prescribed, or purchased over-the-counter.

CONCLUSIONS

In order to maintain an adequate vitamin D status, one must consume the minimum recommended daily allowance (RDA) of vitamin D. Since very few food sources consist of vitamin D and since UV-B absorption is season- and location-dependent, vitamin D supplementation is the recommended alternative to vitamin D consumption.

Supplements that contain vitamin D may be prescribed by a physician or purchased over-the-counter due to the recommendation of a physician or own one's own decision. However it is believed that physicians have greatly influenced the trends in the consumption of supplements containing vitamin D. Trends in consumption seems to correlate with the reasons for consumption being that of one taking the physician's advice to consume. But results from this study show that similar trends are found in reasons of the respondent's own when making the decision to consume the supplements. Because of this, the extent of the role that physicians play in the reasons why supplements containing vitamin D are consumed, cannot be concluded.

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CARDIOVASCULAR RESPONSES FOLLOWING DIFFERENT TYPES OF BREATHING EXERCISES

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ABSTRACT

Purpose: The purpose of this study was to determine the effect of different types of breathing exercises on blood pressure, heart rate, and respiratory rate. **Subjects:** The study was performed on 45 healthy volunteer subjects ranging from 21-50 years of age. **Methods:** Subjects were randomly divided into three equal groups: control breathing (C, n=15), shallow breathing (SB, n=15) and combined breathing ({diaphragmatic and pursed-lip breaching techniques used together} CB, n=15). Blood pressure (BP), heart rate (HR), and respiratory rate (RR) were recorded before and after the breathing exercises. Each subject was instructed and given a demonstration of their specific breathing exercise. Each subject successfully performed the assigned breathing exercise for fifteen minutes. All data were analyzed using repeated analysis of variance. **Results**: The mean RR in C group before and after breathing exercises were 14.27±2.84 and 14.53±4.61, respectively. The mean RR in SB group before and after breathing exercises were 15.27±3.26 and 17.47±4.07, respectively. The mean RR in CB group were 14.33±3.75 and 12.67±2.50, respectively. SB had significantly increased RR ($P<0.05$) and CB had significantly decreased RR ($P<0.05$). There were no significant changes in blood pressure and heart rate. **Conclusion:** This pilot study indicates that 15 minutes of breathing exercises has no effect on BP and HR, but SB significantly increases respiration and CB significantly decreases RR. **Clinical relevance:** It has been shown that an increase in RR is associated with an increase in stress and a decrease in respiratory efficiency and endurance. Therefore, this pilot study results may suggest that CB breathing technique could be used to decrease RR, to relieve stress and to increase respiratory efficiency. Future study is needed to determine the long term effect of CB breathing on cardiovascular and stress responses, especially for patients with respiratory problems and those patients in stress induced situations.

Keywords: Breathing exercise, blood pressure, heart rate, and respiration rate

INTRODUCTION

In physical therapy practice, breathing exercises have commonly been used to treat patients with respiratory problems to decrease the cost of oxygen consumption, increase respiratory efficiency, and increase endurance during physical activity. A report from Jones et al. and others (1-9) have demonstrated that diaphragm breathing (DB), pursed-lip breathing (PLB), and a combination of DB and PLB significantly lowered oxygen cost in patients with chronic obstructive pulmonary disease (COPD). Schein et. al (10) has shown that slow, regular breathing guided by a device called BIM (Breath with Interactive Music) significantly lowered the high blood pressure by approximately 10 to 15 mmHg. However, lack of conclusive information regarding the effects of traditional breathing exercises on cardiovascular response is evident. Therefore, the purpose of this study was to investigate the effects of different types of breathing exercises on blood pressure, heart rate, and respiration rate.

METHODS

The study was performed on 45 healthy volunteer subjects ranging from 21-50 years of age according to our standard protocols unless otherwise indicated.

Experimental Approach:

All subjects were screened for medical problems that would prevent their participation. All subjects included in this study their age ranged between 21-50 years old. The exclusion criteria implemented for those subjects that have a history of: (1) respiratory problems, (2) cardiovascular problem, (3) neurological problem, (4) metabolic syndrome, (5) those subjects who are currently on medication which that might affect heart rate, blood pressure and mental status, (6) those subjects who are pregnant, and (7) those who failed to answer questions in a consistent manner. All subjects voluntarily signed an informed consent document and confirmed that they understood the procedure of all tests and the risks that might be involved.

Subjects were randomly divided into three equal groups: control breathing (CR), shallow breathing (SB) and combined breathing (CB). Prior to the breathing exercise, all subjects were asked to rest for 5 minutes. Then blood pressure, heart rate, respiratory rate were recorded.

Subjects in the CR group sat on the chair quietly and

took spontaneous breathing test for 15 minutes at their regular breathing pace following standard protocols.

Subjects in the SB group sat on the chair and took fast shallow breathing for 15 minutes. Subjects were allowed to rest at any time when they felt so short of breath.

Subjects in the CB group sat on the chair and performed CB for 15 minutes. CB was the combination of diaphragm breathing (DB) and pursed-lip breathing (PLB). DB occurred when there was a conscious appreciation of inspiring air to the lung bases with slight forward abdominal displacement and passive relaxed expiration. PLB consisted of each subject's normal pattern of inspiration, but expiration was performed by gently exhaling through "pursed" lips.

Blood pressure, heart rate, respiratory rate were recorded and saliva was analyzed for cortisol levels at the end of breathing exercise. The tolerance of stress as a measure of cortisol levels was conducted following standard laboratory procedures and as instructed by kits manual.

Statistical analysis between the groups was achieved using repeated ANOVA ($P < 0.05$ was considered as a judgment for statistical significant difference). Sigma Stat software was utilized for descriptive and test analysis. Slide write software was used to tabulate and construct the results.

RESULTS

As shown in Figure 1 the mean RR in C group before and after breathing exercises was 14.27±2.84 and 14.53±4.61, respectively. The mean RR in SB group before and after breathing exercises was 15.27±3.26 and 17.47±4.07, respectively. The mean RR in CB group was 14.33±3.75 and 12.67±2.50, respectively. SB had significantly increased RR ($P<0.05$) and CB had significantly decreased RR ($P<0.05$).

The mean systolic blood pressure in all groups before and after breathing exercise were demonstrated in Table 1. The results revealed that the mean systolic blood pressure slightly decreased among the control group after the breathing exercise, however, no significant difference noted in SB and CB groups compared to control.

The mean diastolic blood pressure in all groups before and after breathing exercise were demonstrated in Table 2. The results revealed similar trend to that observed in ventricular ejection (systolic) measurements.

The mean heart rate in all groups before and after breathing exercise were demonstrated in Table 3. The results revealed similar trend to that observed in blood pressure measurements. Overall results, there were no significant changes in systolic blood pressure, diastolic blood pressure and heart rate between pre and post breathing exercises among any of these groups ($P>0.05$).

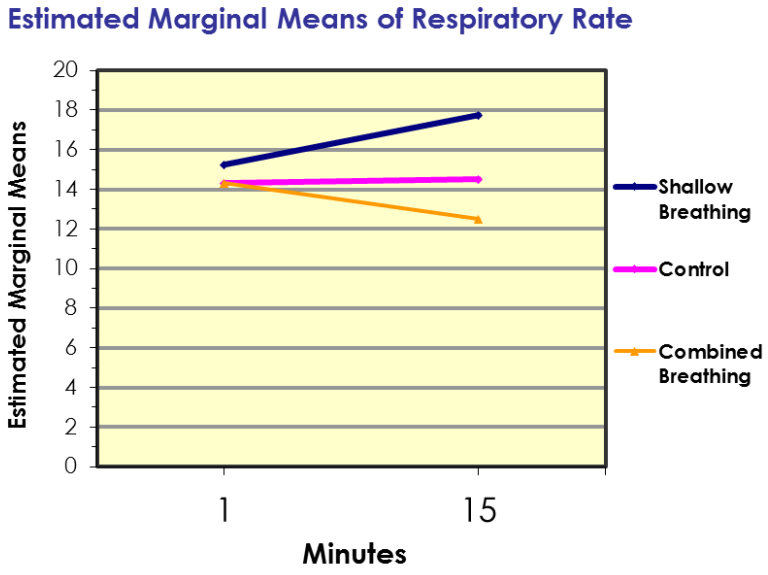

Figure 1: the effect of different breathing exercise on respiration rate among the three different groups. See methods for more details.

Table 1. Quantitative measure of the mean ± SD systolic blood pressure (S-BB) in all groups before and after breathing exercise (n = 45).

Groups	S-BB at base time	S-BB after test	Significant Difference
Control (C)	125.20±20.64	121.20±11.60	
SB	117.73±12.61	116.33±13.06	P>0.05
CB	118.53±11.94	117.87±11.20	P>0.05

Table 2. Quantitative measure of the mean ± SD diastolic blood pressure (D-BB) in all groups before and after breathing exercise (n =45).

Groups	D-BB at base time	D-BB after test	Significant Difference
Control (C)	78.40±15.56	71.6±7.40	
SB	69.93±8.19	68.27±9.81	P>0.05
CB	76.13±5.87	76.13±5.87	P>0.05

Table 3. Quantitative measure of the mean ± SD heart rate (HR) in all groups before and after breathing exercise (n= 45).

Groups	HR at base time	HR after test	Significant Difference
Control (C)	73.60±15.99	76.20±13.29	
SB	68.40±10.07	67.47±12.30	P>0.05
CB	72.13±10.40	72.13±10.40	P>0.05

DISCUSSION

In the present study, we investigated the direct effects of different traditional breathing patterns on blood pressure, heart rate and respiration rate. Compared with spontaneous breathing, combination of diaphragm breathing and pursed lip breathing (combined breathing) significantly decreased respiration rate, and shallow breathing significantly increased respiration rate. However, neither combined breathing nor shallow breathing affected blood pressure and heart rate significantly (P>0.05).

To the best of our knowledge this study is considered the first study to be reported in the literature in which assessed the effects of different traditional breathing pattern on blood pressure, heart rate and respiration rate in healthy adults without preexisting respiratory problems.

Many form of breathing exercises are widely used as an intervention to reduce major disturbances such as dyspnea, short of breath, depression, anxiety, hypertension, angina, functional chest disorder, chronic obstructive pulmonary disease (COPD) and cardiac rehabilitation (3,4,5,7,9). In physical therapy rehabilitation traditional program, breathing exercises are commonly used to treat patients with respiratory problems (8). It was well documented that the common clinic symptoms of respiratory disorders are dyspnea and fatigue. Fatigue is usually induced due to the hypoxia in which resulting from different entities including: (i) an obstruction of the air ways, (ii) an increase in respiratory activities and (iii) lack of quality of breathing. Previous research reported by Breslin et.al (2) showed that an increase in fatigue severity lead to an increased disorder in pulmonary performance. Consequently, further reduced endurance for activity and resulting in sever fatigue

conditions. Pharmacologically, there is still no effective cure for dyspnea and fatigue that have been scientifically documented. It is well known that pulmonary rehabilitation and respiratory exercises are actions usually taken to control the symptoms and to improve functional performance of life activities. A research conducted by Zakerimoghadam et.al (11) showed the change in respiratory pattern caused reduction of fatigue intensity in COPD patients. Patient with COPD has a high tendency to have a shallow, fast and insufficient breathing. Our results confirms that PLB exercise improved patient to diaphragm respiration pattern which is a slow, deep and relaxing breathing pattern. PLB and DB are the methods of choice of slow and deep breathing exercise. However there is no agreement which favors one over the other. This was the main reason CB pattern was selected in this study. This also could be attributed to a slow deep breathing exercise. The SB group was designed to simulate the breathing pattern of COPD and to explore the harmful effect of this breathing pattern. The results of the present study clearly demonstrated that combination DB and PLB significantly slowed the rate of respiration, indicating that CB can be an effective tool for the reduction of fatigue, dyspnea, anxiety, short of breath and increasing endurance for daily activity and improving quality of life. On the other hand, SB significantly increased the rate of respiration, indicating this type of breathing can increase pulmonary symptoms and further impair the quality of life of patients.

In addition to the management of dyspnea and fatigue, a slow and relaxed breathing exercise can be a choice of nonpharmacological invention of hypertension. Schein et.al (10) reported that slow, regular breathing guided by a device called BIM (Breath with Interactive Music) significantly lowers the high blood pressure by 10 to 15 mmHg. A recent view article made by Brandani et.al (1) showed that Yoga breathing exercise can decrease blood pressure by 4 to 21 mmHg. However, the results of the present study showed that there were no significant changes in HR and BP between pre and post measurements in any of the groups. The difference between previous studies (**1,10**) and our study is that our study only performed for a duration of 15 minutes of breathing excise in healthy subject without pulmonary problems and hypertension. Further study is needed to determine the long term beneficial effects of CB exercise on blood pressure, heart rate, and respiration rate in patient population.

Overall conclusion: a duration of 15 minutes of CB exercise significantly decreased the rate of respiration, suggesting CB can be a useful tool to reduce fatigue, dyspnea, anxiety and short of breath caused pulmonary disorders.

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DOSIMETRIC PREDICTIVE MODEL FOR ESOPHAGITIS INDUCED FROM RADIOTHERAPY OF LUNG CANCER PATIENTS

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ABSTRACT

Purpose: To establish a predictive model for the incidence of esophagitis for lung cancer patients treated with radiotherapy. **Methods and Materials**: 139 treatment charts of lung cancer patients treated with radiation therapy or combined chemo-radiotherapy from January 01, 2014 to June 30, 2017 at University of Mississippi Radiation Oncology were retrospectively reviewed. Mean esophagus dose (MED) and the endpoints of esophagitis grade 1 and 2 based on Radiation Therapy Oncology Group (RTOG) definitions were derived from the Pinnacle treatment planning system (TPS) and the EPIC electronic medical record (EMR) system, respectively. Binary logistic regression and Probit statistical analysis were used to determine the relationship between the probability of grade 1 and 2 esophagitis with the mean esophagus dose. **Results and Conclusions**: The regression model of the incidence of grade 1 and 2 esophagitis was established. The results suggest that MED is a good predictor of the risk of radiation-induced esophagitis. The mean esophagus doses associated with a 50% incidence probability (TD_{50}) for grade 1 and 2 esophagitis were determined as 1,510 cGy and 4,594 cGy, respectively. The parameters, n, m and TD_{50} as described in the Lyman Kutcher Burman (LKB) model were fitted and compared with other published findings. Our findings may be useful as additional clinical guidelines in treatment planning and plan evaluation, as well as obtaining informed patient consent.

Key Words: Radiation Therapy, Lung Cancer, Dose Volume Effect, Esophagitis, Radiation Dosimetric Parameters, Mean Esophagus Dose, LKB Model

INTRODUCTION

Since the late 1980s, radiation therapy was considered the standard of care for patients with lung cancer.[1] Although radiation is beneficial at killing the tumor cell, it is harmful at detriment of the healthy organ.[2] An ideal practice of radiotherapy involves optimization of treatment plans to maximize the radiation dose to the tumor and minimize the dose to the critical organs and structures around the tumor.[3] Unfortunately, this is often impossible in radiotherapy due to the anatomic distribution and the limitation of the beam delivery. The aim of radiation therapy is to achieve an uncomplicated loco-regional control of cancer.[2] In order to reach this goal, the precise knowledge of tumor control radiation dose and the tolerance dose (TD) of normal tissue to radiation is required.[4]

Esophagus is one of the critical organs at risk (OAR) when the lung cancer is treated by radiation. Acute esophageal toxicity is seen as one of the main complications for lung cancer radiation treatment and it is known to be a significant dose-limiting factor.[5] Severe esophagitis can cause patient in hospitalization, and treatment breaking, which will reduce the tumor control probabilities.[5] The late esophageal complication may induce stricture formation and ulceration, etc. It is prudent to understand and correlate the dosimetric parameters to esophagitis in order to prevent or reduce the incident of esophagitis. Normal tissue complication probability (NTCP) modeling plays an important role in optimizing treatment plan and predicting the normal tissue radiation injury.[6,7] Lyman Kutcher Burman (LKB) model is probably the most useful method of NTCP modeling in radiotherapy.[8] The purpose of this retrospective study was to establish a dose predictive model of the incidence of esophagitis for lung cancer patients treated with radiotherapy. The parameters of LKB model were fitted using the treatment outcome data.

METHODS

This research was approved by the Institutional Review Board (IRB) of university of Mississippi medical center. All patients with lung cancer treated with Low Modulated intensity modulated radiation therapy (LM-IMRT) in department of Radiation Oncology from January 01, 2014 to June 30, 2017 were included in this study. A total of 139 charts of lung patients, who underwent radiation therapy or with chemo-radiotherapy in this period were

collected and reviewed.

Patients were placed in the supine and head first position on a wing board with both arms placed above the head for the treatment planning computed tomography (CT) scan with 3 mm slice thickness on a Philips Brilliance Big Bore CT with 16 multi-slice capabilities. The Philips respiratory bellow was placed around the patient at the level of the xiphoidal tip. A four dimensional (4D) CT protocol that entailed a free breathing planning CT was selected. Then, a three dimensional standard helical CT from the chin to 5 cm below the diaphragm of the patient and a 4DCT scan of the lung region that covers 2 cm superior and inferior to the tumor were acquired. The isocenter was set on the free-breathing 3D scan using the Philips virtual simulation software. The isocenter coordinate was then sent to the LAP laser system for isocenter displaying on the patient skin where the therapists tattooed the isocenter.

Gross tumor volume (GTV), clinical target volume (CTV), skin, lungs, esophagus, spinal cord, and heart, etc. are contoured by the radiation oncologists on the axial view of the 3DCT data set. Internal target volume (ITV) was created from the 4DCT based on the maximal intensity projection (MIP) and expanded with a margin of normally 0.5 to 1 cm to create the planning target volume (PTV). LM-IMRT treatment plans were generated with Pinnacle treatment planning system, which is one of treatment techniques implemented at our department for lung cancer treatment.

There has been a significant increase using intensity modulated radiation therapy (IMRT) for conventional fractionated lung irradiation because of its ability to spare organs at risk such as the spinal cord, heart, esophagus, and healthy lungs.[9] However, fully modulated IMRT possesses unique challenges for lung cancer treatment mainly due to the interplay between lung tumor motion and small segment MLC movements.[10] When the trajectory of the MLC and the target is not synchronous, blurring of the dose may occur around the target.[11] In contrast, LM-IMRT can be seen as a sparsely intensity modulated radiation therapy technique used by limiting the degree of modulation, number of segments per beam, and keeping larger segment sizes during the inverse planning process. The dosimetric criterion of the plan is $V_{95\%Rx} \geq 95\%$, which means that the 95% of the PTV should receive more than 95% of the prescription dose. Our institution recommended guideline for LM-IMRT is to start the optimization with minimum segment size of 40 cm^2 and minimum monitor units (MU) of 20 per beam; however these values can be iteratively modified. LM-IMRT technique has been implemented uniquely at our radiation oncology department since 2010 and used mainly for stage II or III lung cancer patients to generate a better conformal dose distribution around tumor and minimum radiation dose to the surrounding healthy tissues while most of other institutions ignore or debate the effects of interplay between lung tumor motion and small MLC segments movements. The selected effective dose prescription is normally 59.4 or 60 Gy (or 66Gy) with 1.8 Gy or 2 Gy per fraction daily with 6 MV photon beams.

The mean esophagus dose of all patients treated with LM-IMRT technique was collected from Pinnacle TPS, together with endpoints of esophagitis from EPIC electronic medical record system. The grade of esophagitis was based on radiation therapy oncology group (RTOG) classification listed in Table 1 and determined by our institution's attending radiation oncologists for each patient. The treatment plans were delivered to the patients using Elekta Synergy Linear Accelerator.

The mean esophagus dose for each individual lung patients were correlated with esophagitis grade 1 and 2 using binary logistic regression and probit statistical analysis. All the statistical analyses were performed using IBM SPSS software version 24 at the 5% significant level (α=0.05). LKB model was fitted by using mean esophagus dose (MED), which was implicitly assumed *n* equals to 1 in the model.

Table 1: RTOG classification about acute Esophagitis [5]

Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
No change over baseline	Mild dysphagia or odynophagia/may require topical anesthetic or non-narcotic analgesics/may require soft diet	Moderate dysphagia or odynophagia/may require narcotic analgesics/may require puree or liquid diet	Severe dysphagia or odynophagia with dehydration or weight loss(O15% from pre-treatment baseline) requiring N-G feeding tube, I.V. fluids or hyperalimentation	Complete obstruction, ulceration, perforation, fistula

RESULTS

Figure 1 shows a logistic regression of probability of esophagitis grade 1 and 2 versus MED calculated based on the regression models (p=0.005 and p=0.18 for esophagitis grade 1 and grade 2 respectively). The general trend was that the esophagitis incidence probability increased with increasing MED. MED was associated with esophagitis. This quantitatively showed that the MED was a useful predictor for the incidence of esophagitis (grade 1 and 2) for lung cancer patients treated with radiation therapy. It revealed that a typical sigmoid shape of the probability of esophagitis grade 1 vs. MED, with *m* as the slope of the curve being determined as 0.17. By transferring the sigmoid dose response curve of esophagitis grade 1 to a straight line using probit analysis, the TD_{50} of the incidence of esophagitis grade 1 and grade 2 were determined as 1510 cGy and 4590 cGy, respectively.

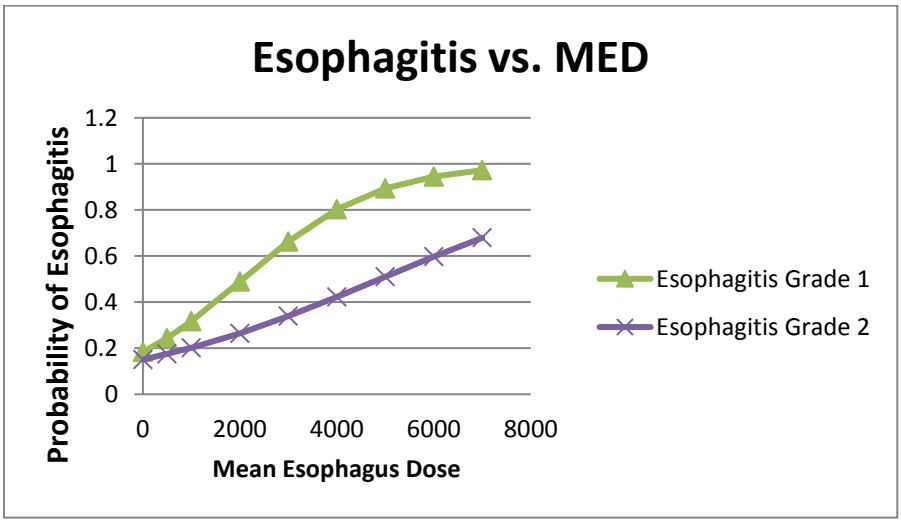

Figure 1: Probability of esophagitis vs. mean esophagus dose for both grades

DISCUSSION

The following three equations are used in LKB model.[12,13] Equivalent Uniform Dose (EUD) is the dose at which a given uniform dose to the entire organ would produce the same normal tissue complication probability as the original dose distribution by assuming that any two dose distributions are equivalent if they cause the same radiobiological effects.[14] Equation (2) and (3) represent a sigmoidal curve that is determined by three parameters, TD_{50}, *m* and *n*. For each organ, NTCP depends in a very complex way on the dose and the irradiated volume of an organ. When $n = 1$, the model reverts EUD to mean dose (MD) of an organ. Although TD_{50} is strongly dependent on the grade of the organ toxicity, *n* is often considered as a tissue characteristic. The MD model is widely used due to its simplicity and effectiveness. It was the metric used by the large multi-institutional analysis and often performs as well as more complex model.[15]

$$EUD = \left(\sum_i v_i D_i^{1/n} \right)^n$$

$$t = \frac{EUD - TD_{50}}{m\ TD_{50}}$$

$$NTCP = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{t} e^{\frac{-x^2}{2}}\, dx$$

The results in this work displayed clearly that the esophagitis incidence probability increased with increasing MED. This qualitatively revealed that the MED is a useful predictor for the incidence of esophagitis (grade 1 and 2) for lung cancer patients treated with LM-IMRT. We attempted to compare our results with previous results from three institutions regarding the LKB model parameters. It can be seen that the TD_{50} of 68 Gy[13] is quite different compared to our values and other two investigators. This could be because the endpoint in their findings considered very severe toxicity due to non-advanced treatment techniques at the time of 1991. However, our findings of TD_{50} for esophagitis grade 2, which is 45.9 (33.9-71.4) Gy agreed with other two investigators[16,17], which are 51 (20-82) Gy and 47 (45-60) Gy. These results elucidate that the implementation of the LM-IMRT treatment technique at our institution improved the dosimetric parameters of esophagus for lung cancer patients treated by radiation.

CONCLUSIONS

In this study, the dosimetric predictive model of esophagitis for lung cancer patients treated with LM-IMRT was established. We found that the mean esophagus dose (MED) is a useful predictor of radiation induced esophagitis. TD_{50} of esophagitis grade 1 and 2 were determined using the predictive model established in this study. In addition, all treatment data for this study is from a single institute, therefore the variety of volumetric metrics of the esophagus from different institution's published data are avoided. Findings from this study can be used as a clinical guideline in treatment planning and plan evaluation, as well as for patient consent information.

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TEACHING AND LEARNING IN AN ACTIVE LEARNING CLASSROOM: A MIXED-METHODS CASE STUDY

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Abstract

In the era of ubiquitous use of technology, education in all settings is impelled to incorporate technology into classroom settings in order to meet student needs and facilitate student learning. A southeastern academic medical center took the initiative and transformed a traditional classroom (TC) into a technology-advanced active learning classroom (ALC). In this case study, we conducted a mixed-methods cohort study aiming to find out what was taking place in the room and how students and faculty perceived it. To find the answers, we conducted a year-long observation of a dental hygiene professor teaching two consecutive courses. Along with gathering observational notes, we recorded and transcribed 19 sessions. Towards the end of the academic year, we interviewed the professor following a 13-question guide and surveyed students with a 25-question questionnaire. Our multi-perspective data indicated that approximately 63% preferred to take classes in the ALC rather than TCs. They especially enjoyed spaciousness, mobility, unobstructed views, and ease of information sharing and engagement in class. In the meantime, the ALC presented both faculty and students with sensory and technique challenges.

Keywords: active learning classroom, traditional classrooms, technology, physical features, information sharing, strengths, challenges

INTRODUCTION

Technology has the potential to accelerate and strengthen the impact of effective teaching practices, and technology-enabled learning spaces allow learners to reimagine their learning experiences and learn the skills they need to learn for academic success [1]. Experiments with technology-enabled learning spaces began in the early 2000s. Pioneering work in the field includes North Carolina State University's SCALE-UP (Student-centered Activities for Large Enrollment Undergraduate Physics) project [2, 3], Massachusetts Institute of Technology's TEAL(Technology-Enabled Active Learning) project [4, 5], and the University of Minnesota's ALC (Active Learning Classroom) research [6, 7]. These learning spaces are equipped with advanced technology, movable round tables and comfortable chairs, multiple flat-panel screens or projectors to allow connectivity, flexibility and 360-degree visibility. The research showed that students immersed in these spaces demonstrated better knowledge retention and improved conceptual understanding.

In 2014, the School of Health Related Professions at University of Mississippi Medical Center (UMMC) led the institution and built its first active learning classroom named the Collaboratory (Figure 1). The Collaboratory is equipped with multiple large interconnected flat panel screens on each wall and on each of semicircular and rectangular tables. Power outlets and smart device hookups are provided on each table to allow easy screen sharing among in-room and personal devices. There are also portable white boards on rolling stands placed in different corners in the room. Since the opening of the Collaboratory, faculty and administrators were interested in what was actually taking place in the room, how the room shaped teaching and learning, and how faculty and students perceived the room. In this case study, the researchers examined two Dental Hygiene courses taught in the Collaboratory using mixed-methods research design.

Figure 1 Collaboratory

Dental hygiene is a profession defined as the science and practice of the recognition, treatment , and prevention of oral diseases [8]. At UMMC, the Dental Hygiene Program has enrolled approximately 20 students each year across its 47-year existence. Students take all required courses in lockstep. The two courses under study are Dental Radiology I and II, which are two consecutive courses taught in the first year aiming to teach students the use of radiology as a dental diagnostic aid.

Mixed methods research (MMR) popularity has increased in the last decade [9, 10]. The approach allows researchers to combine quantitative and qualitative research techniques into a single study, drawing strengths from and minimizing the weaknesses of both types of research [11, 12]. With MMR, data collection can be sequential or concurrent, and collected quantitative and qualitative data can be given equal or unequal priority in analysis [13]. The current study employed concurrent design with equal priority given to both. This design was considered useful for confirming, cross validating, and corroborating study findings [13].

METHODS

To examine a complex classroom phenomenon, the researchers determined that a mixed-methods research (MMR) design was the most appropriate approach. The rationale was that the combination of quantitative and qualitative methodologies would allow us to collect data from multiple angles, and the richer and more inclusive data collection would help us develop more holistic views, gain deeper understanding, and generate more accurate inferences [11, 12].

Data Collection

Upon UMMC IRB approval, the first author observed a professor who taught two consecutive dental hygiene courses (Dental Radiology I and II) in the 2015-2016 academic year as a nonparticipant observer. Observation is considered the best research tool to record an activity or event as it is happening and is often used in conjunction with interviews or documents to draw trustworthy inferences [14]. In total, the researcher observed and recorded 19 classes, and took 95 pages of field notes of verbal and non-verbal natural classroom happenings.

Towards the end of spring semester in 2016, the researcher interviewed the professor following a semi-structured 13-question guide adapted from the Learning Spaces Research team at the University of Minnesota [6, 7, 15]. The interview was video-recorded and transcribed. Interviews are one of the most commonly used data gathering techniques in qualitative research. The purpose of interviewing is to find out what is going on in study participants' minds and how interpret a phenomenon under investigation, which by no means can we directly observe [14].

The researcher also administered a 25-question online survey to 38 students who took classes from this professor in the Collaboratory, which included the same students (n=19) who were being observed and ones (n=19) enrolled in another dental hygiene course from this professor in the Collaboatory the

previous year. Adapted from Park and Choi's study [16], the survey consisted six demographical and academic attitudinal questions, fifteen questions on five-point Likert scale (strongly agree=5, strongly disagree =1), comparing the educational effects of the Collaboratory and TCs, and four questions related to students' experiences with the Collaboratory. Among the last four questions, three were open-ended. Participation in the survey was voluntary. A total of 32 completed survey responses were garnered.

Data Analysis

Qualitative data, including faculty interview and student open-ended survey questions [1. Do you prefer to take classes in the ALC or TC? Explain your answer. 2. What features in the ALC do you like the best? 3. What can we do to improve your learning experiences in the ALC? Any suggestions?] were analyzed using the constant comparative method (CCM). CCM is a methodology widely used in all forms of qualitative studies to compare data from open-ended questions, interviews or focus group discussions [17]. A defined process suggested by Savin-Baden [18] was followed (Figure 2). We also referred to field notes of real happenings from the classroom to complement and support qualitative findings.

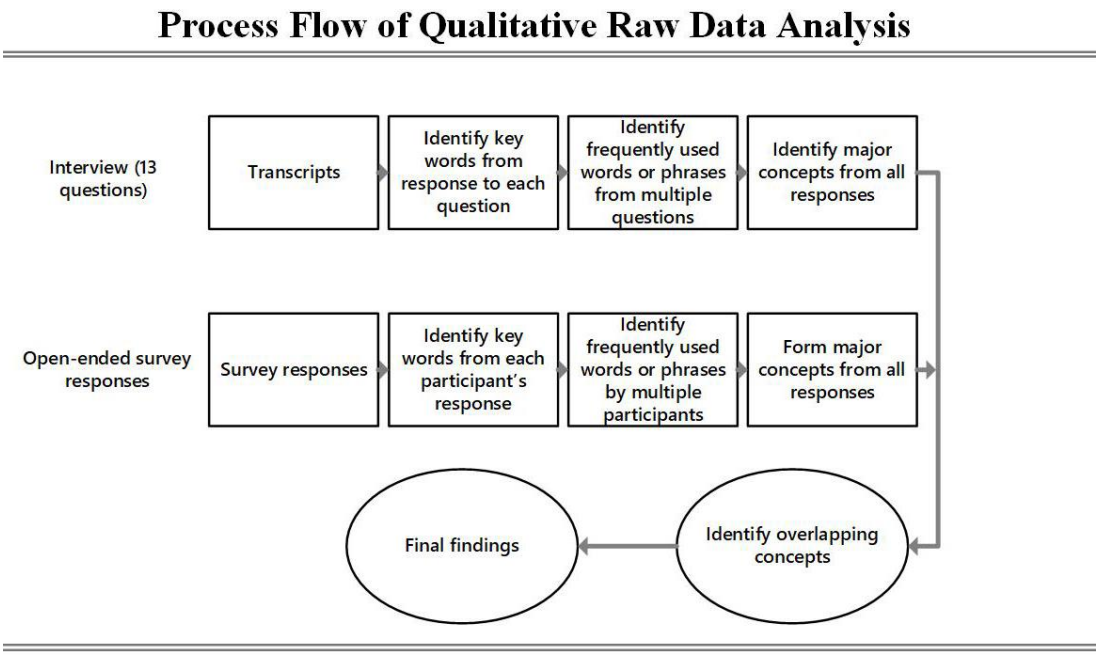

Figure 2 Process flow of qualitative raw data analysis

The student survey data was exported in SPSS format from the online survey tool. All quantitative analyses were conducted using IBM SPSS Statistics 23. Independent-samples t-tests were performed to compare students' perceptions regarding educational effects of the Collaboratory and TCs. The level of significance was set at 0.05. All statistical tests were two-sided.

RESULTS

Results from both qualitative and quantitative data are presented in this section. The majority of students enrolled in the program were females, white, with an incoming GPA in the range of 3.5 to 3.9.

Qualitative findings - strengths and challenges of the Collaboratory

The physical features in the Collaboratory provided both students and faculty with comfort, mobility, and unobstructed view from anywhere in the room.

The students specially embraced the spaciousness, circular seating arrangement, and multiple screens that made it easy to view instructional content from anywhere in the room. Typical quotes include:

> **Student 1**: Active learning classrooms are better because we don't have to be crammed in and can

move around. [I like] TVs all over so that we can see.

Student 3: [I prefer to take class in the] ALC because I like being able to see the screen from anywhere in the room.

Professor: It freed me up from being stuck at the front of the room. And also I felt like the students are freed up too because they can look at any screen anywhere.

The advanced technology in the Collaboratory enabled easy information sharing and classroom engagement.

All screens in the Collaboratory were interconnected and controlled on a user-friendly interface. The professor could easily take over control of all or designate all or only certain ones to students based on the undergoing activities. Students responded:

Student 1: [I like] using the computers and having the capability to show what's on our computers on the TV screen. I feel like we are more interested in the class when it's active learning. I think getting more classrooms like the Collaboratory would be great.

Student 2: [I prefer to take classes in the] ALC. I like the interaction between the students and the instructor. It is a very good environment and helps me to learn better

Student 3: [I prefer to take classes in the] ALC. It is more hands-on and the technology makes it more engaging.

Students 4: [I like] how each seated section has its own computer monitor. Everyone participates in activities and there are different ways for everyone to interact with the material being taught.

From classroom observations, we noticed that on many occasions the professor asked students to google the topic of interest and gave students control of screens so that they could share, discuss and present what they had found on the topic from their smart devices. Oftentimes, observation notes indicated, "students were engaged", "students appeared to be on task", or "students are all paying attention".

The Collaboratory presented sensory as well as technical challenges.

From the professor's perspective, the main sensory challenge was not being able to make visual contacts with all. Unlike in the TCs where all students sit facing forward, in the Collaboratory students sat in circles. **The professor said**:

I had about half of the class with their backs to me…So, I would, you know, walk beside them, stand there, they still wouldn't turn around, and then I would touch them on the back, they still wouldn't turn around, so finally I had to change the way that I lectured…I had to change all of it to say 'if you are not looking at me…I am assuming you are not listening'.

The student responses showed that about 63% of the students preferred to take classes in the Collaboratory. However, some students found it distracting and would rather to take classes in TCs.

Student 1: "[I prefer to take classes in the] traditional classrooms. Active classrooms sometimes distract me."

Student 2: "[I prefer to take classes in the] traditional classrooms. I feel as though there are less distractions."

Getting the technology to work properly and consistently was another challenge. From class observations, we noticed that sometimes the iPad that was used to control all the screens was dead, the computer in the room was not responding, cords to hook up personal devices were lacking or did not fit properly.

Quantitative findings - student perceptions of the Collaboratory and TCs

Five survey items corresponding to the qualitative findings were included for analysis. The students were asked to respond to one statement twice, once regarding ALC, once TC. From table 1, we can see that the mean scores of students' responses to ALC were higher across the board. Students' perception regarding clear view in the ALC was 0.81 higher than the mean score with classroom type TC (p = 0.001). Students deemed that the instructor tended to devote more time to discussion or group work than lecturing in the ALC, which obtained a mean score of 4.13 whereas TC gained 3.31 (p = 0.001). More students agreed with the statement that the learning space of the ALC provided effective space for group work (p = 0.001) and helped enhance the efficiency of group work (p < 0.001). As to information sharing, ALC again gained a mean 0.57 higher than TC (p = 0.012). These quantitative results support and reinforce the qualitative findings.

Table 1 Survey responses (ALC - Collaboratory; TC - traditional classrooms)

Survey Questions	Classroom Type	Mean	ACT-TC	P-value
I have a clear view of the screen from anywhere in the room.	TC	3.75	0.81	0.001
	ALC	4.56		
The instructor devotes more time to discussion/group activities than lecturing.	TC	3.31	0.82	0.001
	ALC	4.13		
The learning space provides effective space for group activities.	TC	3.59	0.85	0.001
	ALC	4.44		
The learning space enhances the efficiency of group projects.	TC	3.44	1.03	<0.001
	ALC	4.47		
It's easy to exchange information and share different viewpoints with other students.	TC	3.71	0.57	0.012
	ALC	4.28		

DISCUSSION

The design of classrooms should enable, not inhibit, different styles of teaching and learning [19, 20]. Current case study indicated that many students enjoyed the Collaboratory due to its physical characteristics such as spaciousness, flexibility and clear view. From psychological point of view, physical characteristics in a learning environment could affect learners emotionally followed by cognitive and behavioral reactions. Environments that induced positive emotions could potentially lead to enhanced learning, whereas environments that caused discomfort could interfere with learning [21, 22]. Per empirical research from the last century, the reason was that emotions had a vital effect on cognitive processing including the processing of information, communication, negotiation, decision-making, categorization and problem-solving [23, 24]. People in a positive mood were found more cognitively capable of making connections among stimuli and demonstrated broader scope of attention and action [25, 26]. The positive feelings experienced by both faculty and students in the Collaboratory from spaciousness and freedom from rows and columns should position them on a path more conducive to learning.

The ease of information sharing in the Collaboratory was recognized in both qualitative and quantitative findings. This feature of the Collaboratory was given more acclaim than any other features in the data. In education setting, screen sharing is not just a trendy technology; it offers students and faculty an opportunity to connect and learn through collaboration that was not possible previously [27, 28]. According to existing literature, when a learning environment is conducive to information exchange, students are more likely to build upon new ideas assimilated from peers and deepen their current understanding on the subject matter [29, 30]. Moreover, it enables students to acquire competence in communication and collaboration that they can benefit from in their lifetime career experiences [31].

Our observation notes indicated that through information sharing, students appeared to be more engaged in class. According to educational

psychologists, when students are actively engaged in learning, they are more likely to gain deeper understanding and achieve potential knowledge transfer and innovation [32]; they are also found to be more satisfied with their academic experiences [33, 34]. The observed increased level of engagement in the Collaboratory should generate positive impact on student learning.

Our results showed that the Collaboratory presented sensory challenges to the professor and some of the students. The non-traditional seating arrangement in the Collaboratory, on the one hand, created challenges for the professor since she could not make eye contacts with all students; on the other hand, facilitated peer interaction as they were sitting face-to-face. Making eye contact is considered an important nonverbal factor that affects students' learning process, such as knowledge retention, classroom engagement, span of attention, and even attitudes towards the teacher [35, 36]. In the situation where direct eye contact is lacking, it is suggested that the professor use kinesics behavior (e.g. posture, movement, gesture) as well as vocal cues (e.g. pitch, tempo, pauses) to draw students' attention [36].

Regarding technical challenges, experienced educators including the professor under study suggested [37, 38]: first, have a low-tech plan B that is almost an analog version of scheduled instructional content and activities. Second, walk through the technology before teaching in the room. It will help teachers effectively trouble-shoot should issues arise. Third, utilize resources such as the technical support staff, tech-savvy students, or other teachers.

While this case study provided detailed investigation of the active learning classroom and insight for future research, it has limitations. First, with inherent nature of any case study, the inferences drawn could not be generalized to general population. The small sample size is the second drawback. We only followed one professor of one discipline. Since the program only enrolls approximately 20 students a year, our survey sample size was very small. Third, the Collaboratory is the first in the institution. The novelty of non-traditional classroom design and advanced technology might have contributed to some positive findings. Educators are encouraged to conduct further research into active learning classrooms with a larger sample size, a longer research duration, and more precise research questions.

CONCLUSION

Our qualitative data indicated that the majority of students preferred to take classes in the ALC rather than TCs. They especially enjoyed spaciousness, mobility, unobstructed views, and ease of information sharing and class participation. In the meantime, the ALC presented both faculty and students with sensory and technique challenges. It is suggested that teachers use motions or vocal cues to overcome sensory challenges, and familiarize with technology, properly use available resources and have a low-tech backup plan to overcome technical challenges.

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ATTITUDES AND KNOWLEDGE OF NURSES' TOWARDS EDUCATION ON COMPLEMENTARY & ALTERNATIVE MEDICINE USING THE STATE OF MISSISSIPPI UNIVERSITIES AS A MODEL

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ABSTRACT

The growing consumer demand for complementary and alternative therapies (CAM) in health care has had an effect on all health professionals. The discipline of nursing is rooted in many holistic processes but the role of providing such services has not been fully defined, including the state of Mississippi. Nurses are the members of the healthcare team who often initiate such a conversation with patients about CAM. For a starting point, Mississippi nurses and their feelings on CAM instruction in school curriculia or during professional continued education programs was investigated. This was a proactive descriptive quantitative study, with a sample size of 116 participants representing 16 higher education institutions. The survey was conducted during the Mississippi Nurses Association's Annual Meetings & Conventions. The findings of this study demonstrated that overall 80% of nurses felt that their higher education institution did not provide adequate education on CAM. Fifty three percent reported taking a course that covered a component of CAM, suggesting that 95.6% feeling that the physician should be the one to communcate to patients about CAM use. Our findings highlighted the fact that Mississippi nurses feel unprepared in CAM education when communicating with their patients. Our findings also highlighted the associations between Mississippi nurses "feeling comfortable talking to their patients about CAM" and nurses attending a higher education institution within the state of Mississippi, with a P=0.857 (Chi Square). This study shine light on the need of higher education institutions to revisit nursing school's study curricula to fit the continuously changing healthcare system and the popularity of CAM among patients.

Keywords: Complementary & Alternative Medicine, Nurses (CAM) & Complementary and Alternative Medicine, Complementary & Alternative Medicine Education in Nursing School

INTRODUCTION

Complementary and alternative medicine (CAM) is defined as a large and diverse set of systems of diagnosis, treatment, and prevention based on philosophies and techniques other than those used in conventional Western medicine. Such interventions may be described as alternative, existing as a body separate from and as a replacement for conventional means of treatment. CAM is characterized by (1) its focus on the whole person as a unique individual, (2) on the energy of the body and its influence on health and disease, (3) on the healing power of nature and the assembling of the body's own resources to heal itself, and (4) on the treatment of the underlying causes. Many of the techniques used to implement CAM are the subject of debatable conversations and have not been validated by controlled studies [1].

Several reports often use "alternative" and "complementary" interchangeably, but the two terms refer to completely different concepts. If a non-mainstream practice is used together with conventional medicine, it's considered "complementary". If a non-mainstream practice is used in place of conventional medicine, it's considered "alternative". However, when conventional and complementary approaches come together in a coordinated way the term "integrative" medicine is used. The use of integrative approaches to health and wellness has increased within care settings across the United States [2].

Numerous ongoing studies focused on exploring the potential benefits of integrative health in a variety of situations, including pain management, relief of symptoms in cancer patients and survivors, and programs to promote healthy behaviors. Chronic pain is considered a common problem among veterans and active-duty military personnel in the United States. Several agencies are sponsoring research to explore whether integrative approaches can be utilized. For example, many NCCIH-funded studies are testing the effects of adding mindfulness medication, self-hypnosis, or other complementary approaches to pain management programs for veterans. The goal of such program and other programs is to help patients feel and function better

and reduce their need for pain medicines that often have serious side effects. In addition, cancer treatment centers with integrative health care programs may offer services such as acupuncture and meditation to help manage symptoms and side effects for patients who receive conventional cancer treatment [3, 4]. Although research on the potential value of these integrative programs are in the early stages, some studies have had promising results. For example, NICCIH-funded research has suggested that:

- Cancer patients who receive integrative therapies while in the hospital have less pain and anxiety.
- Massage therapy may lead to short-term improvements in pain and mood in patients with advanced cancer.
- Yoga may relieve the persistent fatigue that some women experience after breast cancer treatment.

The integration of CAM with traditional medicine is still in its infancy and more educational programs need to be implemented. Health care providers such as physicians, nurses, therapists, and others considered key elements in prompting CAM and reducing the suffering of patients. The specific objective of this study was to identify the attitudes of nurses towards education on CAM using Mississippi Universities as a model. This is a descriptive quantitative study, with a sample size of 116 active nurses in the state of Mississippi (MS).

Materials & Methods

Population: This study used a proactive survey based quantitative study which was administered to active nurses in the state of MS. The survey solicited to acquire information regarding nurses' beliefs, knowledge, and attitudes of education on CAM in major Mississippi universities and colleges. A detailed 31-questions survey was constructed and administered to MS nurses whom attended MS Nurses Association annual Conventions & Meetings throughout FY 2016 & 2017. Application for Internal Review Board (IRB) Approval was granted by the University of Mississippi Medical Center (approved on November 29, 2016.)

Mechanism of Survey Delivery: Three events were utilized to administer the survey. First, with assistance and approval from the Mississippi Nurses Association, a booth was set-up on the first day of the annual event. Volunteer participants had the opportunity to register for a number of drawings in which survey participants had an opportunity to win a variety of CAM services donated by CAM practitioners within and around the state. Participants were provided with a number ticket upon completing the survey which was later used to select the prize winner at the end of collection day. The definition of CAM was provided in the survey's introductory letter, which also served as a waiver. Participants were also provided with a district map so that their location of employment can be located based on the MS Nurses Association districts definitions. Surveys were conducted via paper based or lab-top computer. Option two was also provided an invite via email during the conventions of which participants were forward link immediately and allowed to complete the survey at any given time. Last page of completion of survey was then required to be shown at research booth for participants to receive ticket for drawings. Most of survey questions were closed-ended; there were some questions that allowed participants to write an additional comments or information. The second and third events utilized for data collection were the MS Annual Nurses Summit and the Annual Nurses' Practitioners Conference. The MS Annual Nurses Summit was held in Jackson Mississippi at the Jackson Convention Center on February 2017. The Annual Nurses' Practitioners Conference was held in Oxford, MS at the Oxford Conference Center on April 2017. Nurse professionals from around the state attended both events, and it is worth mentioning that the nurses' summit had a larger turnout of nursing student, of who did not meet the criteria to complete the survey (excluded).

RESULTS

There was a total of 116 nurses (met the criteria) that completed the survey, of the 116, 112 (96.55%) were females and only 4 (3.45%) were males. The female to male ration is considered a standard in the nursing profession worldwide. Participants' ages ranged from 21 to over 61, with the majority of participants in the age group of 30-39 (32.76%). The second largest age group was between 40-49 (23.28%), followed by age group 50-59 (21.55%), the second lowest group were the 21-29 (12.93%), and the lowest group was between 60 or older (9.48%). See Figure 1. Only 1 participant was of the ethnicity Hispanic or Latino. By races, participants were described as 58 (50%) being Caucasian or white, 55 (47.41%) as Black/African American, and the remaining 3 (2.59%) as Asian.

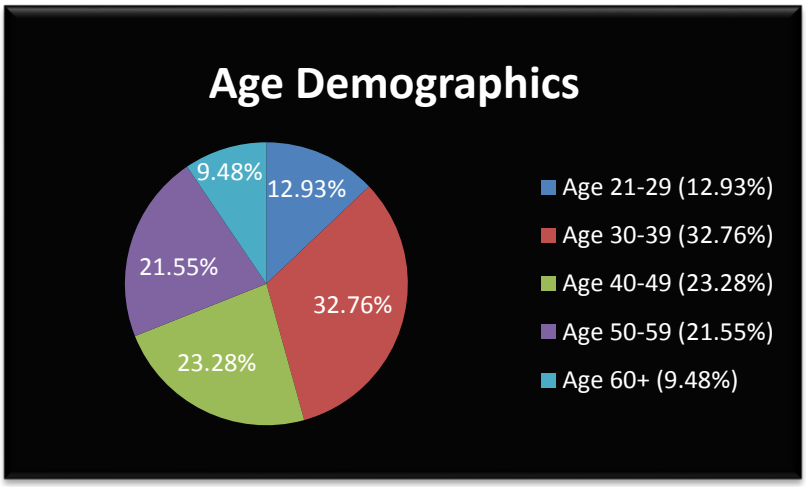

Figure 1: Participants Age Demographics

Among participants, 99 (84.34%) attended a higher education institution in the state of Mississippi, while the other 17 (14.68%) attended institutions outside of the state. Eighty percent of the survey participants reported not feeling adequately educated on CAM within their chosen nursing programs, for both in and out of state educated participants and only 20% reported feeling adequately education on CAM from institutions of choice. Table 1 illustrates the attended According to the collected surveys, the participants represent 16 universities and colleges and nineteen participants declined to share their institution of higher education.

The results revealed that over half (53.04%) of surveyed participants reported taking courses during their education journey with a CAM education component, and 39.13% have indicated that their curricula lacking courses with a CAM education component. A total of 7.83% stated that they were not sure or not remembering if any course was taken that cover a CAM education component (See Table 1). In exploring the possibilities of ever attending a lecture, workshop or seminar on any form of CAM, a total of 80/116 (68.97%) reported never done so and only 36/116 (31.03%) reported attending a variety of lectures/seminars on CAM (See Table 2).

Table 1: Findings on participants input by age groups on survey question regarding educational courses on CAM during Education Journey at various institutions.

Age Groups	CAM education component	Lack of CAM Education Component	Not Sure	Total
21-29	5.17%	6.03%	1.72%	12.92%
30-39	15.52%	13.79%	2.59%	31.9%
40-49	11.21%	9.48%	2.59%	23.28%
50-59	15.52%	6.03%	0.86%	22.41%
60+	5.17%	4.31%	N/A	9.48%
All Ages	53.04%	39.13%	7.83%	99.99%

Table 2: Reported findings on participants input by age groups on "If participants have attended a Lecture or Seminar on any form of CAM?"

Age Groups	Lecture/Seminar on CAM	Lack of Lecture/ Seminar on CAM	Total
20-29	2.59%	10.34%	12.93%
30-39	6.05%	26.72%	32.77%
40-49	5.17%	18.10%	23.27%
50-59	12.07%	9.48%	21.55%
60+	6.9%	2.59%	9.49
All Ages	68.97%	31.03%	100%

Other aspect of the survey was to explore the nurses' potential on conveying the impact of using CAM to patients. Participants were asked about the level of when talking to patients, and a total of 66.37% reported feeling comfortable and 33.63% reported not feeling comfortable talking to patients about it (See Figure 2). In retrospective, a total of 95.61% feeling that it is the job of the physician to talk to the patients about CAM use, and only 4.39% felt that it's not the physician's job (See Figure 3). When asked how important they felt that CAM education among nurses? The majority of participants (97.39%) felt it was important (with choices being very important, important, somewhat important, not important, no interest). Eighty seven percent of surveyed participants supported the idea of providing additional training to nurses to become CAM educators. Seventy two percent of participants reported future plans of continuing their education and were interested to obtain form information on integrative medicine.

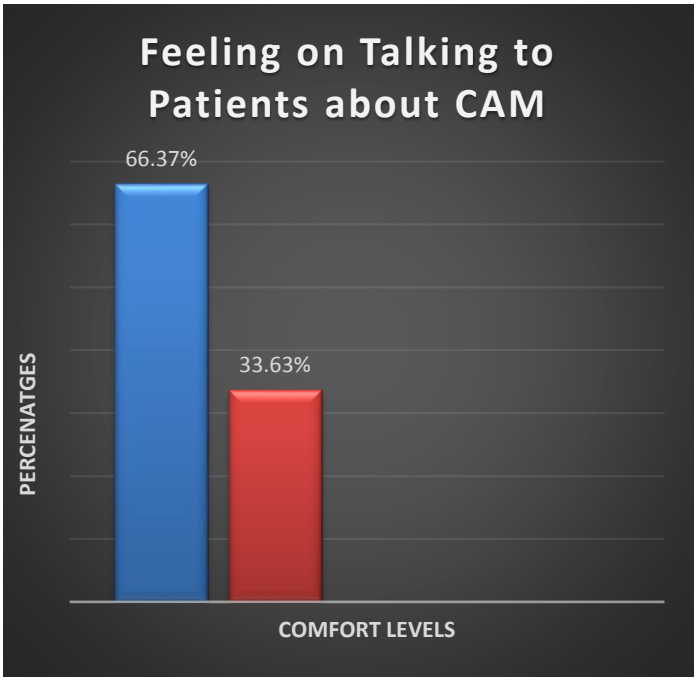

Figure 2: Mississippi Nurses Feelings on "Talking about CAM with Patients" by percentages. N=116

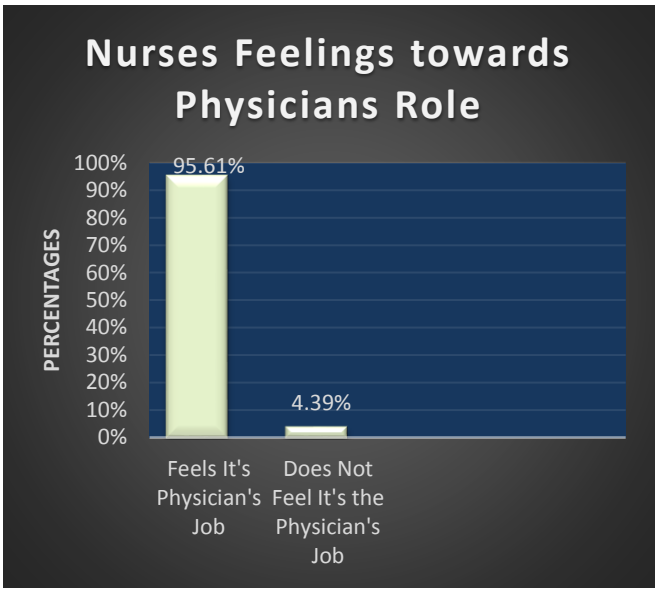

Figure 3: Mississippi Nurses Feelings on "If the Physicians Should Talk to Patients" in percentages (N=116)

A Chi Square test was conducted to identify if associations exist between nurses' feelings of institutions providing adequate education on CAM and nurses feeling comfortable talking to patients about CAM. Overall, there was a negative association between nurses' feelings of whether the institutions provided adequate education and nurses feeling comfortable talking to patients about CAM ($p=0.007$). Data analysis (Chi Square) was also conducted to identify if associations exist between nurse attending a higher education institution in the state of Mississippi and nurses feeling comfortable talking to patients about CAM. With a $P=0.875$, there were no associations between attending a higher education institution in Mississippi and feeling comfortable talking to patients about CAM. Regardless of school attended, the nurses surveyed did not feel adequate in introducing or discussing CAM with their patients.

DISCUSSION

In reviewing the literature, it is quite obvious that consumers are using CAM therapies to help improve health and well-being or to relieve symptoms associated with chronic and terminal illnesses in which conventional therapy failed to do so. Consumers also utilize such therapies because of the high demand on the new holistic healthcare model [5,6,7,8]. As demands for CAM grows, services being addressed by nurses in the medical community will need to be enforced. In order to address the gap between what CAM consumers are utilizing and what the medical community has to offer, the need to include CAM therapy as part of clinical practice as well as training nursing through curriculum changes and continuing education offerings [1].

Data obtained from this study highly suggest that integrative medicine curriculum will ultimately need to be incorporated into medical education just to keep up with an evolving medical system. Many of the core principles of integrative medicine reaffirm the fundamental principles which are already support in many institutions providing medical education such as nursing degrees [9]. Integrating CAM can only happen if health care professionals at all levels allow CAM education to take a position in medical education as fast as CAM has taken the conventional medical system by storm. A storm that is supported by the National Institute of Health/National Center for Complementary and Alternative Medicine, which is currently offering financial support to a number of higher education nursing schools to develop, implement, and evaluate educational programs on complementary and alternative medical therapies. There is no doubt that funding such projects will increase nursing knowledge of CAM therapies and will also provide nurses with expertise to assess the use of CAM therapies safely among diverse populations.

Previous studies have also found that significant patients with major complications are continuously and actively seeking communication about CAM in their health care settings. Berman *et. al.* reported that 59% of patients, in one rural practice, believed that their healthcare provider should discuss CAM with them and more than a third would like to be able to discuss their CAM use with their healthcare providers

[10]. It is likely that patients desire to discuss CAM use with their provider goes way beyond a desire for information to a desire for use [11]. Several studies reported controversial findings that there is a high level of uncertainty about CAM's efficacy and doubts about its potentially adverse interactions when used with conventional medicine among patients [12]. Other researchers suggested that that patients have a desire to discuss such uncertainty with confidence in the information provided by their healthcare providers [6]. Globally, significant studied agreed on the notion that not only will CAM education provide such information, but it can put an improved value into the patient-centered services. The results of the study and others provide us with the evidence to support a need of curricula change in various clinical programs to meet the fast-changing healthcare system. This study also reveals that nurses, using Mississippi as a model, feel unprepared in the area of CAM education and strongly admit their unpreparedness. It also demonstrates an association between nurses attending Mississippi higher education institutions and nurses not feeling comfortable enough with their training to talk about CAM with patients. Nurses who participated in this survey expressed a significant positive feeling towards learning more about CAM knowledge. They showed an interest in being able to provide more advice to their patients on CAM modalities, with an additional interest in physicians also being able to provide such advisement. The limitations of this specific study include the small sample size in comparison to the large number of nurses practicing in the state, as well as, the low male response rate. Future research should be conducted with a larger sample size from various states in mind and including the views of physicians. Future research should also investigate patient's feelings regarding communication on CAM use to their healthcare providers (nurses or physicians). The increase use of CAM by patients dictates the increase need for nurses to feel comfortable with their knowledge and training on CAM, in order to address questions with patient treatment and prevent patients from using unsafe or ineffective CAM therapies. In addition, health care providers must ensure the CAM be used by the patient will not interfere with the traditional therapies administered and result in harm or ineffective treatment to the patient.

CONCLUSION

This study suggests that there is a need to revisit nursing school's study curriculum to fit the continuously changing healthcare system and the popularity of CAM among patients. Our findings demonstrate the fact that Mississippi nurses feel unprepared in CAM education when talking to their patients. Findings also highlighted the associations between the feelings of unpreparedness among Mississippi nurses and attending a higher education institution in the state of Mississippi. Such findings could and should pave the way of higher education institutions directions in program curricula planning to better serve a continually evolving healthcare system.

ACKNOWLEDGEMENTS

A special thank you to Mississippi Nurses Association, Massage Envy, & Rainbow Coop. for their assistance and donations.

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THE MODEL OF INTERDISCIPLINARY COLLABORATION IN PERIOPERATIVE SETTING

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ABSTRACT

Introduction: High-reliability and continually technologically innovative environment of perioperative setting (PS) places increasing demands on adaptability of the interdisciplinary teams (IDT) engaged in its activities. Continual concerns for improvements in safety and facilitation of quality process and outcomes drive the efforts in improving the effectiveness and efficiency of interdisciplinary collaboration (IC) to match or supplant changes in PS brought by ongoing innovation integral to it. The model of ICPS has not been empirically described using objective methodology in peer-reviewed literature.
Purpose: The purpose of this research was to explain the model of ICPS from the perceptions of the interdisciplinary team (IDT) of PS. Questions guiding this research were: What are the significant components of ICPS? What are the interrelation patterns of the ICPS factors in the perceptions of IDT professionals? **Materials and Methods:** This research was conducted under the approval of the internal review board (IRB) of the University of Mississippi Medical Center. This research was based on mixed-methods survey design engaging the population of perioperative professionals at educational and professional meetings. The model of ICPS was defined from the literature review. The assumptions of the model were tested with the partially confirmatory factor analysis (PCFA), exploratory factor analysis (EFA), and the analysis of specific correlations of IC level and its likely significant factors, which were derived with identification of common themes in peer-reviewed literature of theoretic and empiric research on collaboration and convergent in concept teamwork and collegiality. The data input was received from IDT professionals through survey in Research Electronic Data Capture (REDCap). **Results:** The data were statistically analyzed using SPSS. Five primary factors of ICPS as identified by IDT members include: *Collegial Support of Adaptability* (r=0.478), *Reflexive Decision Making* (r=0.457), and *Process Development* (r=0.495). Three factor components with eigenvalues >1 and factor loading of >0.40 were extracted Generalize Least Square (GLS) and Varimax rotation in SPSS; 74.606% of variance was explained with this model. Guardianship-Stewardship Motivational Conflict Model of ICPS was tested with analysis of correlations of the ICPS factors with IC level. ICPS assumptions were adjusted using the empirical findings. **Conclusions:** In this study, we were able to identify significant covariates of ICPS pertinent in describing its model. Guardianship-Stewardship ICPS motivational model was adjusted using these findings. Further research could focus on detailing the effects of ICPS factors on levels of IC and technical outcomes.

Keywords: interdisciplinary, collaboration, perioperative, model, surgery, postoperative recovery, team, multidisciplinary, perceptions

INTRODUCTION

High-reliability environment of perioperative setting (PS) along with resource constraints in healthcare of today place increasing demands on adaptability of the interdisciplinary teams (IDT). Shifting paradigms brought by newer and developing surgical technology and techniques may present additional challenge to teamwork, amplifying the need for team self-adjustment skills. Safety culture is thought to interdepend with the interdisciplinary collaboration (IC) [1, 2, 3, 4, 5]. Continual concerns for patient safety and facilitation of improvements in process and quality outcomes drive the efforts to support interdisciplinary collaboration in perioperative setting (ICPS) to match or supplant changes brought by ongoing innovation integral to PS. Description of ICPS model is necessary for better understanding of the phenomenon and factors influencing it and for validation of the development of effective interventions to improve collaboration, in order to pursue the aims of improving surgical process and outcomes.

Earlier original investigation in modeling collaboration, conducted through the interviews of professionals of several industries, including healthcare resulted in the trilevel model with the *inner core* of person- centered community of *collaborative ethic or culture*, causally interrelated with the *greater sphere* related to professional role (collegiality) and yet greater *outer sphere* of congruent system and coherent intent [6], where IC is influenced by cultural and personal characteristics and collaboration history [7,8]. Five core components of IC in Bronstein's Model include: newly created interprofessional activity, flexibility, interdependence, collective ownership of goals and reflection on process [7]. The trilevel model was appreciated in application to ICPS [9,10,11]. However,

the models of ICPS have not been empirically validated in PS using objective methodology according to our review of literature. The purpose of this research was to explain the model of ICPS from the perceptions of IDT professionals. Questions guiding this research included:

What are the significant components of ICPS?

What are the interrelation patterns of the ICPS factors in the perceptions of IDT professionals?

Stewardship-Guardianship Motivational Conflict Model of ICPS: IC can be described as a favorable solution to work process related problems manifested in a conflict. We adapted to perioperative context the approach of the high-impact conceptual frameworks of conflict management, including *Managerial Grid Model (MGM)* [12], *Thomas-Kilmann Conflict Mode Instrument* (TKI) [13,14,15,16,17], which was also adapted in Kraybill "Style Matters" Conflict Style Inventory (KCSI) [18]. Concerns for patient safety or *Guardianship* in PS is the primary independent factor that is represented in the horizontal axis with the ultimate *Error Risk Reduction* goal (error→0') at its extreme. The motivations of cost reduction and resource conservancy and support of institutional legacy existing within the constraints of organizational sphere could become orthogonal to safety and quality. One example of such orthogonality is evident in the information sharing in the best interest of patient safety vs. information security, where a bedside care provider is limited in the means of information transfer to a specialist (cardiologist) about change in patient status, such as ST elevation. The media means may exist (personal smart phones), but their use is disallowed by the organizations in compliance with privacy laws. Thus, utilization of personal media means in the best interests of Guardianship would interfere with the Stewardship interests of protecting the legacy of organization. The motivations of cost reduction and resource conservancy or *Stewardship* are represented in ICPS model coordinate system in the vertical axis with the *Cost Reduction* (cost→0') at its extreme. *Avoiding*, *Silo*, or *Indifference* at the lower level of horizontal and vertical spectra of motives of Guardianship and Stewardship is an adverse alternative to IC. Himmelman's synergy continuum (*Conflict Avoidance* => *Networking*=> *Cooperation*=> *Coordination* => *Collaboration* [19] *used in TKI and* applicable in PS context, is represented in the diagonal axis of Guardianship-Stewardship coordinate system. In this sense, the motivations driving collaboration are related to higher synergistic levels of the concern for safety and the concern for cost reduction (Figure 1). In its extrapolated position, collaboration is believed to introduce additional resource due to process optimization through the conflict-catalyzed group decision making and problem solving. Structural, pattern, and conceptual modeling of the ICPS collaborative process is likely to inform an approach influencing interactions between representatives of the major professional groups of the perioperative realm: surgeons/radiologists/ICU physicians, ACT, perfusionists, intraoperative neuromonitoring providers (IONMP), perioperative nursing and allied health personnel, and administration and leadership enhancing the improvements in safety of surgical patient. The assumptions of the ICPS model are that the interactions of the motivational conflicts between pro- safety and quality Guardianship and pro-efficiency Stewardship are shaping the synergistic path toward higher levels of teamwork and collegiality, and ICPS. Model assumptions were tested in this study.

MATERIALS AND METHODS

University of Mississippi Medical Center Institutional Review Board (IRB) exempt approval was obtained prior to commencement of data collection. This research was based on mixed-methods survey design engaging the population of perioperative professionals at educational and professional meetings. The model of ICPS was defined and study questionnaire was constructed from the literature review [20]. Data were collected by survey administration from 10/07/2017 to 11/01/2017 among the perioperative professionals attending educational conferences and meetings within areas of Central Alabama, Mississippi, and Louisiana who volunteered to participate. Data collection was based on survey, designed for administration via Research Electronic Data Capture (REDCap) platform, developed by the REDCap Consortium, hosted by Vanderbilt University (REDCap, 2014). "Study data were collected and managed using REDCap electronic data capture tools hosted at [UMMC].1 REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources." [21] (Harris et al, 2009). Potentially significant covariates of ICPS were identified in literature and included in the questionnaire with the assessment of ICPS extent in the perceptions of IDT professionals. The survey questionnaire was

accessible online. However, most participants preferred the paper version of the questionnaire. Specific descriptions of ICPS were assessed for discriminatory refinement of the model.

The assumptions of the model were tested initially with partially confirmatory factor analysis (PCFA) and the analysis of specific correlations of IC level and its likely significant factors, which were derived with identification of common themes in peer-reviewed literature of theoretic

RESULTS

Thirty-two (32) response sets met the inclusion criteria for the aims of this research and were included in statistical analysis. Adequate sample size was verified with Kaiser-Meyer-Olkin (KMO) coefficient (0.725). Factor analysis (FA) was used in delineation of significant latent variables impacting ICPS: HYPOTHESIS 1. Cumulative survey data variance of 80% would be explained with IC factor model fit with eigenvalue greater than 1.0, factor loadings greater than 0.40.

With Generalize Least Square (GLS) extraction, 74.606% of variance could be explained with four factor components with eigenvalues >1 (Table 1). We extracted four components with factor loading of >0.40 using GLS, which is built on regression model, with attained goodness-of-fit (GOF) significance of p=1.000 (df=116)

Table 1 SPSS output for GLS of IC and Covariates

Total Variance Explained

Factor	Initial Eigenvalues			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	10.626	53.130	53.130	5.110	25.550	25.550
2	1.626	8.132	61.262	2.828	14.138	39.689
3	1.466	7.329	68.591	2.737	13.686	53.375
4	1.203	6.015	74.606	2.379	11.897	65.273
5	.998	4.988	79.594			
6	.675	3.374	82.967			
7	.642	3.211	86.179			
8	.512	2.561	88.739			
9	.469	2.346	91.085			
10	.398	1.992	93.077			
11	.360	1.800	94.877			
12	.257	1.286	96.163			
13	.215	1.074	97.237			
14	.178	.890	98.127			
15	.131	.655	98.782			
16	.085	.425	99.207			
17	.064	.320	99.527			
18	.042	.209	99.736			
19	.034	.168	99.903			
20	.019	.097	100.000			

Extraction Method: Generalized Least Squares.

Factor Score Covariance Matrix

Factor	1	2	3	4
1	.996	.005	.001	.000
2	.005	.989	.002	.002
3	.001	.002	.991	.002
4	.000	.002	.002	.991

Extraction Method: Generalized Least Squares.
Rotation Method: Varimax with Kaiser Normalization.
Factor Scores Method: Regression.

Table 3 ICPS component orthogonality in GLS

and empiric research on collaboration and convergent in concept teamwork and collegiality. The data input was received from IDT professionals through survey in REDCap. ICPS context and features from the fill-in blank descriptions of IDT were analyzed using qualitative analytic techniques. Purposiveness of sampling to obtain qualitative data was attained by targeting maximal interdisciplinary inclusion of the population of perioperative professionals of a variety of settings.

in SPSS (Table 2) and estimated normed fit index (NFI) of 0.849, however the root mean square error of approximation (RMSEA) was calculated to be only 0.211, indicating poor model fit [22]. Therefore, only the results of exploratory FA were included in discussion. Satisfactory component orthogonality was achieved with GLS extraction and Varimax rotation with Kaiser Normalization (Table 3). Interpretation of the final factor components of ICPS (Table 4): 1-*Collegial Support of Adaptability* (0.478); 2-*Reflexive Decision-Making* (0.457); 3-*Process Development* (0.495). The relevance of Component 4, interpreted as *Specialization and Mutual Support* (professionalization), to ICPS was not supported by FA results, where it was shown to be secondary to IC through other IC covariates, such as BTD and SE.

Table 2 Model Goodness of Fit

Chi-Square	df	Sig.
68.367	116	1.000

Table 4 Component of ICPS Covariates

Rotated Factor Matrix[a]

	Factor			
	1	2	3	4
Adaptability	.884	.434		
CDPS	.709		.406	
SSA - error recogn	.686			
Collegiality	.631			
SE	.616			.527
Teamwork	.595		.410	
Spealization	.520			
Organizational Loyalty	.506			
Adaptability and Reflexivity	.483	.409		
CCF	.445			
Team Dynamics	.439			
Balanced Leadership	.429	.425		
CRR		.831		
SE	.401	.637		
Coordination of Resources		.596		
Reflexivity			.910	
Briefing-Debriefing			.566	
POM	.401			.763
SSA				.693
IC level	.478		.457	.495

Extraction Method: Generalized Least Squares.
Rotation Method: Varimax with Kaiser Normalization.[a]

a. Rotation converged in 10 iterations.

The factor components relevant to ICPS in this extraction seem to reflect the dynamic rather than static nature of ICPS and its covariates. However, IC loaded only modestly on these components. We compared the correlations between the levels of IC factors with the levels of IC and convergent in concepts of collegiality and teamwork in perceptions of IDT professionals. The exact correlations between the elements of safety and efficiency with collaboration, teamwork, and collegiality deferred from those theoretically assumed. Pearson *r*, where normality of distribution was confirmed with Levene's test ($p>0.05$) and Spearman *rho*, where it was not, were utilized in testing the assumptions about ICPS and its factors arising from the model. This testing was necessary in order to recreate the shared mental model of IDT professionals of IC, teamwork, and collegiality in relation to the *Guardianship-Stewardship* system of coordinates a priori of any facilitation of theoretic assumptions in IDT perceptions. HYPOTHESIS 2. Higher process coordination and management (PCM) – IC level and PCM - teamwork correlations, compared to PCM - collegiality correlations, are expected to be observed. H0: Spearman *rho* (PCM – IC level) ≤ Spearman rho (PCM – collegiality) and Spearman rho (PCM 1 – teamwork) ≤ Spearman rho (PCM – collegiality); HA: Spearman *rho* (PCM – IC level) > Spearman *rho* (PCM – collegiality) and Spearman *rho* (PCM – teamwork) > Spearman *rho* (PCM – collegiality).

Significant moderate positive correlations (Spearman *rho*=0.580, $p<0.025$) were observed in PCM – teamwork, which was less than that of PCM–collegiality (Spearman *rho*=0.601, $p<0.025$); PCM – IC level (extent) correlations were the highest (Spearman *rho*=0.703, $p<0.025$). The alternative hypothesis was partially rejected. Similar comparison testing was performed with the ICPS features of management of disruptions, error recognition (safety), shared situational awareness (SSA) in conflicting situations (safety), commitment to organizational values (loyalty), and focus on specialized tasks. The correlations of SSA, shared collaborative decision-making and problem-solving capacity (SCDPC), adaptability, reflexivity (RA), and balanced team dynamics (BTD) with IC level were specifically assessed due to the critical nature of these features in PS, based on teamwork theory and empirical evidence [23,24]. We also tested specific correlations of professional identity and PCM with IC level, in order to find their position in Guardianship-Stewardship coordinate system. Analysis of these results is included in the Discussion section (following).

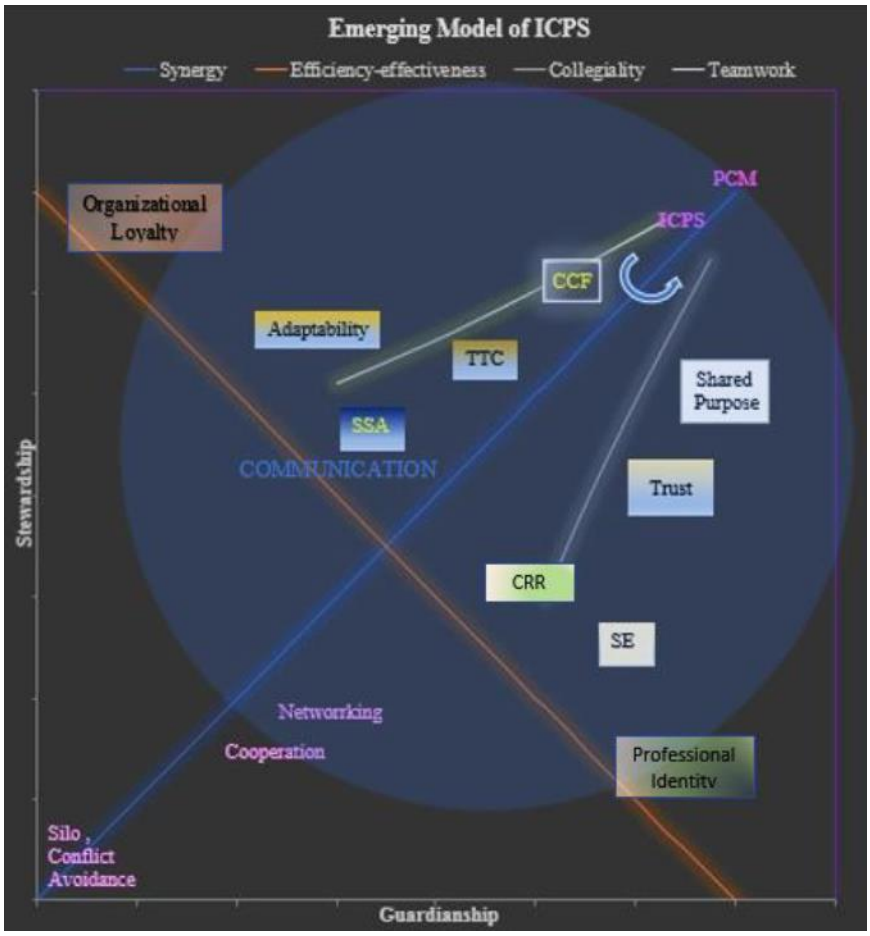
Qualitative inputs were marked by succinctness, but a few common themes have been identified. The following major features essential to ICPS were identified by the participants: IC knowledge ([technical] competence [3]), IC attitudes (respect [2], compliance [1], and commitment [1]), IC skills (communication/critical communication [13], feedback [1], response to critical situations [1]), and organizational environment (quiet setting/environment [2], balanced leadership [1]). We adjusted the Motivational Model of ICPS to include the identified positions and trends (Figure 1).

DISCUSSION

From the perceptions scores of IDT professionals and by means of PCFA, ICPS was found to be related to the following groups of features: *Collegial Support of Adaptability*, including adaptability, SCDPC, SSA in error recognition, collegiality, loyalty to organization, SE, Specialization focus; *Reflexive Decision-Making*, including reflexivity, formal means of communication (briefing-debriefing), SCDPC, and teamwork; and *Process Development*, including PCM, SE, and SSA. Predicted location of IC and convergent to it teamwork and collegiality and their significant IC factors in the Guardianship- Stewardship coordinate system were supported partially. Specifically, three out of seven correlation assumptions tested for the ICPS model were supported; two were supported partially. Unlike that predicted, loyalty to organization was highly interrelated with both collegiality and teamwork, indicating less conflict between organizational motivations of supporting teamwork and those of professional affinity. SSA in conflicting situations, an important safety feature, was correlated more with collegiality than teamwork as it was expected, but less with the level of IC, compared to teamwork. IC was found to have greater relevance with error recognition (SSA), than with IDT adaptability. IC extent was seen even less relevant to reflexivity of error risk in this dataset. At the same time, management of disruptions was seen to have greater relevance with collegiality and IC extent than with teamwork, as it was expected. Communication was the single most important factor of ICPS, confirmed with triangulated methodology in this study. Consistent with input- process-output (IPO) framework, shared expertise (SE), critical communication and feedback (CCF), and shared situational awareness (SSA) were directly correlated with ICPS; CCF is a form of communication, essential in SSA. Both, communication and CCF, were confirmed to be significant factors in ICPS. Therefore, *Networking*, with its emphasis on communication, is positioned higher on *Synergistic Continuum*, compared to *Cooperation*.

Effective communication could, however, be argued to be more than just a factor, but the environment, the medium, upon which teamwork, collegiality, and collaboration are striving in organizations. It is, therefore, the single most important lever in supporting ICPS. Trust, purpose, mutual respect, and shared expertise directly aligned with IC levels and were reiterated as essential antecedents qualitatively in this study. ICPS correlations with Professional Identity were positive and higher than those with Focus on Specialized Tasks, which, in turn, correlated unexpectedly higher with teamwork and less with collegiality. Succinctness in qualitative responses could be indicative of high uncertainty in decision-making and possible interference of high-stress [JDS1] milieu common in PS with IDT locus of control.

Figure 1. Guardianship-Stewardship ICPS



The model of ICPS emerged in this study supports the course from silo mentality to higher levels of ICPS to better PCM as the outcome, guided by motivations of Guardianship and Stewardship. This model supports increasing ICPS magnitude along the synergistic continuum, coursing away from silo mentality to greater ICPS levels, where PCM is supported as ICPS output. PCM scores positively correlated with IC extent. With added PCM as the outcome of IC, adaptation of Himmelman's synergistic continuum seems to be a valid inclusion in modeling ICPS. Further research could detail the differences in the effects of the elements of teamwork, collegiality, and collaboration on technical outcomes in specific surgical procedures, situations, and settings. Though represented here in 2-D planar coordinate system, the motivations for support of *Innovation* are likely orthogonal to efficiency (resource stewardship) and effectiveness (safety) axes. Technical competence was found to be significant in ICPS with the implication of likely significant role of motives supporting innovation. Creating sociocultural conditions supportive of collaborative culture and coordinated adaptations is a significant component of the sociotechnical system geared for reducing errors associated with introduction of technological innovations [24]. We propose the three-dimensional scaling of ICPS process within organizations consisting of *Guardianship* (safety) x *Stewardship* x *Innovation*, which is to be tested in further research. The fourth dimension to be considered in ICPS model is *Temporal Dynamics*, which could be assessed in longitudinal applications of ICPS instrument [20]. Ilgen et al (2005) described temporal team existences through three stages or processes of formation, functioning (bonding, adapting, and learning), and dissolution with the accompanying affective, behavioral, and cognitive attributes approachable in the input-mediator-output- input framework, which is arguably converging the diverse perspectives of theories of teams and "small groups in general" and applicable to ICPS modeling [25].

Several limitations could have interfered with the validity of the results. IDT members were selected not directly in operating rooms, but among the professionals attending education meetings to reduce interference with OR processes and any pressure to participate. Such approach to participant selection was deemed to be appropriate, considering commonly encountered IDT fluidity. Nevertheless, participant sampling representativeness could be affected by engagement bias. The data were collected from the retrospective recollection of IDT members outside of PS, thus being subject to possible issues with recall. Participant subjective biases could have influenced the results and conclusions. Individual healthcare provider levels of familiarity with the concepts included in the questionnaire, level of interest in the subject of collaboration and teamwork and engagement motivations could have introduced an extraneous variance. Attrition bias could have influenced the accuracy of responses. Interference by survey instrument item sequence could not be eliminated. Overall, response bias risk in this study must be assumed to be as in average survey environment. Attained small sample size could limit generalizability of the results and retesting would improve validity of conclusions. Larger sample size and number of items testing specific aspects of ICPS in future research could provide better definition of the construct.

CONCLUSIONS

Conceptual clarification and structural and pattern modeling of ICPS in this study is likely to provide the insights into further research of interactions between the representatives of the interdisciplinary groups collaborating in the perioperative realm. Results of this research supports well-evidenced centrality of communication in ICPS. Further research detailing the effects of specific ICPS factors, particularly SSA, adaptability, and reflexivity, would allow refinement of effective means to improve it. Overall, study results support the nonlinear nature of collaboration and its features with likely culpability of the phenomenon with fuzzy logistics and collaboration networks in further ICPS modeling [25, 26]. Deeper understanding of ICPS could potentiate the innovation in PS and the efforts of perioperative professional communities, the leadership of healthcare organizations, and professional societies endeavoring quality standard development. This model demonstrates how the safety of patients undergoing surgery could be improved with ICPS, which was the primary aim of this research.

ACKNOWLEDGMENTS

We would to express our great gratitude to the study participants in Central Mississippi and the Association of periOperative Registered Nurses (AORN) chapters of Acadiana (Lafayette, LA) and Birmingham, AL.

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BLUETOOTH ENABLED SMARTPHONE APPLICATION FOR WIRELESS PHOTOPLETHYSMOGRAPHY MONITORING DEVICES

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ABSTRACT

This article presents the design, prototyping and testing steps of a wireless heart rate monitoring device based on photoplethysmography (PPG) technology. PPG monitoring devices use optical sensors to collect vital signs such as heart rate, blood pressure, oxygen saturation and cardiac output. The collected data must wirelessly be transferred to a smart device in real time for additional processing. Therefore, wireless connectivity plays an important role in such biomedical instruments. A typical reflection-mode PPG monitoring device is made of an infrared sensor and a photodetector to illuminate the tissue and detect the variations in the light intensity of illuminated tissue, a signal conditioning stage to prepare the captured signals through amplification and filtering, a low-power microcontroller to control and manipulate the analog PPG signals, and a Bluetooth module to transmit the PPG data to a Bluetooth-based smart device such as a tablet. A user-friendly mobile application (App) is then designed and developed using MIT App Inventor 2 in order to acquire and visually display the received analog PPG signals in real-time on the smart device. The aim of this article is to provide a precise step-by-step procedure of designing a PPG monitoring device and detailed procedure of developing a user-friendly app.

Keywords: PPG Sensor, Real-Time Wireless Monitoring, Smartphone Application

INTRODUCTION

Real-time monitoring of heart rate is a vital component of cardiovascular fitness assessment and training programs. For many years, the Electrocardiogram (ECG) has been used as the oldest and the simplest technique to assess the electrical activity of the heart and diagnose cardiovascular diseases. Although, currently used ECG monitoring systems are equipped with flexible electrodes and portable wireless ECG measurement systems to provide user flexibility and portability [1-2], they are still not very comfortable for daily physical activities. In order to be effectively operable, a number of electrodes must be placed at specific body locations. This implication greatly restricts the flexibility of users especially during daily activities. To overcome the mentioned disadvantage, PPG technology can be used as an alternative. A PPG sensor can be used to replace the ECG electrodes. A PPG sensor consists of a source usually an infrared light and a photodetector to acquire blood volume changes in the microvascular bed of tissue [3]. PPG sensor is used to detect the variations of light intensity via transmission through or reflection from the tissue [3]. Based on these variations in the light intensity, heart-related information can be extracted from the cardiovascular system. Generally, PPG sensors operate in two distinct operable modes: the transmittance mode and the reflectance mode [3-5]. In the transmittance mode, the light that is transmitted through the tissue is detected by a photodetector that is placed on the opposite side of the tissue, while in the reflectance mode, the infrared light transmitter and detector are placed on the same side where the light from the transmitter is reflected back from the tissue to the detector. Transmittance-mode PPG sensors can usually acquire good quality signals but in order to be effective, they must be placed at specific body locations such as earlobe or fingertip.

However, the reflectance-mode PPG sensors could be placed at a variety of places on the body such as forehead, chest and wrist. These various measurement sites provide more wearing options for the users and are more convenient places for sensor placements. They can be used as ideal measurement sites for daily routine activities.

With the rise of popularity of wearable fitness technologies, this work is intended to present the design, prototyping and testing steps of a wireless heart rate monitoring device based on PPG technology. The proposed PPG-based heart rate monitoring device is able to collect an individual's PPG signals and wirelessly transfer them to a smartphone as well as displaying these signals on a smartphone application in real time.

Proposed Heart Rate Monitoring Prototype

A wireless PPG-based heart rate monitoring device was designed on a breadboard to collect the arterial pulse signal. The proposed device, as shown in Fig. 1, consists of an infrared sensor to detect the arterial pulse in the finger, a signal conditioning unit to prepare the acquired signals through filtering and amplification for an Analog-to-Digital Conversion (ADC) stage, a low-power Arduino-based microcontroller to digitize the analog PPG signals, and a wireless Bluetooth module to transfer the digital data to a smart device. An Android app based on MIT App Inventor 2 was also designed and developed to enable the smart device to obtain and graphically display the received analog PPG signals in real-time on the smart device [4-5].

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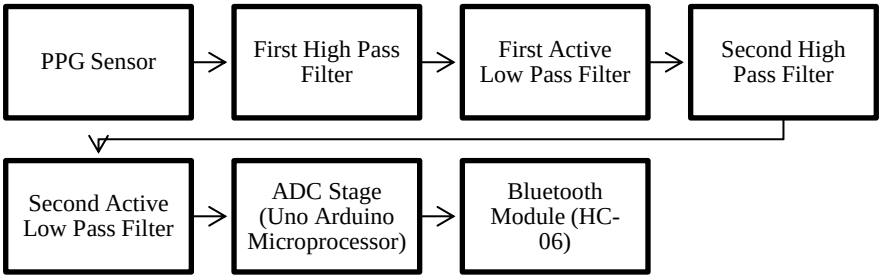
Fig. 1. Block diagram of the proposed heart rate monitoring device.



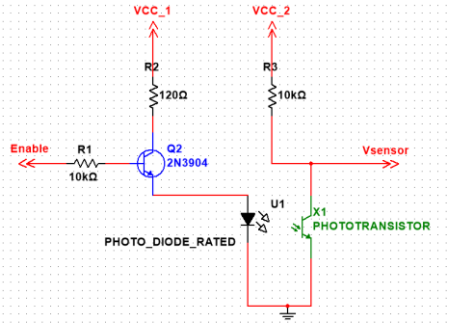


Fig. 2. Schematic circuit diagram of the PPG sensor.

A. Photoplethysmography Circuit Design

An electronic circuit was designed on a breadboard consisting of a reflectance-mode PPG sensor called TCRT1000 IR. As shown in the circuit diagram in Fig. 2, the PPG sensor uses an infrared light and a photodetector. By placing one's fingertip over the TCRT1000 sensor, the incident light will be reflected and the amount of light that is reflected back from the fingertip is detected by the photodetector. The output of the sensor as shown in Fig. 3 is fed to a passive high pass filter with a cut-off frequency 0.5Hz, which can be calculated using equation 1.

$$\boldsymbol{F} = \frac{\mathbf{1}}{\mathbf{2}\boldsymbol{\pi RC}} \qquad \text{(Eq. 1)}$$

Then the output from the passive high pass filter is fed to an active low pass filter with cut off frequency of 3.4 Hz and with gain of about 34 dB. This means that any signal that is not in the 0.5-3.4 Hz band will be attenuated while the frequencies that are in the passband are amplified by 34 dB. This low pass filter is attached to a potentiometer that leads to the second filtering stage. The last stage has the same cut- off frequency and gain and creates an overall second-order filter with a slope of 40dB/decade. This allows the circuit to be more precise in attenuating signals near the stopband. A reference voltage circuit is added in between both filters. This allows the signal to achieve a full swing at the output. The fully connected circuit is shown in Fig. 3. The PPG signal is captured on an oscilloscope as shown in Fig. 4.

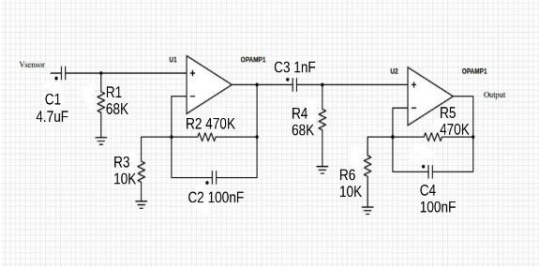


Fig. 3. Full PPG circuit

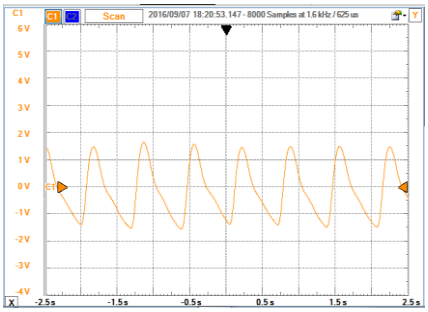


Fig. 4. Real-time PPG signal is captured on an Oscilloscope

B. Establishment of a Wireless Connection Between the PPG Circuit and Smartphone

Setting up a wireless connection between the PPG circuit and the smartphone was achieved by using an Arduino Uno and a Bluetooth module [8]. To do this, the Arduino Uno circuit board was used along with a HC-06 Bluetooth module. The Bluetooth module is a four pins device: the VCC which is used to power the module and it was connected to the Arduino 5v pin, the GND which is the ground pin and grounds the Arduino, the TX pin which was connected to the Arduino's RX pin whenever data were to be transmitted to the Arduino, and the RX pin of the module which was connected to the TX pin of the Arduino to send data through Bluetooth from the Arduino. The Arduino's TX line runs at 5V while the RX pin of the Bluetooth module runs at 3.3V causing the Arduino to supply a higher voltage that may damage the Bluetooth module. To overcome this problem, a voltage divider circuit was created such that the TX pin was connected to a resistor R1 (20K), R2 (10K) and ground and the output taken from the connection between R1 and R2, was calculated by equation 2.

$$V_{out} = V_{tx} * \frac{R_2}{R_1+R_2} \quad \text{(Eq. 2)}$$

1) Arduino Uno Code

The Arduino code provided the connection between the PPG sensor and the Arduino Uno board for data reading. The code first recognized if data were received from the sensor in order to transmit the data via the Bluetooth module. In that case, then the data were read with a delay of 75 milliseconds to avoid errors during reading. The code also established the serial connection for the transmission of data at a bit rate of 9600 per second and turned on the analog pin 0 in the Arduino board since the PPG sensor was connected to this pin. The data received from the PPG sensor once the initial check was confirmed was set to a variable and printed internally. The information feed was slowed down to every 75 milliseconds due to errors occurring between the smart device and board when no delay was implemented.

C. Smartphone Application

An Android Galaxy tablet and an Android Galaxy 5 Samsung smartphone were used to display the PPG graph. They were used to test for accuracy and detection of Bluetooth signals. The application was coded using a code block building creator called MIT App Inventor [9]. The MIT App Inventor is an open source web application that allows beginners to learn and experience computer programming to create fully functional software applications for Android operating systems. To develop an application using the App Inventor, one is going to have to use two main components: App Inventor Designer and App Inventor Blocks Editor. The App Inventor Designer is used to design the application's user interface for arranging both on-screen and off-screen components. The App Inventor Designer has three main elements: Viewer, Palette and Properties. The Palette contains main components such as Buttons, CheckBoxes, TextBoxes, etc. When the users want to use any of these components, they simply select the element and drag to the Viewer. The Viewer lets the users know what the application will look like and allows them to drag the elements and the components they need to use to develop the application. The Properties section is used to change the color, size, behavior, etc. of a component. The App Inventor Blocks Editor also contains the Viewer section and another section called Blocks. The Blocks section contains blocks for general behaviors the user might want to add to the application and also contains component-specific blocks to control the behavior of a button.

1) Proposed Smartphone Application

The application developed for the PPG sensor signal was created using a block code interactive program called MIT App Inventor. The application functionality can be represented through the flowchart as shown in Fig. 5.

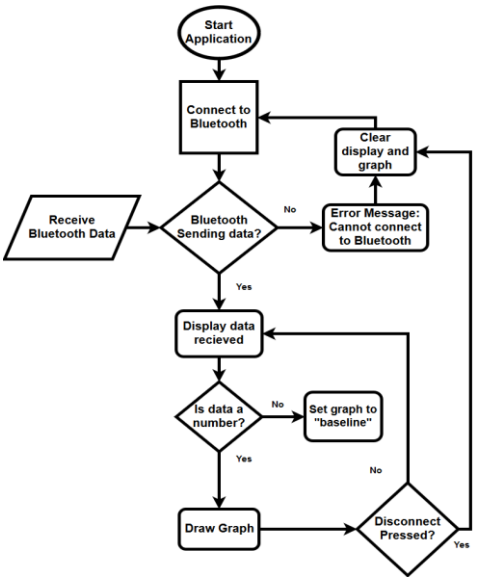


Fig. 5. Flowchart of the application's functionality.

In order to initiate a connection with the "server", the Arduino, the application was set to act as a client as shown in Fig. 6. For this connection to be made, our device had to be connected to Bluetooth or an error message was to be displayed. Since the application at its current state was unable to create paired devices connections, the Arduino had to be made a paired device for the Android device before the initiation of the application. The listpicker block was used to look up all the Bluetooth clients by address and name that have been setup as paired devices for the current Android device. Once a device was picked, the next block, Devices, connected the device selected via Bluetooth to the application. The *Button1* block was used to disconnect the Bluetooth device once pressed and ended the connection.

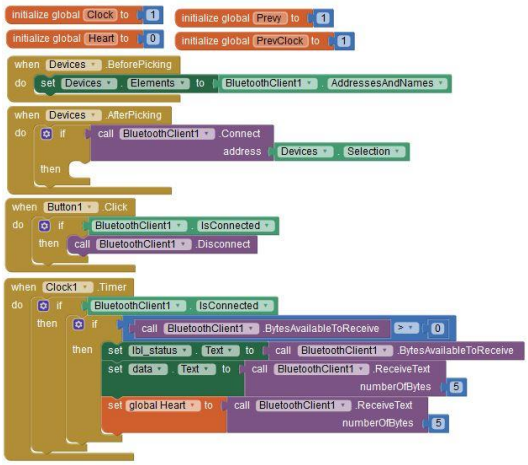


Fig. 6. Code used for initiation of connection between application and Arduino. Global variables: clock, Heart, Prevy, and PrevClock are given initial values.

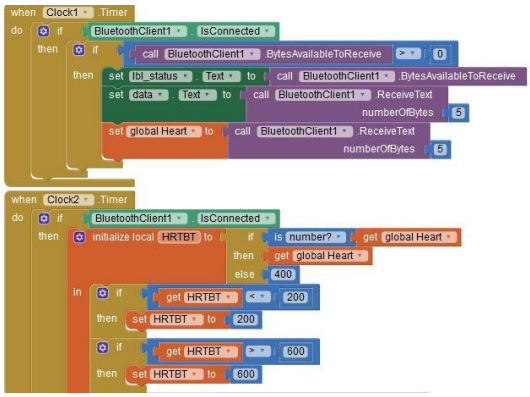


Fig. 7. Clocks used for the timing of different aspects of the application. The range of the graph is also set to avoid out of bounds.

The *Clock1* variable was used to create a loop calibrated to the timer on the tablet to receive information from the Bluetooth device. In the block shown in Figure 7, the algorithm checked for a valid connection to a Bluetooth client. Once connection was verified, it checked to see if there were data to receive and set variable *lbl_status* to read the bytes the application could receive. It set the *data* variable to a 5 byte number received by the Bluetooth client, and it set the variable *Heart* to this number. *Clock2* was used to time the speed of the graph after ensuring connection to the Bluetooth Arduino. Once this was true, the algorithm initialized a local variable *HRTBT*. If the variable Heart was a number, it set *HRTBT* to *Heart*. If it was not a number, then it set the local variable to 400. This check was created due to errors where the received variable was an empty string causing the graph to crash. The number 400 was used as it was the resting number when the PPG was not reading a heartbeat. If the number received from the PPG was above 600 or below 200, it set the ceiling and floor as 600 and 200, respectively to ensure that the graph was within the given bounds.

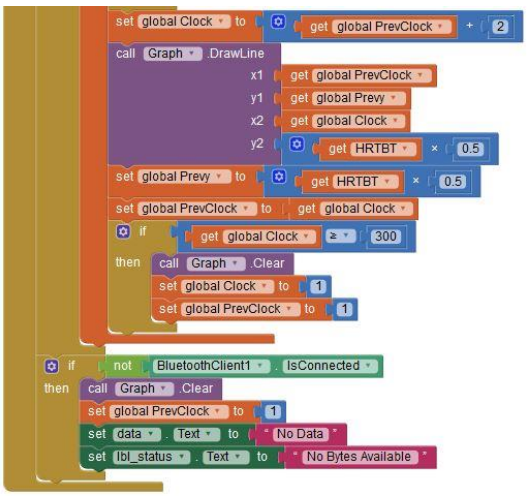


Fig. 8. Code used for the creation of the real-time graph scaled to 0.5 of the received data. The variable Clock is set to PrevClock plus 2 for a real-time effect.

The Graph.*DrawLine* function is called with the variables *PrevClock* and *Clock* as the x-variables and *prevy* and *HRTBT* as the y-variables. *HRTBT* was multiplied by 0.5 in order to scale the data to fit properly on the graph as seen in Figure 8. *Prevy* was set to *HRTBT* and *PrevClock* to *Clock*. This caused the line graph to continuously move on the x-axis as the y-values display the data from the PPG signal. Once the line graph hits the right side of the screen, which is at about 300, it cleared the graph and set the x-values to 1 to return to the left side of the screen. The second if statement cleared the graph, set the graph back to the left of the screen, and cleared the data from the text boxes once the Bluetooth client was disconnected.

RESULTS

The PPG HRM was successful in capturing and displaying the PPG signals and measuring an individual's HR. There were challenges with noise in the acquired PPG signals that required further robust signal processing. These were considered and resolved partially at this stage of development.

A. Data Collection

In order to obtain PPG data, 3 of the authors volunteered as subjects and sat down and elevated their right hand slightly upwards. The PPG sensor was placed on the tip of the index finger. These PPG signals were observed through both the Arduino graph plotter as well as the Android application. The sensor was connected for about 5 minutes in each one of the subjects with no deterioration of the signal or synchronization issues with the graphs. Experimental data were recorded through screen capturing on the Arduino.

B. Experimental Data

The application shows the detection of the PPG signals from the transmission device. These signals were detected in real time and displayed within milliseconds on the graph represented. The application read at a slower rate than ideal in order for it to be more stable on the Android application as shown in Figure 9.

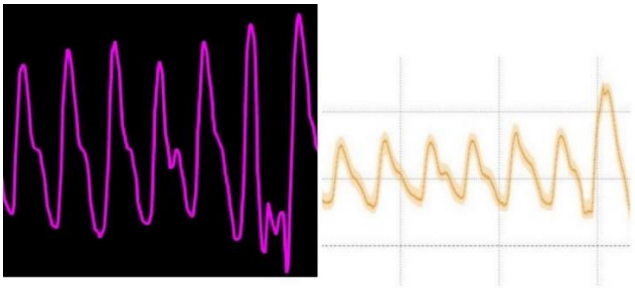


Fig. 9. Resulting graph of a test run of the application and PPG sensor.

DISCUSSION

As the Arduino application in its current state does run and display a PPG line graph, there is notable room for improvement. The application has a tendency to run off sync from the bits the Bluetooth sends and what the application reads. This reading problem can lead to producing errors in the HR determination. The team is currently fine tuning the system and is experimenting with byte delimiters or with a two-way communication approach in order to prevent the application from overloading with data. Another possible improvement is to decrease the circuit size in order to make the PPG HRM a more practical wearable device. At the moment, the Arduino is relatively bulky and contains loose wires that can easily be unpinned with sudden movement. A more advanced filtering scheme to more effectively remove PPG signal due to sensor movements will enable making the entire setup more wearable. Future implementations will include more sophisticated signal processing and more accurate data display for HR in beats per minute and calculation and display of the Heart Rate Variability (HRV) signal.

CONCLUSIONS

An electronic circuit was successfully designed and implemented to sense PPG signals to provide information about the heart, sending these signals to an Arduino, which accurately converted them into a digital signal and transmitted them to an Android smart device through a Bluetooth module. The application was successful in pairing with the Bluetooth module and receiving the PPG signal to graph it and use it to extract information about the heart rate and its variations.

ACKNOWLEDGMENT

Authors thank the National Institutes of Health (NIH) Diversity Program Consortium for support through BUILD award number 8UL1GM118970-02.

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EFFECT OF PEDICLE-SCREW FIXATION IN LUMBAR SPINE AT L3-L5 LEVEL: A FINITE ELEMENT STUDY

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ABSTRACT

Deformities in the spine often require pedicle screw insertion, which alters the mechanical environment in the spine resulting in implant loosening and failure. This study examined the alteration of the state of strain at the bone-implant interface in the lumbar spine after pedicle screw insertion. Three-dimensional (3D) geometry of the lumber region (L1-S) and CAD model of pedicle screw was subjected at L3-L5 level to finite element (FE) analysis to examine the strain condition at the 6 selected locations at the bone-implant interface using different screw diameters (5 mm and 6 mm), implant materials (stainless steel and titanium), bone conditions (very strong, strong, normal, weak and very weak) and loading conditions (420 N, 490 N and 588 N). Screw diameter was observed to be the most crucial factor in determining the strain environment. The 6 mm diameter screw did not alter the strain environment significantly ($p<0.05$) as compared to un-implanted bones. Stronger bones, smaller loads and stainless steel did not alter strain environment significantly after the implantation. This preliminary analysis will help in understanding the effect of different physiological and implant parameter on the mechanical environment at the bone-implant interface and will help to design better pedicle screw implants.

Keywords: lumbar vertebra, spinal implant, pedicle screw, finite element analysis, bone condition

INTRODUCTION

Surgical mediation is frequently a need for the treatment of degenerative diseases in spine area [1]. A standout amongst the most widely recognized surgical system utilized is the insertion of pedicle screws into the spine, where the flexibility of the affected area is partially preserved [2]. Pedicle screws are gaining its popularity for treating conditions like Degenerative Disc Disease (DDD), Lumbar spinal stenosis (LSS), Lumbar spondylolysis and Spondylolisthesis and post-trauma instability [3,4,5].

Some studies explored the pedicle screw breakage and loosening are the most common obstacle for successful implantation [3,6,7]. The risk of pedicle screw loosening has been found to be highly correlated with bone mineral density (BMD) in the investigation of osteoporotic cadaveric bone [8]. However the cases of loosening and breakages of the pedicle screws are observed in case of physical overload of the repaired spine and inadequate strength of the implant, especially in patients with osteoporosis [9]. As a result some patients experience non-union of the vertebral body which leads painful kyphosis [10]. Due to surgical fixation of the pedicle screw, stress concentration is developed at the implant-bone interface [3,11].

Insertion of the implant in the bone results in a change in its mechanical environment and the post-operative success depends on how the altered condition at the implant-bone interface performs in terms of load bearing. Load transfer mechanism and the changes in the mechanical environment at the bone-implant interface is important for understanding the failure mechanism and better design for the pedicle screws [12]. Halvorson et al. [13] also concluded that bone mineral density (BMD) is primarily related to pull-out strength of pedicle screw fixation. Poor BMD causes to decrease the pull-out strength of the pedicle screw in osteoporotic bone that leads to loosening of the screw [13,14,15].

In case of bone remodelling, strain at the bone-implant interface is an important factor [16,17]. The generated strain at adjacent bone depends on the local bone density, which varies widely among human population [18,19,20].

The Young's moduli of vertebral cancellous bone and cortical bone vary around 100 MPa and 10 to 12 GPa respectively [21, 22]. There are very few studies that give significance to the patient bone quality for designing specific implant [23,24].

Finite element (FE) analysis is a very useful tool for analyzing stress-strain distributions in implant–bone constructs. FE analysis allows the flexible alteration of parameters to obtain the full field of mechanical responses and gaining more and more acceptance and popularity [25,26]. The parameters could be physical dimensions and material for the implant, as well as bone and loading condition. Chen et al. studied load transfer mechanisms of the screw-vertebra complex using various screw lengths along with bonded and contact interface conditions between screw and vertebra by varying coefficient of friction between 0.01 and 0.4 [25]. In the lumbar spine, the L3-L5 are the most clinically relevant sites [27,28,29,30], even though the effect of insertion of a screw and rod construct extend to the adjacent levels as well. Therefore, FE analysis of the entire lumbar spine (L1-S) has considerable importance [31,32,33]. Lim et al. analyzed the change in the mechanical environment in intact and fixated bone using an FE model [34]. Previous studies often involved analysis on a single vertebra, but in physiological conditions vertebrae being connected entities, these studies cannot provide the complete scenario. As the bone condition largely varies patient to patient [19,20], the optimum design of implant should also vary accordingly to achieve more favourable mechanical environment at the bone-implant interface for better bone remodelling i.e. ossiointegration.

The present study is aimed to examine the mechanical environment (stress, strain) in lumbar spines (L1-S) before and after inserting the pedicle screw at the L3-L5 level under parametric variations in bone condition, loading, and diameter as well as material for the screws.

METHODS

Pre-processing

In the present study a set of two-dimensional (2D) format High-Resolution Computed Tomography (HRCT) scan data in DICOM (0.208 mm×0.208 mm, 146 slices, 1 mm gap) were processed using MIMICS® software (Materialise Inc, Belgium) to generate a three-dimensional (3D) reconstructed the geometry of the L1-S region (Figure 1). The set of fully threaded pedicle screws has been modeled in the commercial CAD software Pro/Engineer®. Three screws on each side of the spine have been designed which were connected by two connecting rods (Figure 2).

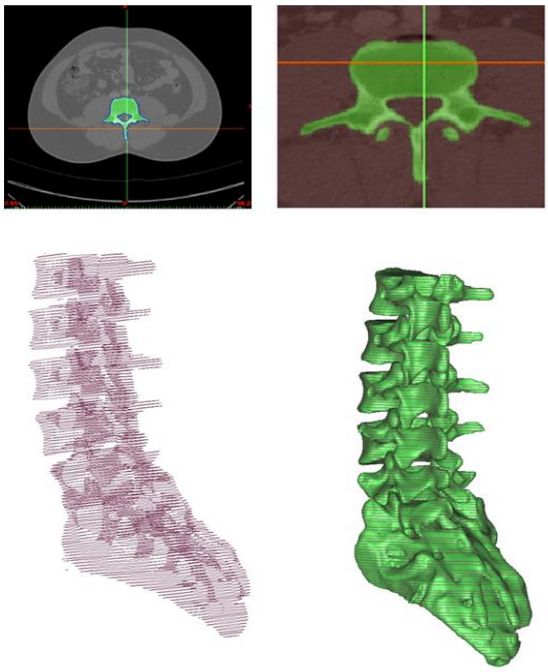


Figure 1 Figure 1: Thresholding and stacking of CT scan images to construct the 3D model of the lumbar spine (L1-S).

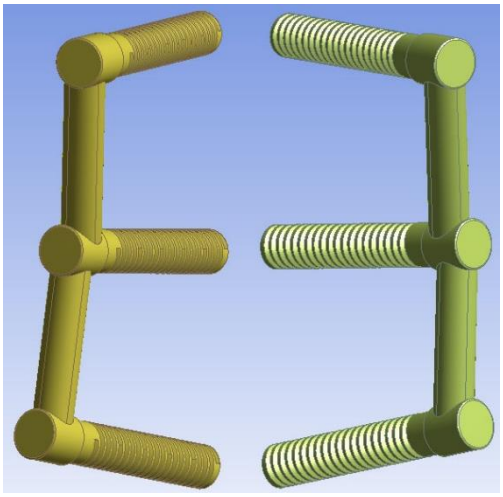


Figure 2: Solid model of L3-L5 vertebra and inter-vertebral discs along with pedicle screws.

Finite Element Modeling

Both the reconstructed geometry of the spine and the pedicle screw model have been imported into the environment of the FE analysis software ANSYS® for subsequent analysis. Considering the complex geometry and quadratic displacement behaviour of the solid model, the SOLID 187 element was used since it is well suited for meshing irregular bodies. The element is 10-noded and has 3 degrees of freedom at each node.

The mechanical property of the normal bone was considered to be linearly elastic. Young's modulus was assigned from MIMICS based on CT scan data [25,35]. A change in the elastic modulus for 'normal bone' at a step of 10% on either side has been considered to represent the bone conditions other that the normal one, (very strong, strong, weak and very weak), where an increase in the elastic modulus is considered for stronger bones and vice versa. Seven types of ligaments were modeled considering tension only node to node link elements. The ligaments are anterior longitudinal (ALL), posterior longitudinal (PLL), ligamentumflavum (LF), interspinous (ISL), supraspinous (SSL), intertransverse (ITL), and Facet capsulary (FCL). The mechanical properties of these ligaments were adapted from Biswas et al. [36].

Boundary Condition

In this study, two sides of the sacrum vertebra were constrained to move in any direction and the load was applied such that all the nodes of the topmost surface of L1 vertebra were loaded. Three cases of compressive loads (420 N, 490N and 588N) were applied in the downward direction which accounts for variations in the patient body weight (Figure 3). These are equivalent to three body weights (Light: 70 kg; Medium: 90 kg; Heavy: 120 kg), taking into account of the upper body part including trunk, head and arms [37,38].

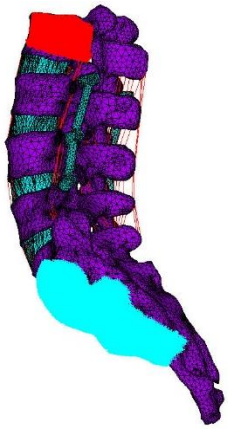


Figure 3: FE model of L1-S vertebra with ligaments and boundary conditions.

For nodal contact, the upper surface of the screw is selected as the contact surface and the screw hole in the vertebrae is selected as the target surface. The coefficient of friction was set at 0.3 for non-linear analysis [25].The FE model consisted of 301320 elements connected through 450757 nodes. A total of 60 FE simulations were performed using the parameters (i) screw diameter (5 mm and 6 mm), (ii) screw materials (stainless steel (SS) and titanium (Ti)), (iii) Bone type (very strong, strong, normal, weak and very weak) and (iv) loading condition (420 N, 490 N and 588 N).

RESULTS

The equivalent strain and its three components, viz., 1st, 2nd and 3rd principal strains at the 6 selected bone-implant interface locations (Figure 4) have been compared to that of the corresponding locations in the un-implanted spine for different sets of conditions. It has been observed that the 2nd principal strain did not vary significantly ($p<0.005$) in any of the cases

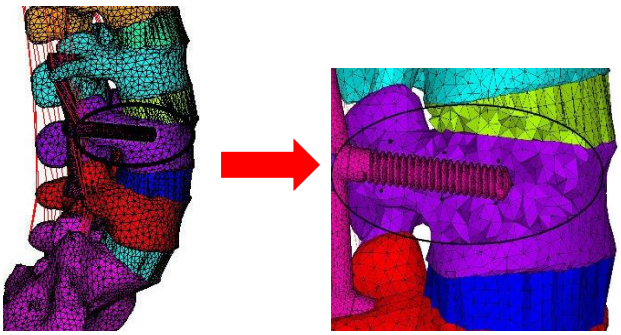


Figure 4: Six selected bone-implant interface locations for which the results will be compared to that of the corresponding locations in the un-implanted spine.

Effect of screw diameter

The changes in the equivalent strain at the selected bone-implant interface points for the 6 mm diameter implant screw did not vary significantly from that in the corresponding points in the un-implanted spine in most cases, while for the 5 mm diameter screw the variation was significant (63.33% as compared to 3.33% insignificant cases; Table 1).

The variation in the 1st principal strain at the selected bone-implant interface points was observed to depend solely on the screw diameter. For the 6 mm diameter implant screw the equivalent strain at the corresponding points did not vary significantly from that in the corresponding point in the un-implanted spine, while for the 5 mm diameter screw the variation is significant (100% as compared to 0% insignificant cases; Table 1).

The variation in the 3rd principal strain at the selected bone-implant interface points for the 6 mm diameter implant screw the equivalent strain did not vary significantly from that in the corresponding points in the un-implanted spine in most cases, while for the 5 mm diameter screw the variation was significant (40% as compared to 0% insignificant cases; Table 1).

Effect of screw material

The equivalent strain at the selected bone-implant interface points varied from the un-implanted spine for both titanium (Ti) and stainless steel (SS) as screw material (23.33% and 43.33% insignificant cases respectively; Table 1). The equivalent strain for 5 mm diameter implant varied significantly for both the implant materials (0% and 6.67% insignificant cases for Ti and SS respectively, Table 2), while for 6 mm diameter implant the deviation for Ti was more than that of SS (46.67% and 80% insignificant cases respectively, Table 2).

The screw material did not have any effect on the 1st principal strain. It varied significantly from the un-implanted spine in 50% of the cases for both Ti and SS implants, only if the screw diameter is 5 mm (Table 1 and 2).

The 3rd principal strain for at the selected bone-implant interface points varied much more for titanium (Ti) than stainless steel (SS) from the un-implanted spine (6.67% and 66.67% insignificant cases respectively; Table 1). The 3rd principal strain for 5 mm diameter implant varied significantly for both the implant materials (0% insignificant cases for both Ti and SS respectively, Table 2), while for 6 mm diameter implant the variation was more for Ti than that of SS (13.33% and 66.67% insignificant cases respectively, Table 2).

Effect of bone type

The variation in the equivalent strain at the selected bone-implant interface points were moderate for most of the bone conditions (41.67%, 33.33%, 41.67% and 33.33% insignificant cases for very strong, strong, normal and weak bones respectively, Table 1), except for very weak bone, where the variation is largest (16.67% insignificant cases, Table 1). When the variations observed for different screw diameter, it is observed that the deviation in equivalent strains from the un-implanted bone in different bone conditions arises mostly for 5 mm diameter implant (0% insignificant case for all the bone conditions, Table 2). The equivalent strain for 6 mm diameter implant varied mostly for weak and very weak bone conditions (33.33% insignificant cases for both, Table 2), followed by normal and strong bone condition (66.67% insignificant cases for both, Table 2), while the very strong bone has the least variation from the un-implanted bone (83.33% insignificant cases for both, Table 2).

The bone condition did not have any effect on the 1st principal strain. It varied significantly from the un-implanted spine in 50% of the cases for both Ti and SS implants, only if the screw diameter is 5 mm (Table 1 and 2).

The variation in the 3rd principal strain at the selected bone-implant interface points were larger for normal, weak and very weak bone condition (8.33%, 8.33% and 16.67% insignificant cases respectively, Table 1) than that of strong and very strong bone condition (25% and 41.67% insignificant cases respectively, Table 1). Most of this variation is accounted for 5 mm implant diameter (0% insignificant cases for all the bone conditions, Table 2). For 6 mm diameter implant the 3rd principal strain varied mostly for normal and weak bone condition 16.67% insignificant cases for both, Table 2), while for strong and very strong bone condition that variation was less (50% and 83.33% insignificant cases respectively, Table 2).

Effect of loading

The equivalent strain varied at the selected bone-implant interface points from that of the un-implanted bone increasingly with loading (45%, 30% and 20% insignificant cases for 420 N, 490 N and 588 N respectively, Table 2). When the variations observed for different screw diameters, most of the deviation from the corresponding un-implanted condition was found to be due to 5 mm screw diameter (0% insignificant cases for all the loadings, Table 2). The equivalent strain for 6 mm diameter implant varied moderately for 490 N and 588 N loading (50% insignificant cases for both, Table 2), while the variation is less for 420 N load (90% insignificant cases, Table 2).

The loading condition did not have any effect on the 1st principal strain. It varied significantly from the un-implanted spine in 50% of the cases for both Ti and SS implants, only if the screw diameter is 5 mm (Table 1 and 2).

The 3rd principal strain varied at the selected bone-implant interface points from that of the un-implanted bone mostly for all the loading conditions (20% insignificant cases for all the cases, Table 2). The variations due to loading condition were largely due to 5 mm screw diameter (0% insignificant cases for all the loadings, Table 2), while deviations from the corresponding un-implanted condition for 6 mm screw

diameter were moderate for all the loading conditions (20% insignificant cases for all the cases, Table 2).

Table: 2: Pattern of variations in the equivalent strain: 1st principal strain and 3rd principal strain at the 6 selected bone-implant interface locations with the strain at the corresponding locations in the un-implanted spine. Significant variations in strain ($p<0.05$) is observed in most of the cases when the screw diameter is 5 mm and this was less for 6 mm screw diameter.

Table: 1: Pattern of variations in the equivalent strain: 1st principal strain and 3rd principal strain at the 6 selected bone-implant interface locations with the strain at the corresponding locations in the un-implanted spine. Significant variations in strain ($p<0.05$) is observed in most of the cases when the screw diameter is 5 mm and this was less for 6 mm screw diameter.

Parameters		**Total case**	**Non-significant cases (%)**		
			Equiv	**1st**	**3rd**
Screw diameter (mm)	5	30	3.33	0	0
	6	30	63.33	100	40
Screw material	Ti	30	23.33	50	6.67
	SS	30	43.33	50	66.67
Bone type	Very Strong	12	41.67	50	41.67
	Strong	12	33.33	50	25
	Normal	12	41.67	50	8.33
	Weak	12	33.33	50	8.33
	Very Weak	12	16.67	50	16.67
Load (N)	420	20	45	50	20
	490	20	30	50	20
	588	20	20	50	20

Table 2: The pattern of significant variations in 1st principal strain and 3rd principal strain at 6 selected bone-implant interface locations with the strain at the corresponding locations in the un-implanted spine for two different screw diameters. It is observed that the strain variation is significant ($p>0.05$) as compared to un-implanted spine when the screw diameter is 5 mm in most of the cases, but the variation is not significant for 6 mm screw diameter.

Parameters		**Total Cases**	**Non-Significant cases (%)**					
			6 mm			**5 mm**		
			Equiv	**1st**	**3rd**	**Equiv**	**1st**	**3rd**
Screw materials	Ti	15	46.67	100	13.33	0	0	0
	SS	15	80	100	66.67	6.67	0	0
Bone type	Very Strong	6	83.33	100	83.33	0	0	0
	Strong	6	66.67	100	50	0	0	0
	Normal	6	66.67	100	16.67	16.67	0	0
	Weak	6	33.33	100	16.67	0	0	0
	Very Weak	6	33.33	100	33.33	0	0	0
Load (N)	420	10	90	100	40	0	0	0
	490	10	50	100	40	10	0	0
	588	10	50	100	40	40	0	0

Table 2: The effect of equivalent strain and its 3rd principal strain component for different bone type and loading condition. It is observed that 3rd principal strain plays an important role determining the suitability of pedicle screw implants for different bone condition and loading.

	Very strong		**Strong**		**Normal**		**Weak**		**Very Weak**		
	Eqv	**3rd**	**Eqv**	**3rd**	**Eqv**	**3rd**	**Eqv**	**3rd**	**Eqv**	**3rd**	**Metal**
420 N	0.096	0.063	0.055	0.015	0.055	0.013	0.054	0.024	0.061	0.035	Ti
	0.129	0.173	0.064	0.097	0.086	0.050	0.016	0.021	0.069	0.082	SS
490 N	0.049	0.029	0.043	0.031	0.034	0.017	0.055	0.023	0.018	0.004	Ti

	0.081	0.119	0.075	0.122	0.053	0.060	0.058	0.053	0.018	0.014	SS
588 N	0.098	0.053	0.037	0.026	0.039	0.003	0.046	0.013	0.032	0.022	Ti
	0.112	0.132	0.053	0.099	0.052	0.009	0.058	0.040	0.040	0.079	SS

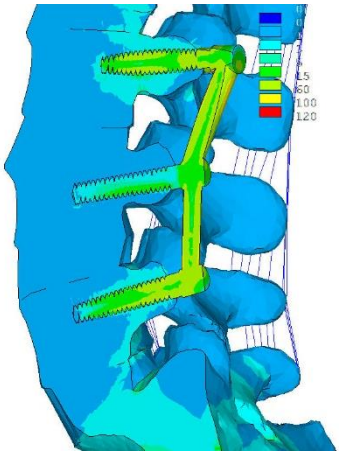
DISCUSSION

The present computational study examines the roles of different implant parameters (screw diameter and screw material) and physiological parameters (bone condition and loading) on the state of strain at the bone-implant interface. In this study the state of strain is measured in terms of the equivalent strain and its components, viz., 1st, 2nd and 3rd principal strain. The desirable condition for a suitable implant is hypothesized to be the least variation of the strains in the bone-implant interface in comparison to the natural bone.

One of the limitations of the study is the selection of 6 points around the implant to estimate the strain environment in the bone-implant interface. Instead, if different zones were chosen around the implant, each containing multiple points, the analysis would have been more robust, and a spatial variation in the interface strain could have also been analyzed. Other limitations are not modeling the nucleus polpusus in the disc which would reflect realistic deformation of the disc. Also the screw threads were not modeled which would replicate the actual implant-bone interface. Muscle forces were also not considered.

To validate the FE model, the results can be compared with the findings of Adams et al. [39]. While investigating the distribution of compressive stress within cadaver intervertebral disc using pressure transducer, they have found that the L3-L4 disc stress to vary from 1.7 to 2.5 MPa under 1 kN load. Whereas, we observed the L3-L4 disc stress to vary from 1 to 2 MPa for the un-implanted FE models under a compressive load of 420 N, 490 N and 588 N. The stresses generated were comparable, while the difference in the stress was mainly due to the lower applied load in comparison to the study of Adams et al. [39]. Also, the material properties assigned to our FE model may not match with the properties of their cadaveric vertebrae.

Chen et al. found a maximum stress of 200 MPa in the neck portion of the screw for the contact type interface with a 30 mm long screw under a load of 400 N [25]. We have obtained the maximum screw stress of 120 MPa for 6mm diameter in the similar location under 420 N load (Figure 5 and Figure 6). This wide gap in the result was due to using of one single vertebra without the modeling the disc and constraining the screw at its end [25]. It is observed that for an increase in compressive load, stress increases accordingly on both the cancellous and cortical part of the bone. The stress contours inside vertebrae did not vary with bone condition.



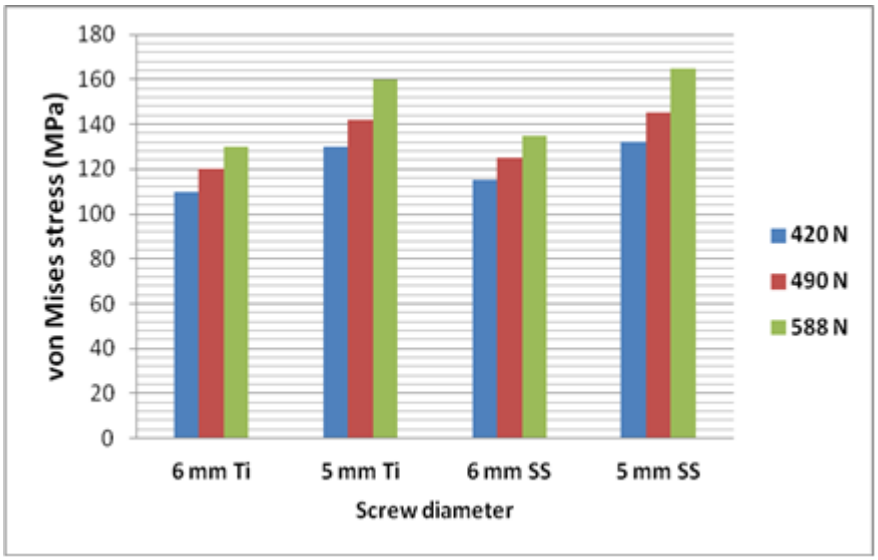


Figure 5: Stress contour plot (MPa) for the 420 N load case

.**Figure 6:** Variation of Stress of the pedicle screw for normal bone condition.

It was observed that the screw material is the primary determining factor of the strain environment at the bone-implant interface. Two different screw diameters (5 mm and 6 mm) were used in the present study. Among the two implants, the implant with 5 mm diameter altered the strain environment significantly from the corresponding un-implanted conditions in all the cases. The equivalent strain varied significantly from the un-implanted conditions, only in 3.33% cases the deviation being statistically insignificant, where the variations in the 1st and 3rd principal component strains were contributed most (0% statistically insignificant cases). So the screw diameter of 5 mm or less screw diameter is not suitable for pedicle screw for the lumbar region.

On the other hand, the pedicle screw implant with 6 mm diameter generated strain environment that deviated less from that of the corresponding un-implanted cases (66.67%, 100% and 40% insignificant cases for equivalent strain, 1st principal strain and 3rd principal strain respectively, Table 1). Stainless steel gives lesser deviation as the pedicle screw material for the 6 mm diameter implant as compared to titanium (80% insignificant cases for stainless steel as compared to 46.67% for the titanium, Table 2). Titanium implant maintains the strain environment.

CONCLUSIONS

The diameter of the screw was found to the most dominant factor determining the mechanical environment at the bone-implant interface. For very strong bone condition both the stainless steel and titanium implant of 6 mm diameter performed satisfactorily (83.33% insignificant cases, Table 2) for different loading conditions, while 5 mm diameter screw altered the mechanical environment significantly. In the case of strong, normal and weak bones, stainless steel implant performed better, while titanium implant performed satisfactorily only for lighter body weight (420 N loading). The weak bone condition gives the least satisfactory performance in terms of the strain environment. The variation in the 3rd principal strain played an important role in deciding the suitability of the implants for different bone conditions (Table 3). The knowledge acquired for the alteration of the mechanical environment at the bone-implant interface from the pre-operative condition can shed light on the failure mechanism of the pedicle screws and their proposed design.

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THE EFFECT OF TROGLITAZONE ON C- REACTIVE PROTEIN IN INDIVIDUALS WITH PREDIABETES: DATA FROM THE DIABETES PREVENTION PROGRAM

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ABSTRACT

Background Inflammation has been clearly recognized as a factor in the development and progression of multiple cardiovascular (CVD) diseases especially atherosclerosis and coronary heart diseases (CHD). The importance of C-reactive protein (CRP) levels in the prediction of future cardiovascular evens has been underscored in multiple studies. Limited studies investigated the effect of troglitazone on inflammatory markers including CRP levels in individuals with diabetes mellitus (DM), very few analyzed this effect on individuals with prediabetes. The Diabetes Prevention Program (DPP) is a large randomized trial which evaluated the effect of different interventions including metformin and intensive lifestyle modifications (ILS) compared to placebo on the prevention of DM. In this study we evaluated the effect of troglitazone on CRP levels in a subgroup of the DPP population. **Materials and Methods:** A subgroup of a total of 3,171 subjects from the original DPP study population was selected for this analysis. The effect of troglitazone on inflammatory markers was measured by analyzing its effects on CRP levels at baseline and at 12 months and compared it to the other three interventions (ILS, metformin, and placebo). **Results:** Overall, the median percentage change in CRP at 1 year from baseline was -20.00 percent in the troglitazone arm (p <0.001 for all between group analysis: troglitazone vs. lifestyle, troglitazone vs. metformin, & troglitazone vs. placebo). Due to the differences in CRP levels with sex where women usually report higher baseline CRP values than men, the effect of the interventions was also reported by sex. In women, the median percent change in CRP at 1 year from baseline was -27.08 percent in the troglitazone arm (troglitazone vs. lifestyle: P = 0.854; troglitazone vs. placebo: P <0.001; and troglitazone vs. metformin: P=0.001). In men, troglitazone reported a median percentage change of -4.64 percent in CRP levels (troglitazone vs. lifestyle: P = 0.012; troglitazone vs. placebo: P = 0.012; and troglitazone vs. metformin: P = 0.33). **Conclusion** This analysis showed a significant decrease in CRP levels due to troglitazone treatment for 1 year, the reduction was significantly greater that what is produced by metformin.

Keywords:Troglitazone, C-reactive peptide, CRP, coronary heart disease prediabetes, fibrinogen, tPA

INTRODUCTION

Prediabetes constitutes a major health problem due to its common presence in high proportion of apparently healthy populations leading to considerable health problems associated with overt diabetes (Ligthart et al., 2016). Prediabetes is a condition typically defined as glycemic parameters above normal but below diagnostic criteria for diabetes, according to the centers for disease control and prevention (CDC) 2014 reports, prediabetes affects 37 percent of United States adults aged 20 years or older, the estimates rises to 51 percent in individuals 65 years or older (CDC, 2014). After adjusting for age differences, CDC reported that the percentage of adults aged 20 years or above affected by prediabetes was similar across ethnic lines, about 35 percent for Hispanic whites, 39 percent for non-Hispanic blacks, and 38 percent for Hispanics (CDC, 2014).

Studies have highlighted the role that inflammatory process plays in the pathogenesis of CVD. C-reactive protein (CRP) is a well-established element of the inflammatory response, blood levels of CRP constitute sensitive marker of the inflammatory response to infection and tissue damage. CRP levels have been considered a powerful predictor of the development of CVD as well as in the development and progression of atherosclerosis. Furthermore, elevated CRP levels have been seen in individuals at

increased risk for cardiovascular morbidity and mortality and also recognized as a factor in the development and progression of multiple CVDs especially atherosclerosis and CHDs (Willerson et al., 2004; & Laveti et al., 2013). The association between inflammatory and coagulation markers including CRP and fibrinogen is well documented in the literature (Han et al., 2002; Festa & D'Agostino et al., 2000; & Ndumele et al., 2006). Coronary incidents have been shown to increase significantly with an elevated high base line CRP levels. In fact, large studies suggested the strength of the predictive value of CRP for future cardiovascular events to transcend that of conventional markers such as LDL, a classic maker for the prediction of cardiovascular risks (Ridker, Rifai, & Rose et al., 2002; Blake, Ridker, 2003; Ridker, Bassuk, &Toth, 2003). Several reports have suggested a more important role of CRP, beyond its role as only a marker of vascular and cardiovascular inflammation. CRP was considered a mediator in the pathogenesis of atherosclerosis and other CVDs (Blake, Ridker, 2003; Ridker et al., 2003; Ballou et al., 1992; & Pasceri et al., 2000), in addition to its role as a powerful predictive and prognostic biomarker for CVDs (Donald et al., 2006; Folsom et al., 2006; & Packard et al., 2008).

The effect of rosiglitazone and pioglitazone on reducing CRP levels in individuals with type 2 diabetes is well documented in the literature (Pfutzner et al., 2005; Stocker et al., 2007; Agarwal 2006; Davidson et al., 2007; Hartemann-Heurtier et al., 2009; Forst et al., 2008; Marfella et al., 2006; Ogasawara et al., 2009; & Yu et al., 2007). Recent metanalyses evaluated the effect of the two agents on both diabetics and non-diabetic individuals and showed a remarkable decrease in CRP levels (Qayyum & Adomaityte, 2006; Zhao Y. et al., 2010; & Chen et al, 2015). Limited numbers of studies were specifically designed to examine the effects of rosiglitazone and pioglitazone on CRP levels in prediabetic individuals were found (Mohanty et al., 2004; Mizoguchi et al., 2011). In subjects with diabetes, troglitazone was found to lower CRP levels in limited number of small studies (van Tits et al., 2005; Chu et al., 2002). To date, no known studies were found which specifically designed to investigate the effect of troglitazone on CRP, fibrinogen and tPA levels in prediabetic individuals.

This study used the Diabetes Prevention Program Research Group (DPP) study data set to investigate the relationship between troglitazone and CRP, tPA and fibrinogen and insulin resistance in a prediabetic population.

RESEARCH DESIGN AND METHODS

The original DPP was a 27-center randomized clinical trial to determine whether lifestyle modification or select pharmacological therapy would prevent or delay the onset of diabetes in individuals with IGT. The protocol for DPP has been previously documented in previous publications which included the study design, recruitment and measurement methods, and main characteristics of the overall population (Diabetes Prevention Program Research Group (DPP), 1999; and DPP, 2000). Inclusion criteria were age ≥25 years, fasting serum glucose (FSG) levels between 5.6–7.7 mmol/l before June 1997 and between 5.3–6.9 mmol/l after that date, and BMI of ≥24 kg/m2 (DPP Research Group, 1999). Our study utilized the data from the DPP. The original study by the DPP group included a total of 3819 prediabetic individuals. Out of the total participants, 585 were assigned to troglitazone.

Our analysis selected a subgroup (n= 3,171) from the original DPP population to analyze tPA and fibrinogen. The total number of participants in this subgroup was determined based on the number of participants with available values for these markers at the end of 1 year from randomization. A total of 291 were in the troglitazone intervention arm (400mg every day), the rest were in the other three interventions including placebo, lifestyle, and metformin (850mg twice a day). The troglitazone arm was discontinued in June 1998 (DPP Research Group, 1999). In this report, we examine the effects of troglitazone on CRP. We also evaluate the effect of changes in selected measures, particularly changes in measures of insulin resistance and glycemia, obesity, and lipid profile; on the changes in this inflammatory marker.

Statistical Analysis

Descriptive analysis at baseline of all variables were generated for each of the four treatment arms. Analysis was performed and presented using SPSS software. Baseline characteristics were reported as means and standard errors. Paired t-tests were conducted to analyze the main effects of troglitazone on CRP. Partial Spearman correlation coefficients and accompanied P values were used to summarize the association between the main dependent variables at baseline with selected independent variables. Partial Spearman correlation was also performed to summarize the association between the changes in CRP levels at 1 year from baseline to understand whether the greater changes in this variable was affected by changes in weight, waist circumference, insulin resistance measures, or hemoglobin A1c (HbA1c). The correlation analysis was shown as unadjusted, followed by adjusted analysis controlling for age, sex, and ethnicity, in attempt to adjust for

these potential confounders. Correlation analysis was performed only on the troglitazone intervention arm.

Multiple linear regression was performed to examine whether the changes in CRP levels due to treatment with troglitazone were explained by a weight and waist circumference changes, and changes in measures of glycemia and insulin resistance. The changes from baseline for CRP levels were shown as mean changes and SE, they were also summarized as the percent change from baseline. The percentage is calculated as [(value at 1 year – baseline value) x 100/baseline value] (Haffner et al, 2005). Median percent changes for CRP levels were tested using nonparametric Wilcoxon's test. Fasting insulin levels along with a pretested model were both used as measures for insulin resistance. The homeostasis model assessment for insulin resistance (HOMA- IR). HOMA- IR was calculated using the following formula (Matthews et al., 1985): HOMA- IR= {fasting insulin μU/ml × fasting glucose (mmol/l)} /22.5

RESULTS

As indicated in Table 1, baseline characteristics for the subgroup selected for the analysis of inflammatory markers includes values for CRP were presented by intervention groups including placebo (n = 956), troglitazone (n = 291), metformin (n = 962), and ILS (n = 962). Values for inflammatory markers were similar in all interventions, women exhibited higher mean values for CRP than men (0.71 for women and 0.32 for men). While the mean values for waist circumferences at run-in visits were the same across interventions, the mean weights at 6 months were lower in the ILs group compared to the average of the means of the three other interventions (87 ± 0.66 vs. 93 ±0.87) (note: weights were taken at 6 months from randomization; waist circumferences were measured at run-in visits which are the visits scheduled after screening visits, prior to randomization). African Americans also showed higher baseline values for CRP compared to males and compared to the overall average.

Table 1 Descriptive baseline characteristics (means ± standard errors) in the inflammatory subgroup displayed by interventions

	Placebo	**Troglitazone**	**Metformin**	**Lifestyle**
n	**956**	**291**	**962**	**962**
Weight*	94 ± 0.72	95±1.23	91±0.67	87±0.66
W.Cir **	105 ± 0.47	105±0.83	105±0.48	105±0.49
HbA1c	5.9 ± 0.02	5.8±0.03	5.9±0.02	5.9±0.02
HOMA-IR	7.0±0.13	6.8±0.23	7.2±0.13	7.0±0.14
Fasting insulin (μu/ml)	26.4 ± 0.47	25.0±0.82	27.0±0.48	26.5±0.5
Fasting glucose (mg/dl)	107.4 ± 0.25	109.0 ± 0.46	107.3 ± 0.25	107.0 ± 0.24
Triglycerides mg/dL	167.3 ± 2.97	161.9±6.2	159.1±2.91	163.0±3.1
CLDL mg/dL	125.1±1.07	122.4±1.83	125.1±1.04	126.2±1.05
CRP (mg/dL) overall	0.59 ± 0.02	0.58 ± 0.05	0.58 ± 0.02	0.58 ± 0.02
Males n	311	110	345	318
CRP	0.31 ± 0.02	0.37 ± 0.08	0.31 ± 0.02	0.33 ± 0.03
females n	657	183	620	650
CRP	0.74 ± 0.03	0.72 ± 0.05	0.73 ± 0.03	0.70 ± 0.03
AA n	203	51	209	186
CRP	0.71 ± 0.10	0.63 ± 0.10	0.69 ± 0.10	0.62 ± 0.10

* measured at 6 months from randomization ** measured at run-in visits W Cir= Waist circumference

Table 2 displays baseline correlations for selected variables with CRP, fibrinogen, and tPA in the inflammatory subgroup in the troglitazone intervention, Table 3 presents these baseline correlations values after adjusting for age, sex, and ethnicity. As shown in Table 2, both weight at 6 months and waist circumference at run-in visit along with fasting glucose and HOMA-IR were all significantly and positivity correlated with all three inflammatory and coagulation markers. Mean HbA1c values were also significantly correlated with fibrinogen and tPA, the correlation did not reach statistical significance with CRP. Fibrinogen and CRP showed a strong and significant correlation (r =

0.53, *P* <0.001). Table 3 provides the outcomes for the partial correlation analysis after adjusting for age, sex, and ethnicity. The adjusted correlational analysis showed similar results as the unadjusted analysis with slight decrease in the correlation coefficient values. Table 4 displays the mean changes from baseline by treatment group for the overall DPP population.

Table 4 illustrates the changes in the mean values of selected anthropometric and metabolic variables resulting from the effect of the different interventions. It appeared that troglitazone significantly reduced the levels of fasting insulin and HOMA-IR at 1 year from baseline, these values reflect on the magnitude of the effect of this agent on insulin resistance.

Troglitazone showed greater reductions compared to metformin in the mean values of both fasting glucose levels (-4.06 vs -3.60, respectively, both P <0.001) and HOMA-IR (-1.22 vs –1.19, respectively, both P <0.001), while ILS exceeded these values. On lipid profile measures, all four interventions significantly reduced triglycerides levels, troglitazone resulted in the highest mean reductions followed by ILS (-27.28 ± 3.88 vs. -25.78 ± 2.29, respectively, both P <0.01), while metformin exhibited the lowest mean reductions (-5.34 ± 2.16, P = 0.01). Troglitazone did increase the C-LDL levels, different from all other interventions which showed a decrease, however this change was statistically insignificant.

Figure 1 shows the mean changes in CRP, all interventions demonstrated statistically significant decrease in CRP levels (P < 0.001), except placebo which failed to reach a statistical significance. Overall, the median percentage change in CRP at 1 year from baseline was -20.00 percent in the troglitazone arm, shown in Figure 2 (p <0.001 for all between group analysis: troglitazone vs. lifestyle, troglitazone vs. metformin, & troglitazone vs. placebo). Due to the differences in CRP levels with sex where women usually report higher baseline CRP values than men, the effect of the interventions was also reported by sex. In women (Figure 4), the median percent change in CRP at 1 year from baseline was -27.08 percent in the troglitazone arm (troglitazone vs. lifestyle: P = 0.854; troglitazone vs. placebo: P <0.001; and troglitazone vs. metformin: P=0.001). In men (Figure 3), troglitazone reported a median percentage change of -4.64 percent in CRP levels (troglitazone vs. lifestyle: P = 0.012; troglitazone vs. placebo: P = 0.012; and troglitazone vs. metformin: P = 0.33).

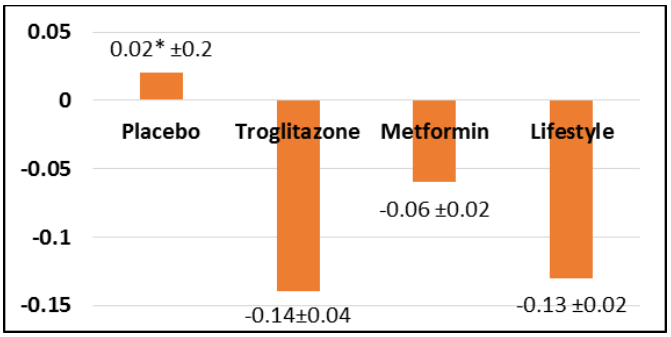

Figure 1 presents the mean changes in CRP levels after 1 year of follow-up.*statistically non-significant (P value is <0.05 for all other values)

Table 2 Partial spearman correlation coefficients of baseline values (P-values) of CRP, tPA, and fibrinogen with selected metabolic and anthropometric variables (troglitazone arm) in the inflammatory subgroup

	CRP	tPA	Fibr	W Cir*	FSG	FSI	HOMA-IR	HBA1c	Weight**	TRIG	CHO	CLDL	UCRE	UALB	SCr	eGFR
CRP		0.04 (NS)	0.53 (<0.001)	0.25 (<0.001)	0.22 (<0.001)	0.05 (NS)	0.22 (<0.001)	0.07 (NS)	0.27 (<0.001)	0.03 (NS)	0.02 (NS)	0.10 (NS)	0.10 (NS)	0.17 (0.004)	0.16 (0.009)	0.05 (NS)
tPA	0.04 (NS)		0.13 (0.04)	0.20 (0.001)	0.22 (<0.001)	0.16 (0.006)	0.23 (<0.001)	0.12 (0.04)	0.22 (<0.001)	0.13 (0.03)	0.4 (NS)	0.04 (NS)	0.09 (NS)	0.14 (0.2)	0.01 (NS)	0.01 (NS)
Fibr	0.53 (<0.001)	0.13 (0.04)		0.31 (<0.001)	0.16 (0.006)	0.11 (NS)	0.18 (0.003)	0.22 (<0.001)	0.26 (<0.001)	-0.03 (NS)	0.08 (NS)	0.10 (NS)	0.02 (NS)	0.09 (NS)	0.16 (0.007)	0.01 (NS)

CRP= C-Reactive Protein (mg/dL), Fibr = Fibrinogen, tPA = Tissue Plasminogen Activator (ng/dL), W Cir = Waist Circumference (cm), FSG= Fasting Serum Glucose (mg/dL), FSI = Fasting Serum Insulin (micro units/mL), HOMA-IR = Homeostasis Model Assessment for Insulin Resistance, HBA1c = Glycosylated Hemoglobin Type A1C (%), TRIG = Triglycerides (mg/dL), CHO = Total Cholesterol (mg/dL), CLDL = Low Density Lipoprotein Subfraction (mg/dL), UALB = Urine Albumin (mg/dL), SCr = Serum Creatinine (mg/dL), eGFR = Estimated Glomerular Filtration Rate * waist Circumference was measured at run-in visits per DPP study ** weight was measured at 6 months visits

Table 3 Partial spearman correlation coefficient of baseline (P-values) for CRP, tPA, and fibrinogen with selected metabolic and anthropometric variables in the inflammatory subgroup adjusted for age, sex, and ethnicity

	CRP	tPA	Fibr	W Cir*	FSG	FSI	HOMA-IR	HBA1c	Weight**	TRIG	CHOL	CLDL	UCRE	UALB	CREA	eGFR
CRP		0.07 NS)	0.49 (<0.001)	0.29 (<0.001)	0.18 (0.003)	0.05 (NS)	0.18 (0.003)	0.09 (NS)	0.29 (<0.001)	0.06 (NS)	0.003 (NS)	-0.001 (NS)	0.12 (0.05)	0.21 (<0.001)	0.02 (NS)	0.03 (NS)
tPA	0.07 (NS)		0.17 (0.005)	0.21 (<0.001)	0.24 (<0.001)	0.16 (<0.001)	0.26 (<0.001)	0.12 (0.05)	0.25 (<0.001)	0.13 (0.04)	0.17 (0.004)	0.03 (NS)	0.08 (NS)	0.13 (0.03)	0.07 (NS)	-0.01 (NS)
Fibr	0.49 (<0.001)	0.17 (0.005)		0.35 (<0.001)	0.14 (0.02)	0.12 (0.05)	0.15 (0.01)	0.25 (<0.001)	0.28 (<0.001)	0.003 (NS)	0.08 (NS)	0.11 (NS)	0.06 (NS)	0.11 (NS)	0.01 (NS)	0.02 (NS)

CRP = C-Reactive Protein (mg/dL), Fibr = Fibrinogen, tPA = Tissue Plasminogen Activator (ng/dL), W Cir = Waist Circumference (cm), FSG= Fasting Serum Glucose (mg/dL), FSI = Fasting Serum Insulin (micro units/mL), HOMA-IR = Homeostasis Model Assessment for Insulin Resistance, HBA1c = Glycosylated Hemoglobin Type A1C (%), TRIG = Triglycerides (mg/dL), CHOL = Total Cholesterol (mg/dL), CLDL = Low Density Lipoprotein Subfraction (mg/dL), UALB = Urine Albumin (mg/dL), SCr = Serum Creatinine (mg/dL), eGFR = Estimated Glomerular Filtration Rate * waist Circumference was measured at run-in visits per DPP study ** weight was measured at 6 months visits

Troglitazone also showed significant reduction in triglycerides levels exceeding ILS and metformin in this same DPP population (Mokhtar et al., 2017). A close association between CRP and coagulation markers such as tPA and fibrinogen in the DPP population was also documented in that same previous study (Mokhtar et al., 2017). Similar results underscoring the close association between CRP and fibrinogen was shown in previous investigations, these studies suggested similar association between these two variables and with the component of insulin resistance (Festa & D'Agostino et al., 2000; Han et al., 2002; Juhan-Vague et al., 1993; Festa & D'Agostino et al., 2000; & Ndumele et al., 2006).

Table 5 and table 6 presents the results from the partial spearman correlational analyses between the inflammatory and coagulation markers in relation to the changes in HBA1c from baseline to 1 year in each intervention, before and after adjustment for age, sex, and ethnicity.

These results revelaed an inverse relationship between changes in HBA1c and changes in CRP along with other coagulation markers such as fibrinogen and tPA. Even though the magnitude of the correlation coefficients was moderate, and statistically nonsignificant, it may still suggest an association between the teggects of TZDs on glycemic control and their effects on inflammatory and coagulation markers.

Based on the observed results, it seemed like the decreases in the levels of CRP and fibrinogen neither correlated with improvement shown by troglitazone in insulin sensitivity and glycemia, nor with the changes in weight and waist circumference, although the changes in the two latter variables did not meet a statistical significance.

Multiple linear regression analysis was performed to explain whether the effect of troglitazone on CRP could possibly be explained by changes in selected demographic, anthropometric, or metabolic variables. Results from our regression analysis produced statistically nonsignificant models. Therefore, we may assume that our finding regarding the effect of troglitazone on CRP were not affected by changes in these selected metabolic and anthropometric measures.

Table 4 Mean changes ± SE (P-values) of selected variables from baseline for the overall DPP population by interventions

change	Placebo	Troglitazone	Metformin	Lifestyle
n	962	527	958	961
Weight[a]	0.34 ± 1.01 (ns)	***3.22 ± 2.33 (ns)	0.22 ± 0.96 (ns)	-0.28 ± 0.9 (ns)
W Cir[b]	-0.97 ± 0.71 (ns)	****-1.61 ± 1.71 (ns)	-2.12 ± 0.67	-6.8 ± 0.71
HbA1c	0.09 ± 0.01	0.02 ± 0.02 (ns)	0.01 ± 0.02 (ns)	-0.09 ± 0.01
HOMA-IR	0.35 ± 0.15 (0.02)	-1.22 ± 0.16	-1.19 ± 0.12	-1.58 ± 0.15
Fasting insulin (µu/ml)	0.88 ± 0.53 (ns)	-4.06 ± 0.58	-3.60 ± 0.41	-5.24 ± 0.53
Fasting glucose (mg/dl)	0.28 ± 0.44 (ns)	-4.10 ± 0.52	-4.52 ± 0.34	-5.29 ± 0.35
Triglycerides mg/dL	-8.75 ± 2.34	-27.28 ± 3.88	-5.34 ± 2.16 (0.01)	-25.78 ± 2.29
CLDL mg/dL	-1.97 ± 0.78	1.10 ± 1.07 (ns)	-4.52 ± 0.75	-6.13 ± 0.75
cholesterol	-3.70 ± 0.85	-2.61 ± 1.2 (0.03)	-5.0 ± 0.82	-9.84 ± 0.83

All p values are < 0.01 except when indicated in parenthesis [a] weight differences at 1 year from 6 months after randomization
[b] waist circumference was taken at run-in visits (visits taking place after screening visit and prior to randomization
n number of participants, * n = 165, ** n = 285, *** n=218, and **** n = 274 W Cir= Waist Circumference

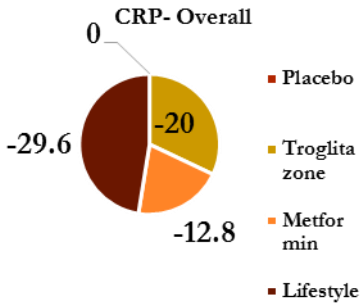

Figure 2 Median percent change in overall CRP levels after 1 year of treatment with troglitazone

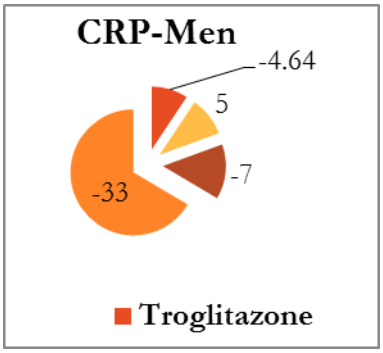

Figure 3 Median percent change in overall CRP levels in men after 1 year of treatment with troglitazone

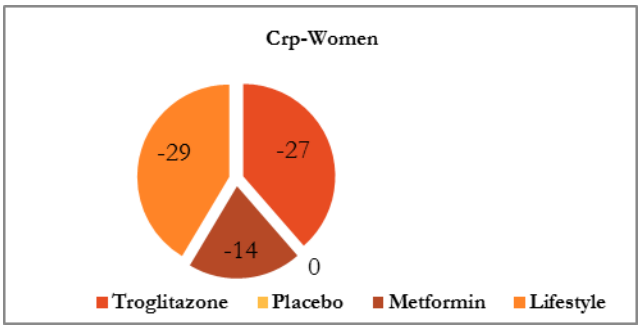

Figure 4 Median percent change in overall CRP levels in women after 1 year of treatment with troglitazone

(Data for placebo, metformin and lifestyle were already published by Haffner et al., 2005, and were reproduced in this analysis).

Table 5 Partial spearman collations of year 1 changes from baseline (P-values) in the inflammatory and coagulation markers with metabolic and renal variables (troglitazone intervention) in the inflammatory subgroup

	CRP	**tPA**	**Fibr**	**W Cir***	**FSG**	**FSI**	**HOMA-IR**	**HBA1c**	**Weight****	**TRIG**	**CLDL**	**CREA**	**eGFR**	**ACR**
CRP	1.0	-0.05 (NS)	0.50 (0.04)	0.04 (NS)	-0.11 (NS)	-0.31 (NS)	-0.12 (NS)	-0.22 (NS)	0.14 (NS)	-0.5 (NS)	0.5 (NS)	0.10 (NS)	-0.07 (NS)	0.5 (0.05)
tPA	-0.05 (NS)	1.0	-0.13 (NS)	0.41 (NS)	0.52 (0.05)	0.62 (0.01)	0.55 (0.04)	0.34 (NS)	0.53 (0.04)	0.08 (NS)	-0.19 NS)	0.54 (0.04)	0.42 (NS)	0.06 NS)
Fibr	0.53 (0.04)	-0.13 NS)	1.0	-0.09 (NS)	-0.23 (NS)	-0.31 (NS)	-0.23 (NS)	0.34 NS)	0.09 (NS)	-0.65 (0.009)	0.3 (NS)	0.19 (NS)	0.06 (NS)	0.66 (0.008)

CRP = C-Reactive Protein (mg/dL), Fibr= Fibrinogen, tPA = Tissue Plasminogen Activator (ng/dL), W Cir = Waist Circumference (cm), FSG= Fasting Serum Glucose (mg/dL), FSI = Fasting Serum Insulin (micro units/mL), HOMA-IR = Homeostasis Model Assessment for Insulin Resistance, HBA1c = Glycosylated Hemoglobin Type A1C (%), TRIG = Triglycerides (mg/dL), CLDL = Low Density Lipoprotein Subfraction (mg/dL), UALB = Urine Albumin (mg/dL), SCr = Serum Creatinine (mg/dL)
* waist Circumference was measured at run-in visits per DPP study ** weight was measured at 6 months visits per DPP study

Table 6 Partial spearman correlations of year 1 changes from baseline (P-values) in the inflammatory and coagulation markers with metabolic and renal variables (troglitazone intervention) in the inflammatory subgroup adjusted for age, sex, and ethnicity

	CRP	tPA	Fibr	W Cir*	FSG	FSI	HOMA-IR	HBA1c	Weight*	TRIG	CLDL	SCr	eGFR	ACR
CRP	1.0	0.04 (NS)	0.3 (NS)	-0.12 (NS)	-.22 (NS)	-0.36 (NS)	-0.27 (NS)	-0.33 (NS)	0.07 (NS)	-0.4 (NS)	0.4 (NS)	-0.13 (NS)	0.27 (NS)	0.59 (0.04)
tPA	0.04 (NS)	1.0	-0.01 (NS)	0.23 (NS)	0.49 (NS)	0.58 (0.05)	0.5 (NS)	0.49 (NS)	0.35 (NS)	0.04 (NS)	0.49 (NS)	-0.69 (0.01)	0.58 (0.05)	0.04 (NS)
Fibr	0.3 (NS)	-0.01 (NS)	1.0	-0.17 (NS)	-.33 (NS)	-0.30 (NS)	-0.33 (NS)	.037 (NS)	0.12 (NS)	-0.59 (0.04)	0.14 (NS)	0.6 (NS)	0.27 (NS)	0.75 (0.005)

CRP = C-Reactive Protein (mg/dL), Fibr= Fibrinogen, tPA = Tissue Plasminogen Activator (ng/dL), W Cir = Waist Circumference (cm), FSG= Fasting Serum Glucose (mg/dL), FSI = Fasting Serum Insulin (micro units/mL), HOMA-IR = Homeostasis Model Assessment - for Insulin Resistance, HBA1c = Glycosylated Hemoglobin Type A1C (%), TRIG = Triglycerides (mg/dL), CLDL = Low Density -Lipoprotein Subfraction (mg/dL), UALB = Urine Albumin (mg/dL), SCr = Serum Creatinine (mg/dL)

* waist Circumference was measured at run-in visits per DPP study

** weight was measured at 6 months visits per DPP study

DISCUSSION

This current study demonstrated a significant decrease in CRP levels due to treatment with troglitazone. The decrease in CRP levels demonstrated by our analysis was significantly greater than the effect of metformin or placebo as presented in the previous DPP study which analyzed the effect of ILS, metformin, and placebo on CRP levels in the same population studied in this present analysis, with the exclusion of troglitazone intervention. Since CRP levels differ according to gender, women always reported higher baseline CRP than men (Festa et al., 2000) in conformity with our analysis. Therefore, we analyzed the effect of troglitazone on CRP by sex in addition to the overall analysis.

Results from our current analysis showed that troglitazone produced a 27.8 percent decrease in the median levels of CRP in females and 14.6 percent in males. Comparable results were reported by Haffner et al. on three other interventions included in the DPP study. Haffner et al. reported a 29 percent and 14 percent reduction in CRP levels for females in ILS and metformin, respectively, and 33 and 7 percent for male in ILS and metformin, respectively. Clearly troglitazone exceeded the results demonstrated by metformin, the only other available insulin sensitizer.

Although CRP serum levels can increase multiple times in response to acute inflammation (Pepys & Baltz, 1983), these levels are maintained at specific range in healthy individuals (Shine, de Beer, & Pepys, 1981). CRP has been considered a sensitive and stable marker for subclinical inflammatory state (Ridker, 2001). Slight elevations in CRP levels may be considered clinically significant; in fact, levels from 0.3 to 1.0 mg/L are clinically considered true elevation (Slade et al., 2003; Kushner et al., 2006; Giles et al., 2008; & Vuong et al., 2014). Accordingly, results from our analysis, although much smaller in value compared to some previous research, may be regarded as clinically significant.

Since the changes produced by the treatment with troglitazone on CRP differ according to the sex and ethnicity of the participants. Therefore, we were able to suggest that sex and ethnicity play a role in the main effect of this agent. The outcome from our analysis regarding CRP agreed with multiple other findings from previous research. In a meta-analysis by Qayyum & Adomaityte, TZDs were shown to reduce CRP levels in both diabetic and nondiabetic individuals, the mean reduction in CRP in diabetic individuals surpassed which is shown in nondiabetics. This meta-analysis included much more diabetic subjects than others. Additionally, the analysis did not specify the percentage of prediabetics among those nondiabetics included (Qayyum & Adomaityte, 2006). Results from a recent meta-analysis, Zhao et al. showed similar results to ours as well. Unlike the previous analysis by Qayyum & Adomaityte, Zhao et al. demonstrated a more pronounced reduction in CRP levels in nondiabetics than in diabetic populations (Zhao et al., 2010). Both meta-analyses failed to specifically identify how many of the nondiabetic individuals have prediabetes. A more recent meta-analysis by

Chen et al. published in 2015 which included only patients with diabetes. Still, results from this analysis showed significant reduction by TZDs on CRP levels (Chen et al., 2015). Limited number of studies, all included small number of participants, specifically examined the effect of TZDs on inflammatory markers in prediabetic patients. Mizoguchi et al. studied the effect of treatment with pioglitazone for four months in 56 individuals with impaired glucose tolerance or with diabetes, pioglitazone treated subjects showed a reduction in CRP levels by 0.27 mg/L, which accounted for more than thirty percent reduction from baseline (Mizoguchi et al., 2011). These results demonstrated a much larger reduction when compared to results from our current analysis.

The lack of large studies specifically designed to analyze the effect of TZDs on CRP levels, as in the previously discussed meta-analyses, may give our analysis a distinctive attribute and may give the opportunity for new research to explore this gap, especially since CRP levels were reported to be closely associated with CVD risks (Liuzzo et al. 1994; Thompson et al, 1995; Haverkate et al, 1997; Willerson et al, 2004; Danesh et al. 2000; Ridker et al, 2002; Blake et al., 2003; & Ridker et al., 2003). Several studies have also elucidated on the importance of CRP in the predictions of future CV events and as a prognostic marker. The favorable effects shown by the TZDs on CRP levels, and eventually on the overall cardiovascular risks have been suggested to be independent of their action on glycemia and insulin sensitivity (van Tits et al., 2005). Two major trials, the REVERAL and the PROVE-IT, examined the effect of agents from different therapeutic classes on clinical cardiovascular outcomes through their actions on CRP levels (Ridker et al., 2005; & Nissen et al., 2005).. Moreover, numerous research has gone further suggesting a greater role of CRP beyond its mere role as a marker of vascular and cardiovascular inflammation, to be considered a risk factor involved in the pathogenesis of atherosclerosis and other CVDs (Blake & Ridker, 2003; Ridker et al., 2003; Ballou et al., 1992; & Pasceri et al., 2000).

The design and scope of this current did not allow us to determine the underlying mechanism behind the strong effect produced by troglitazone on inflammatory markers when compared to other interventions, and whether the observed effects were related to the unique characteristics of these agents or simply a function of their ability to lower plasma glucose levels and improve insulin sensitivity. Troglitazone impact on inflammatory markers may be due its unique mechanism of action. TZDS, including troglitazone are strong activators of $PPAR_{\gamma}$ (Qayyum et al., 2006; & Quinn et al., 2008). $PPAR_{\gamma}$ ligands were proven to have strong effects on several other inflammatory markers through their ability to inhibit macrophage activation (Hevener et al., 2007; & Odegaard et al., 2007), interfere with smooth muscle proliferation (Ren et al., 201; & Law et al., 1996), and inhibit or downregulate important proinflammatory protein such cytokines and interleukins (Sigrist et al., 2000; & Ruan et al., 2003; & Fidan et al., 2011). All these actions were suggested pathways which may indirectly impact the levels of the inflammatory and coagulation markers studied in this preset analysis, other possibilities could possibly be due to direct actions of the TZDS on $PPAR_{\gamma}$ (Samaha et al., 2006). Apparently, more in-depth research is needed to explain the mechanisms behind the TZDs action on these markers.

As previously indicated, our study showed marked elevation in the overall mean CRP levels (0.58 ± 0.03) in prediabetic individuals at baseline compared with healthy individuals, comparable to what was reported in previous research. A previous analysis in the same population studied in this report have reported on the elevated CRP and fibrinogen levels at baseline in ILs, metformin, and placebo arms, with the exclusion of troglitazone (Haffner et al., 2005; McMillan, 1981 Festa & D'Agostino et al., 2000; & Festa & D'Agostino et al., 1999). Our results as well as others may lead to the suggestion that improved vascular function brought by TZDs could possibly be related to their ability to suppress inflammation and coagulation markers, as suggested by previous reporting (Tousoulis et al., 2007; & Gada et al., 2013).

Elevated CRP and fibrinogen levels in African Americans at baseline were also observed by our analysis. This pattern agrees with what was shown in preceding research (Carroll et al., 2009; Lin et al., 2007; & Wee et al., 2008). In fact, different reports considered the elevation in the levels of CRP and fibrinogen among African Americans to be a possible explanation for the increased risk for CVDs in this population (Anuurad et al., 2008). Our findings, regarding the reduction in inflammatory and coagulation markers, further support the assumption that TZDs exerts their benefits on cardiovascular

system by reducing these specific inflammatory markers, this extends to such at risk population as the African Americans. African American and Hispanic American are also reported to have higher rates of insulin resistance and obesity than other ethnicities; which elevates the risks for CVDs (Cossrow et al., 2004).

CONCLUSION

The association of prediabetes with CVDs and cardiovascular mortality was demonstrated in multiple research. CRP was shown as a valid marker for CVDs and the associated prognosis. Numerous research has gone further suggesting a greater role of CRP beyond its mere role as a marker of vascular and cardiovascular inflammation, to be considered a risk factor involved in the pathogenesis of atherosclerosis and other CVDs. The decrease in CRP levels demonstrated by our analysis was significantly greater than the effect of metformin or placebo as presented in the previous DPP study which analyzed the effect of ILS, metformin, and placebo on CRP levels in the same population studied in this present analysis, with the exception of troglitazone arm which was not included. The design and scope of this current did not allow us to determine the underlying mechanism behind the strong effect produced by troglitazone on inflammatory markers

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ABSTRACTS

MULTIFUNCTIONAL BIOCERAMIC FOR INNOVATIVE THERAPY

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Silica and calcium phosphate are important ingredients in synthetic bone grafts due to their significant role in new bone formation and vascularization. Of prime importance is for the synthetic bone graft to be able to present its stimulating elements in an amendable format for osteoblasts during new bone formation. Studies on Silica-Calcium Phosphate composite (SCPC) demonstrated that the silica phase provided guided cell growth and bone matrix deposition. Loading porous SCPC granules with antibiotic provided sustained release of a therapeutic dose for more than 28 days. Implantation of the antibiotic-loaded SCPC granules in a critical size calvarial defect in rabbit demonstrated the ability of the graft material to stimulate new bone formation. Moreover, on the cellular level, the SCPC-vancomycin hybrid stimulated osteoblast phenotypic expression and the released antibiotic demonstrated bactericidal effect against Staph aureus.

Session I: Biomaterials-Tissue Engineering

PULSED LASER DEPOSITION OF BIOACTIVE COATING FROM WHITE PORTLAND CEMENT

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Objective. We report the study of feasibility to produce the thing bioactive coating from White Portland cement (WPC) using pulsed laser deposition (PLD) technique. **Methods**. The targets for PLD system (disks 30 mm in diameter × 5 mm thick) were sintered from micronized powder of set Alborg White Portland cement. The parameters for sintering process were chosen based thermo-gravimetric analysis and differential scanning calorimetry (TGA/DSC). The coatings were deposited by PLD on silicon substrates. The effect of laser power on coating crystallinity and morphology was evaluated by scanning electron microscope (SEM) and X-ray diffraction (XRD). The material transfer from target to substrate were evaluated by X-ray fluorescence (XRF) and X-ray energy dispersive spectroscopy (EDS).The bioactivity of deposited films was evaluated by ability produce the hydroxyapatite (HA) layer on a surface of specimen immersed in a simulated body fluid (Dulbecco's Phosphate-Buffered Saline (DPBS). The formation of hydroxyapatite was confirmed by SEM, X-ray energy dispersive spectroscopy (EDS), XRD and micro-Raman spectroscopy. The formation of HA was evaluated after 1, 3, 7, 14, and 21 days of immersion. **Results.** This study demonstrated that White Portland cement can be used as a target material for manufacturing of bio-functional coatings. The films deposited on Si substrates have mainly amorphous structure; the crystallinity of the film can be achieved by increasing the laser power. The biological performance of deposited films was tested by HA forming ability in simulated body fluid. The HA layer was formed on a coated surface after first day of immersion.

VASCULARIZED 3D TISSUE CULTURE MODEL USING MICROFLUIDIC CASSIE-BAXTER SURFACES

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In medical and pharmaceutical research, there is a pressing need for biologically relevant *in vitro* cell culture models that can capture critical aspects of the biochemistry, mechanics and physiology of the *in vivo* microenvironment, such as cell-ECM interactions and vascularized dynamic nutrition delivery. Integrating 3D culture with microfluidic systems makes it possible to mimic the nutrition delivery via microcirculation and can be used to study a variety of complex cellular behaviors such as angiogenesis, chemotaxis and cell migration. In this work, we developed a microfluidic platform that adopts a novel method of using topography-induced vapor entrapment to integrate 3D cell culture with an array of microchannels. This enables barrier free nutrition delivery from the cell culture media flow in the microchannels to the cells in the 3D hydrogel. Also, a removable lid provides physical access to the 3D cell culture which is useful for further biochemical and morphological analysis of the cells. Wettability analysis on the microchannels was performed both theoretically and experimentally, and the dynamic solute exchange between the flow and the 3D cell culture was illustrated. Also, in order to demonstrate the functionality of microfluidic device as a cell culture platform, the viability of MDA-231 breast cancer cells was studied over an extended period of time.

3D HETEROGENEOUS BREAST TISSUE MICROENVIRONMENT USING POLYLACTIDE BEADS

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The microenvironment composition and density critically affect the breast epithelial cells behavior and can promote cancer development and progression. Those parameters are more suitably investigated in 3D *in vitro* culture systems. However, current 3D breast tissue systems poorly account for the heterogeneous density and composition of the extracellular matrix (ECM) observed within breast tissue. Here we investigated whether 3D matrices embedded with polylactide beads more closely mimicked the heterogeneous microenvironment of breast tissue. Briefly, breast epithelial cells were grown in 3D collagen / Matrigel® matrices embedded with polylactide beads and the development of complex structures i.e., acinus- and duct-like structures was monitored over time. Results indicate that

polylactide beads coated with either media (117±4 um) or Collagen I (124±2um) had significantly smaller diameters on average than control beads in PBS (133±3um). The cells formed complex structures surrounding cluster of beads with cell strands migrating outward. The cell strands included both acinus- and duct-like structures. The length and the complexity of the cell strands formed in 3D matrix embedded with polylactide beads differed based on the beads coating. Thus, embedding polylactide beads in an *in vitro* 3D test system may model the density heterogeneity of normal breast tissue. *This study was supported, in part, by a grant from the National Science Foundation EFRI program (CBE0736007).*

HYBRID NETWORK STRUCTURE OF CHITOSAN FOR SOFT TISSUE INJURY WITH ANTIMICROBIAL AND CONTROLLED DRUG RELEASE ATTRIBUTES

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Traumatic injury is a life-threatening prospect for soldiers in combat as well as civilians in serious accidents, such as motor vehicle accidents. Uncontrolled hemorrhage i.e. excessive loss of blood due to injury is a leading cause of death in soldiers and civilians. Hemorrhage can be controlled by applying pressure on the wound, however, this approach might not be suitable for injuries that occur in soft-tissues or organs such as eyes, lungs, liver, kidney, spleen, and neural tissue damage. This research is involved in the formulation of a gel that can be applied on a soft tissue to prevent excessive blood loss while providing anti-inflammatory and antibacterial benefits. The gel was prepared using chitosan, which is considered as the second most abundant biopolymer on this planet. The gel was loaded with a non-steroidal anti-inflammatory drug ibuprofen (Ibu) and silver nanoparticles (AgNPs) for antibacterial properties. Tri-polyphosphate (TPP) was used to prepare the cross-linked chitosan nanoparticles, and the degree of crosslinking was varied in order to understand the effect of cross-linking density on the microstructure of the gel. Our results suggest a novel strategy and potential biomaterial for soft tissue engineering applications.

FIBROUS BRANCHED-CLUSTERS AS MODULAR BUILDING UNITS FOR TISSUE REGENERATION TEMPLATES

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Electrospun templates are often limited with regards to cell infiltration and microenvironment tailorability. Thus, there is a need for a flexible 3-D template system that allows cells to freely reorganize their microenvironment. In this study, electrospun templates were processed into fibrous branched-clusters and then separated by size via centrifugation. Fibroblasts were combined in culture with various amounts of fibrous clusters, and either centrifuged down or allowed to settle under gravity. After 3 weeks of culture, the fibrous clusters and cells formed three-dimensional tissue-mimicking constructs. Pycnometry was used to measure construct density, and found the densities (1.2-1.6 g/mL) were comparable to those of native soft tissues. Cryosectioning and DAPI staining revealed uniform cell distributions throughout all construct types (1.3-3.2 mm in diameter). Immunostaining for Ki67 indicated little ongoing proliferation throughout the construct after 3 weeks. These results demonstrate the ability of these branched-clusters to serve as building blocks for cells to create constructs with homogenous cell distributions not yet realized from traditional electrospun templates. These experiments are currently being replicated using chondrocytes to explore the potential of this template system in creating cartilage analogues.

Session II: Patient Rehabilitation

THE EFFECT OF TROGLITAZONE ON C - REACTIVE PROTEIN IN INDIVIDUALS WITH PREDIABETES: DATA FROM THE DIABETES PREVENTION PROGRAM

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Background: Limited studies investigated the effect of troglitazone on CRP levels in individuals with prediabetes. The Diabetes Prevention Program (DPP) evaluated the effect of different interventions including metformin and intensive lifestyle modifications (ILS) compared to placebo on the prevention of DM. The DPP included 3,234 subjects with prediabetes. In this study we evaluated the effect of troglitazone on CRP levels in a subgroup from the DPP population. **Materials and Methods:** The effect of trolgitazone on CRP levels at baseline & at 12 months was studied in a subgroup of a total of 3,171 subjects from the original DPP study population and compared to the other three interventions. **Results:** Overall, the median percentage change in CRP at 1 year from baseline was -20.00% in the troglitazone arm ($p < 0.001$ for all between group analysis. The effect of the interventions was also reported by sex. In women, the median percent change was -27.08% in the troglitazone arm ($P<0.001$ for troglitazone vs. placebo & troglitazone vs. metformin: $P=0.001$). In men, troglitazone reported a median percentage change of -4.64% in CRP levels ($P<0.05$ for troglitazone vs. lifestyle & troglitazone vs. placebo: $P = 0.012$). **Conclusion** The decrease in CRP levels demonstrated by our analysis was significantly greater than the effect of metformin or placebo as presented in the previous DPP study which analyzed the effect of ILS, metformin, and placebo on CRP levels in the same population studied in this present analysis, with the exception of troglitazone arm which was not included.

THE EFFECTS OF MIRROR THERAPY ON UPPER EXTREMITY FUNCTION POST-STROKE: A SYSTEMATIC REVIEW

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Background and Significance: Impairment in UE function post stroke is detrimental to independence in activities of daily living. During mirror therapy (MT), the patient watches the movement of the unaffected hand in the mirror giving the patient the illusion of correctly moving the paretic hand. The purpose of this study was to determine if mirror therapy improved upper extremity function in patients post-stroke. **Methods:** A systematic search of the literature was performed to include randomized control trials comparing conventional treatment and/or sham treatment to MT for UE weakness/paresis and independence in activities of daily living. Five articles met the inclusion criteria. Study quality was evaluated using the PEDro scale, a 10-point scale developed to assess the internal validity of clinical trials in physical therapy. Studies were also scored using the 2011 Centre of Evidence Based Medicine (CEBM) scale, a 5-level scale, in which lower numbers indicate higher levels of evidence. **Results:** Evidence in four out of the five articles demonstrated that MT led to statistically significant improvements in function ($p<0.05$), as shown on the Functional Independence Measure (FIM). The mean PEDro score was 6.2 with a range of 5 to 7. The CEBM levels of evidence included three level II studies and two level III studies. **Conclusion:** This systematic review provides evidence to support the use of MT as a therapeutic intervention in patients with chronic or subacute stroke to increase UE motor function and functional skills.

THE EFFECTIVENESS OF DRY NEEDLING ON THE REDUCTION OF PROXIMAL UPPER QUADRANT PAIN USING COHEN'S D: A SYSTEMATIC REVIEW.

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Purpose: To determine if Dry Needling treatment in patients with proximal upper quadrant pain reduced pain intensity in comparison to controls/interventions using Cohen's d for effective clinical difference. **Number of Subjects:** 11 articles. **Methods:** Literature search was performed using Pubmed, Embase, and PEDro databases. Inclusion criteria required intervention of DN into the trapezius muscle, clinical trials published within 10 years, musculoskeletal pain in the proximal upper quadrant, and comparing effects of dry needling to alternative treatments for pain reduction. PEDro risk of bias assessment was used. Information taken from the articles includes population, intervention group, comparison group(s), and post-intervention and follow up pain outcomes (VAS). We calculated Cohen's d of each article. Number of participants, mean, and standard deviation between two groups were used in the calculation of effect size (ES) and 95% confidence interval (CI). **Results:** Eleven randomized clinical trials were reviewed. The mean PEDro score of the studies was 6.5 with a range of 4 – 8. Using Cohen's d to measure efficacy of DN treatment, DN had a large effect and significant, on pain control in five studies compared to the controls/interventions (ES range of 0.81 to 17.46; CI of 0.08 – 1.50 and 15.19 – 19.50 respectively); moderate effect in one study which was not significant (ES of 0.52; CI: -0.15 – 1.8). **Conclusions:** DN is more effective for pain reduction than controls and other interventions in five of the eleven studies; inconclusive in three studies. It is suggested that DN be taken into consideration pain management.

ATTITUDES OF MISSISSIPPI NURSES' TOWARDS EDUCATION ON COMPLEMENTARY & ALTERNATIVE MEDICINE IN MISSISSIPPI UNIVERSITIES

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The growing consumer demand for complementary and alternative therapies (CAM) in health care has had an effect on all health professionals. The discipline of nursing is rooted in many holistic processes but the role of providing such services has not been fully defined in many states, including the state of Mississippi. Nurses are the members of the healthcare team who often initiate such a conversation with patients about CAM. We took a look at the state of Mississippi nurses and their perception of such a growing consumer demand, with effective healthcare services in mind. This was a descriptive quantitative study, with a sample size of 116 Mississippi Nurses. Participants in attendance to the 2016 MS Nurses Association Annual Meetings & Conventions voluntarily completed a questionnaire. Results found that 66.39% of participating nurses felt comfortable talking about CAM with patients, but only 20% of participating nurses felt prepared educationally. That left 80% of the nurses feeling unprepared when discussing CAM with patients. Only 38.60% nurses said they actually initiate any type of discussion with patients on CAM. These findings support our hypothesis that there is a lack of congruence between nurses' beliefs and knowledge of CAM, and the incorporation of CAM into their current practice.

Session III: Math and Modeling

USING ORDINAL LOGISTIC REGRESSION WITH PROPORTIONAL ODDS TO ANALYZE HEALTH CARE DATA WHERE THE OUTCOME VARIABLE CAN BE ORDERED

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This study was conducted based on a sample of 384 people to determine how satisfied patients were with their primary care professionals' services based on a Likert scale (1 to 4 with 1 = very dissatisfied and 4 = very satisfied). People in the sample were characterized by site (Clinic A = 1 and clinic B = 0), gender (1= females, 0=males), socioeconomic status (0 = Low class, 1 = middle class, 2 = Upper class), and age. In this study, 384 patients (218 females, 166 males) were available for investigating the association between their ratings of professional health care services and the factors of gender, clinical location, socioeconomic status and age as a covariate. A cumulative odds

ordinal logistic regression with proportional odds was run to determine the effect of these predictors on patients' satisfaction with health care services at these clinics. Power analysis for a multiple regression with four predictors was conducted in G-POWER to determine a sufficient sample size using an alpha of 0.05, a power of 0.80, and a medium effect size (f = 0.15).A thorough description of the results of the OLR models will be presented. IBM Statistical Package for the Social Sciences (SPSS) software version 23 and G-POWER 3.0.10 were used to analyze the data.

META-ANALYSIS USING COMPREHENSIVE META-ANALYSIS SOFTWARE: PRACTICAL APPROACH WITH EXAMPLES

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Meta-analysis is a statistical analysis that combines results of multiple studies. The basic idea is that there is a common truth behind all similar studies, but which has been measured with an error within individual studies. The aim is to use statistical approach to derive a pooled estimate closest to the unknown common truth. Meta-analysis contrasts results from different studies and identify patterns among study results and sources of disagreement. When effect size is consistent, meta-analysis is used to identify this common effect. When the effect varies, meta-analysis is used to identify the reason for the variation. A key benefit is the aggregation of information leading to a higher statistical power and more robust point estimate than is possible from the measure derived from any individual study. Comprehensive Meta-analysis software (CMA) will be used to illustrate this concept. CMA is a powerful computer program with a wide array of computational options and sophisticated graphics. The process begins with a systematic review, which is a lengthy process that includes formulating a research problem, searching the literature using MEDLINE, EMBASE, and other search engines, deciding which studies to include in the synthesis based on objective criteria, and then performing meta-analysis. Once an appropriate group of studies has been identified, the relevant data will be abstracted. This presentation provides a brief overview of important features of meta-analysis with emphasis on concepts and practical applications. Several topics such as fixed and random effects model, potential for bias, and conducting subgroup analyses will be discussed.

DATA ANALYTICS FOR IMPROVED DECISION MAKING AT THE VETERANS AFFAIRS MEDICAL CENTERSDATA ANALYTICS FOR IMPROVED DECISION MAKING AT THE VETERANS AFFAIRS MEDICAL CENTERS

Ajay Mahajan[1], Alex Russell[1], Padmini Selvaganesan[1], Parag Madhani[2], Sanjeevi Chitikeshi[3]
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This paper reports on a data-driven methodology for decision making at the Veterans Affairs (VA) Medical Centers to improve patient outcomes, specifically SMR30 (30-day Standardized Mortality Ratio). The quarterly SAIL (Strategic Analytics for Improvement and Learning) reports are used to visualize the data, study trends, provide actionable recommendations and potential consequences. A case study using more than four years of data is used to demonstrate the power of the methodology. Let us say that after seeing the data and studying the trends of other VAs, a decision is made to reduce the SMR30 by 5%. After running correlation algorithms, IHC (In Hospital Complications) is shown to be the most correlated with SMR30. A regression model is then developed between the two that says that IHC would have to be decreased by 44% to attain the desired result. Data shows that this is certainly feasible, and then a principal component analysis is done to create models between IHC and other metrics to see the consequence of the change. The models then predict that MRSA (Methicillin-Resistant Staphylococcus aureus) infection rate would decrease by 16.9%, but CAUTI (Catheter associated urinary tract infection) and PSI (Patient Safety Indicator) would increase by 17.7% and 7.7% respectively. This then lays the groundwork for a healthy discussion between the executives, staffs and clinicians on the path forward, resources required, and more importantly a progress dashboard that reflects weekly progress (data obtained from IT department) rather than waiting three months for the SAIL report to come.

THE MODEL OF INTERDISCIPLINARY COLLABORATION IN PERIOPERATIVE SETTING FROM THE PERCEPTIONS OF THE IDT PROFESSIONALS

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Background: Interdisciplinary collaboration (IC) is viewed as the product of synchronization and harmonization of team efforts supporting the move away from fragmentation of care. Research about the model of IC in perioperative setting (PS) is primarily qualitative and is not sufficient for developing the interventions with measurable effects. Often the latest developments in team and collaborative theory are not considered. Further elucidation of ICPS is necessary for better understanding of this collaborative process and advancing its measurements. **Purpose:** The purpose of this research was to explain the model of ICPS from the perceptions of the interdisciplinary team (IDT) of PS. Questions guiding this research included: What are the significant components of ICPS? What are the relative convergence and divergence of ICPS concept? What are the relational patterns of the factors of ICPS? **Materials and Methods:** Mixed-methods survey design engaging the population of perioperative professionals. Factor analysis and analysis of correlations were performed in SPSS using survey data obtained in REDCap. **Results:** 74.606% of variance was explained with the model of three factor components (eigenvalues >1, factor loadings of

>0.40): Collegial Support of Adaptability (r=0.478), Reflexive Decision Making (r=0.457), and Process development (r=0.495). The assumptions of initial ICPS Model, constructed from literature review, were partially supported. **Conclusions:** We were able to identify significant covariates of ICPS pertinent in describing its model. Guardianship-Stewardship Motivational Conflict Model of ICPS was adjusted using these findings. Further research could allow detailing the effects of ICPS factors on levels of IC and technical outcomes.

Session IV: Education and Research Training

ADVANCED BIOMEDICAL EDUCATION AND RESEARCH TRAINING

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In an effort to increase the number of well-trained minority health care professionals and basic science researchers, Jackson State University, (JSU, a historically black institution) in partnership with the University of Mississippi Medical Center,(UMMC, a major research-oriented medical center) and consultant biomedical researchers/health care professionals at various professional and academic institutions, established a Collaborative Advanced Level Minority Institutional Research Training Program (MIRTP). The purpose of the MIRTP was to enhance: 1. underrepresented minority graduate students training in cardiovascular, pulmonary and/or hematological related areas, the acquisition of Master's degrees and trainee motivation to seek advanced degrees (doctorate) in the biomedical and health sciences areas and 2. Provide specialized advanced training for minority postdoctoral recipients. The program involved faculty and administrators at each institution in the planning and implementation of all programmatic aspects, including trainee selection, advisement procedures and program activities. JSU MIRTP students were recruited from historically black colleges and universities (HBCU'S) and majority institutions nationwide. MIRTP students were trained in cardiovascular, pulmonary and hematological related research laboratory methodologies, responsible conduct of research concepts, literature survey mechanisms, and scientific writing techniques by mentors at JSU, UMMC and various consulting institutions. Students engaged in specific, mentor supervised, individualized research projects for Masters theses and presented their research findings at local and national scientific meetings e.g., MAS, ABRCMS, FASEB, AHA and the Endocrine Society. The results show that enhanced education and research training in cardiovascular areas can enhance the acquisition of advanced degrees and training by minority students. (HL07635).

THE IMPACT OF STEM FACULTY MENTORING ON MINORITY COLLEGE STUDENTS FOR CAREER SUCCESS

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Historically Black Colleges and Universities (HBCUs) have a long history in providing quality college education to minority students. HBCUs account only for 2% of nation's colleges and universities but award a great number of degrees. The awarded degrees are in social and liberal arts, but there has been an increase in the number of science and engineering degrees and there are efforts to broaden the participation in STEM.

Nationally there is a collective effort to increase minority students in STEM through: 1) student mentoring; and 2) online distance learning. Mentoring can alleviate and help the students to navigate the years of college with success and graduate. Having a mentor is important to any person in the early years of career. A mentor is a person with a lot of experience, professionally stable, someone that will share his experience, insight, and knowledge on how to deal with stress, how to meet deadlines, and how to still take advantage of college life outside of the classroom. The development and implementation of online distance education courses represents an avenue to provide the students to enroll in STEM. The online distance learning is a structured and well-planned teaching and learning approach that uses an array of modern technologies with access by the educator and the student. The online distance learning offers flexibility and virtual interactions that encourages the students to continue education. These activities will advance student's understanding of STEM disciplines and prepare them for STEM careers particularly of graduates from underserved groups.

TEACHING WITH TECHNOLOGY: DOES IT REALLY WORK?

Gloria Miller

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The rise of social media and technology has changed the way educators teach, how students learn, and the way teachers and students communicate. Since students are already interested and engaged in technology, teachers can harness that attention for educational proposes. Leaders in government, business, and higher education are calling for today's students to show a mastery of broader and more sophisticated skills like evaluating and analyzing information and thinking creatively about how to solve real-world problems. This paper will share ideas on ways to utilize technology to: 1) engage students and create active learners, 2) encourage individual learning and growth, and 3) facilitate peer collaboration. We will discuss some of the benefits and limitations of technology in the classroom, best practices for using technology in the classroom, and ways to balance traditional methods and the use of technology in order to maximize effectiveness. Our aim is for the reader to discover that teaching with technology is not just about staying current on the latest tools, rather, it is about knowing how to successfully incorporate the best tools into teaching when and where it makes sense.

TEACHING AND LEARNING IN AN ACTIVE LEARNING CLASSROOM – A MIXED-METHODS CASE STUDY

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In the era of ubiquitous use of technology, education in all settings is impelled to incorporate technology into classroom settings in order to meet student needs and facilitate student learning. A southeastern academic medical center took the initiative and transformed a traditional classroom (TC) into a technology-advanced active learning classroom (ALC). In this case study, we conducted a mixed-methods cohort study aiming to find out how an instructor utilized the ALC, how students perceived the room, and whether the room yielded better learning outcomes. To find answers, we conducted a year-long observations of a dental hygiene instructor teaching two consecutive courses. We recorded and transcribed 19 classes, and took observational notes of classroom happenings. Towards the end of the academic year, we interviewed the instructor following a 13-question guide and surveyed the students with a 25-question questionnaire. We also collected six-year grades of the same two courses by this instructor, which included five-year historical grades earned in the TCs and one-year grades from the ALC. Our multi-perspective data gained a variety of examples of how instructor flexibly utilized different features in the room to facilitate individual or group activities. Student survey data indicated that approximately 50% preferred to take classes in the ALC rather than TCs. They especially enjoyed unobstructed views, spaciousness, and ease of screen sharing and interactions in group activities. Our grades data showed that there was a mean increase in both courses, however, significant differences were not found between grades earned in the ALC and TCs.

Session V: Tissue Engineering II

NOVEL PROTEIN THERAPY TO REVERSE AND INHIBIT CALCIFICATION OF VASCULAR SMOOTH MUSCLE CELLS

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Vascular calcification is an active process related to cardiovascular disease resulting from the osteoblastic differentiation of smooth muscle cells (SMC). This leads to the calcification of the medial and intimal layers of the arterial wall. In order to inhibit and potentially reverse this process, research has focused on the circulating protein, Fetuin-A, that binds to free calcium and phosphate in the serum, preventing mineral deposition. Our goal is to understand the mechanisms behind this protein and determine how it can be utilized as a therapeutic agent for calcification. In this study, we cultured and treated human vascular SMCs to induce calcification and determine the level of fetuin required to reverse it. Cells were cultured in calcification media, treated with bovine fetuin, and analyzed for calcium content using *o*-cresolphthalein kit, PCR, western blots, and staining, using xylenol orange for calcification and DAPI counterstain for nuclei. Preliminary western blot data showed that under calcifying conditions, SMCs lose their native α-SMA marker, representing a change from the SMC phenotype bone-forming osteoblasts. In PCR results, groups that lost α-SMA markers in western blots expressed RUNX2 in 2-fold expression, a gene only found in bone cells. On day 14, treating the cells with high fetuin (15 μM) produced significantly lower calcium/protein (μg/mg) content, with a p-value < 0.05, confirmed through staining. In future research, we want to formulate a uremic *in vitro* model observe and manipulate the effects of fetuin in sites of calcification conditions to determine whether this therapy is effective.

COMBINATION OF NANOTECHNOLOGY AND SPERM BIOLOGY TO ENHANCE REPRODUCTION PERFORMANCE

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Nanotechnology allows for the beneficial use of nanoparticles in reproductive medicine to improve understanding of animal fertility. Here we tested various fluorescent nanoparticles for effective and harmless interactions with mammalian spermatozoa. Boar spermatozoa were adjusted to 2×10^8 /ml of PBS and mixed with 60 μg of fluorescent liposomes, 0.05 nM of copper (CuIS/ZnS), or 0.05 nM of cadmium (CdSe/ZnS) core-shell quantum dots (QD) nanoparticles. After co-incubation, sperm were evaluated for motility and morphology characteristics and fluorescence emission. Data were analyzed by ANOVA-1 with significance set at $P<0.05$. Liposomes and CuIS/ZnS QD significantly increased the proportions of motile spermatozoa, while CdSe/ZnS QDs did not affect the perm motility ($P>0.05$). The proportion of forward progressive spermatozoa was significantly increased by the presence of liposomes, but was decreased by both QDs ($P<0.05$). The proportions of spermatozoa with bent tail and distal cytoplasmic droplets were significantly dereased by the presence of each tested nanoparticles ($P<0.05$). Fluorescence imaging showed successful binding of nanoparticles with the entire sperm length. Contrarily to liposomes, both CuIS/ZnS and CdSe/ZnS QDs showed potential sperm toxicity despite the CuIS/ZnS exhibiting greater enhancement of sperm motility and morphology characteristics. Findings are important for harmeless labeling and tracking of spermatozoa in

physiological conditions to improving fertility outcomes during assisted reproduction. Supported by USDA-ARS Biophotonics Initiative #58-6402-3-018.

COMPARISON OF CELL VIABILITY, MORPHOLOGY AND MINERALIZATION OF MESENCHYMAL STEM CELLS FOLLOWING A SINGLE EXPOSURE TO ELECTROMAGNETIC FIELD OR LOW-LEVEL LASER THERAPY

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Mesenchymal stem cells (MSCs) are multipotential cells capable of differentiating into osteoblasts, adipose cells or neural cells, but they differentiate slowly. Electromagnetic field (EMF) and low-level laser therapy (LLLT) are methods that have been used in vitro and clinically to accelerate this process. Increases cell viability, differentiation and mineralization of mesenchymal stem cells grown in osteogenic medium and exposed to either EMF or LLLT have been reported. Osteogenic medium has been used to enhance osteogenic differentiation of mesenchymal stem cells. The goals of this experiment were: (1) to determine the effects of EMF at a distance of 3 inches for a period 30-minutes on cell viability, morphology and mineralization of murine MSCs grown in osteogenic medium at 7, 14 and 21 days; and (2) to determine the effects of a single dose of LLLT at 10 joules on cell viability, morphology and mineralization of murine MSCs grown in osteogenic medium at 7, 14 and 21 days. At 7 and 14 days the EMF treated cells were more numerous than controls while the LLLT treated cells were fewer in number but larger in size than the controls. At 21 days, both treat cell groups were similar in size, shape and numbers as the control group. While neither EMF nor LLLT exposure at recommended dosages caused a detrimental effect on the viability of the murine MSCs used, both produced increases in proliferation and differentiation. However, at 7 and 14 days, the cells treated with LLLT had a significant increase in mineralization.

USE OF A PROTEIN-BASED INHIBITOR TO REGULATE THE PHENOTYPIC SWITCH OF SMOOTH MUSCLE CELLS

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Cardiovascular complications are one of the leading causes of death in patients with diabetes or kidney disease. Vascular calcification used to be considered a passive process resulting from elevated calcium-phosphate interactions. However, it is now known to be a cell-mediated process. The competition of calcification promoted by proteins and inhibitors which cause arteries to harden is what drives this process. Current research has shown that the hardening of these arteries is resemblant to bone development. When exposed to high levels of glucose, calcium, phosphate, and cholesterol, it is thought that smooth muscle cells (SMCs) in arteries that are healthy experience a phenotypic switch to osteoblast-like cells. Even though this phenotypic switch is known to researchers, the cellular and molecular mechanisms that promote calcification are still unknown. The *in vitro* model that we have developed is used to induce vascular calcification and recognize the switching of healthy SMCs to osteoblast-like cells. The goal of using our *in vitro* model is to analyze the Wnt Signaling pathway that is involved in vascular calcification. We also want to block the activation of the Wnt pathway by using Sclerostin (Sost). By doing this we will be able to examine and determine the effects that Sost and Wnt have on vascular calcification.

A DIME-SIZED HUMAN-IMPLANT-READY MULTI-CHANNEL NEUROSTIMULATION DEVICE MOVES TOWARD NETWORKED WIRELESS CAPABILITY

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The ability to put active recording/ stimulation systems in close proximity of neural sites greatly improves ability to target sites and retrieve neural information with accuracy, lower noise, and a greater multiplicity of sites. Here we describe a networked implantable neuromodulation device with on-the-fly reconfiguration of sensing and stimulation. The system is being developed in three configurations: 1) Passive electrode leads for percutaneous use, 2) Dime-size active modules with electrodes for percutaneous use and networking to an implantable hub, and 3) Wirelessly networked active electrode modules. The system leverages custom ASICs and dense hermetic packaging to reduce the burden of bulky electronics, leads, and connectors. Our systems achieve 1μV RMS noise, selectable single-ended or bipolar referencing, stimulation between 1μA and 6mA, and ISO-14708 and IEC-60601 validation for human use. The system, and lessons learned through for-human-use testing and validation will be discussed**.**

DO THE PROTEINS IN A FINGERNAIL OFFER INSIGHT INTO THE BONE HEALTH OF THEIR DONOR?

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Objective: Do the proteins that constitute human fingernail offer a window into their donor's bone health? **Materials and Methods**: A cross-sectional, multi-centre study, 'Fracture Risk Assessment by Nail correlation study' (FRAN) tested the link between nail keratin and bone health of the nail donors. Raman spectroscopy was applied to nail clippings from 633 postmenopausal women, from six clinical sites, of whom 42% had experienced a fragility fracture. The Raman spectra were recorded by a Sierra Reader (Snowy Range, WY). **Results**: The

differences identified in the spectra of nails sourced from non-fracture and fracture groups can be attributed to changes in the order of the keratotic proteins. Nails sourced from donors who have not experienced a fracture contain highly organised alpha helical structures with ample intra-chain disulphide bonding whereas those sourced from donors who have experienced a fracture exhibit less ordered, 'random' secondary structures with a breakdown in intra-chain disulphide bonding. **Conclusions**: Raman spectra of human fingernails may present a surrogate marker of bone protein structure status. **Disclosures**: Both RB and MT are shareholders in Crescent Ops Ltd, who own intellectual property rights on the relationship between nail structure and fracture risk.

MEDICAL ROBOTS MUST HAVE ARTIFICIAL EMOTIONS

Paul Frenger

A Working Hypothesis, Inc., Houston, TX, USA

Medical robots are establishing well-defined roles in healthcare. These include companionship, supervision and eldercare; medical consultation, autonomous surgery and monitoring of astronauts are coming very soon. Some authorities insist these machines must appear indistinguishable from humans, to elicit believable intellectual, personality and emotional responses (avoiding Masahiro Mori's "uncanny valley"). The author has researched artificial emotions for 45 years, designing analog, digital and microprocessor-based circuitry and software. His emotion simulator of 1973 uses a summing operational amplifier with Schmitt Trigger output. He's designed over 50 types of McCulloch-Pitts and Hebbs artificial neurons. His 2000 ANNIE robot, a biologically-inspired design, emulates many aspects of the human experience: self-awareness, recognition of other persons, a fear reaction and trust-love response, artificial hormones, sexual function, addiction to substances and video games, appetite control, ethics, beliefs and emotions. Every item known to ANNIE contains an emotional value. ANNIE's body has a multiprocessor / multitasking plug-and-play computer network, a complex hand, machine vision, multi-sensory system, facial expressiveness and speech I/O. Recently the author devised a carbon-based brainstem coprocessor for a more human-like robot qualia experience. Taken together, ANNIE's systems provide a smart, emotive quality to which colleagues, patients and other people can react naturally.

Session VI: Molecular and Clinical Markers

siRNA GENE DELIVERY MEDIATED BY MESOPOROUS SILICA NANOPARTICLES TO TREAT CANCER

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siRNA therapeutics have gained popularity for treating cancer, and has shown promising results owing to their target specificity to improve the effectiveness of the treatment. Mesoporous silica nanoparticles (MSNs) has emerged as a promising nanocarrier for the efficient delivery of active pharmaceutical ingredients (APIs). This comes from the properties possessed by MSNs, such as high-surface area, tunable particle/pore size, stability and biocompatibility. Also, the surface of MSNs can be functionalized to carry multiple types of cargo such as DNA, APIs, and other small nanoparticles (e.g., siRNA-nanoconstructs, QDs or superparamagnetic nanoparticles). Taking advantage of these attractive features, we present the synthesis of polyethylene imine (PEI)-coated fluorescent MSN material as efficient delivery vehicle for therapeutic nucleic acids. In our present work, we have synthesized PEI-coated MSNs modified with fluorescein in the interior surface. Meanwhile, the external surface was utilized to load ds-DNA, or ds-siRNA or siRNA nanoparticles through the electrostatic interaction between the positive amine groups from PEI polymer and the negative charge of the DNA/siRNA phosphate backbone. The MSN material was characterized using different techniques. In addition, *in vitro* studies (cellular uptake and cell viability assay) were performed in a breast cancer cell line (MDA-MB-231) to elucidate the behavior of our MSN system. To evaluate the transfection efficiency of our MSN platform, gene silencing studies in MDA-MB-231 cell line overexpressing GFP was carried out. As a future endeavor, to develop a combinatorial system, this platform can be used for co-delivering a chemotherapeutic-drug and therapeutic siRNA.

IMMUNOLOGICAL RECOGNITION OF THERAPEUTIC NUCLEIC ACIDS: TRANSLATIONAL FOCUS ON NOVEL CONCEPTS AND THEIR DELIVERY PLATFORMS

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Therapeutic nucleic acids (TNAs) are used for the detection and treatment of a wide variety of disorders. Despite many therapeutic advantages of these materials, their clinical translation is often complicated by the immune-mediated toxicities. Cytokine storm, fever-like reactions, and complement activation are among common dose-limiting toxicities. This presentation will review structure-activity relationship for various types of TNA. It will also discuss the role of physicochemical properties of a variety of nanotechnology carriers of TNA and their contribution to the immunological reactions to these therapeutics. Recommendations for the selection of a nanotechnology carrier suitable for a given type of therapeutic compound will be proposed. Acknowledgements: Supported by NCI contract HHSN261200800001E.

A SIMPLE AND SAFE METHOD TO INCREASE TUMOR BLOOD PERFUSION IMPROVED TUMOR RESPONSE TO RADIATION THERAPY

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Tumors are highly vascularized organoids. However, vascular function is often abnormal. Here, we analyzed the effects produced by an intravenous administration of a hypertonic saline solution (HSS) on tumor hemodynamics through functional imaging studies. Blood velocity (Color Doppler Ultrasound) increased after HSS injection compared to PBS in animals engrafted with B16F10 (p=0,019), SK-MEL-147 (p=0,028) and 4T1-derived tumors (p=0,015). Dynamic Contrast enhanced ultrasound (CEUS) was used to assess functional blood volume in B16F10, HCT-116 and MDA-MB-231 tumor xenografts, kidney and muscle tissues (n=3 per group). Relative tumor blood volume was increased in B16F10 (p=0,022) and HCT-116 (p=0,039) but not on MDA-MB-231 (p=0,186). A non-significant mild change was observed in normal tissues (kidney p=0,957; muscle p=0,104). Dynamic Contrast enhanced MRI (DCE-MRI) was performed in B16F10 tumors (n=4) and showed that contrast distribution increased in the HSS group (p=0,002). Finally, we analyzed the effects of treating animals with either PBS or HSS before exposing tumor-bearing animals to ionizing radiation. Prior HSS increased the efficacy of radiation therapy in 4T1-derived tumor bearing animals. In conclusion, HSS transiently and safely increased blood supply on specific tumors, and therefore could be used to enhance intratumoral delivery of different molecules for either tumor diagnosis or treatment.

FIBER- AND POLYGONS-FORMING RNA-DNA HYBRIDS FOR SIMULTANEOUS ACTIVATION OF MULTIPLE FUNCTIONALITIES

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We introduce a new rational design of DNA-RNA hybrids that depending on connectivity rules can either assemble as fibers or as closed polygons. The hybrids are programmed to activate multiple split functionalities upon their intracellular activation. The functionalities are exemplified in this work by FRET, RNA interference and formation of functional NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) decoys. NF-κB is a transcription factor that plays a critical role in regulating the expression of cytokine secretion. It was reported that NF-κB activation can be altered through gene therapy based interventions.[1] Rationale design of NF-κB decoys mimics the κB consensus sequence in order to prevent NF-κB binding to these sequences into the nucleus, thus stop cytokine production. By this means, during the re-association of RNA-DNA hybrids, the immunogenic response is efficiently decreased while RNAi and FRET are being activated. This work further expands the possibilities of dynamic RNA nanotechnology.

A NOVEL ANTI-MUC1 CAR T CELL DRIVING IMMUNITY AGAINST PANCREATIC CANCER

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Chimeric antigen receptor T cells (CAR-T cells) have shown remarkable success in treating hematologic cancers. However, this success has not been extrapolated in solid tumors. Among them, pancreatic ductal adenocarcinoma (PDA) is the fatal malignancy with extremely poor prognosis. Treatment options are very limited and commonly associated with numerous side effects. Targeted therapy, which only target cancer cells and not the normal cells, have shown promising result regarding lower toxicity and fewer side effects. Mucin 1 (MUC1) which is a glycoprotein expressed on the apical surface of epithelial cells of most of organs in our body including pancreas ducts undergoes some alterations in tumor condition. This aberrant form of MUC1 (tMUC1) is over-expressed in various types of cancers including pancreatic cancer. We have previously developed a patented monoclonal antibody (TAB-004) that only targets tMUC1 and spares the normal MUC1. Here we develop and characterize a novel CAR based on TAB004 antibody specific to tMUC1 which is expressed in many carcinomas. TAB-CAR T cells specifically bind to high MUC1 expressing pancreatic cancer cells and perform robust cytotoxicity against most of pancreatic cancer cell-lines, while spare the normal cells. Moreover, its function in controlling tumor growth in xenograft mouse model is shown. TAB-CAR T cell killing is coupled with IFNγ and granzyme B release. This study demonstrates the specificity and effectiveness of a novel anti-MUC1 CAR-T cell, against variety of pancreatic cancer cells and tumors *in vivo*, which introduces a potential targeted therapy for PDA and other MUC1 positive solid tumors.

COMBINATORIAL THERAPY USING POLYMERIC MICELLE NANOCARRIER FOR AXON REGENERATION AFTER CNS INJURY

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Spinal cord injury results in permanent disruption of axonal pathways that leads to loss of motor and sensory function. The goal of our work is to develop amphiphilic copolymers (PgP) for combinatorial delivery of bioactive molecules targeting different barriers to neurotrauma and axonal regeneration. In this study, we investigated the ability of PgP carrying rolipram (Rm) and RhoA siRNA (siRhoA) to improve axonal regeneration. Rm can reduce the secondary injury following insults. RhoA is a shared target of signaling pathways activated by diverse extracellular molecules present in the injured spinal cord. To evaluate Rm loaded PgP (Rm-PgP) effect on secondary injury, Rm-PgP was injected at the injury lesion and the cAMP level using ELISA Assay, apoptosis

by TUNEL, and inflammatory response by ED1 staining was evaluated. RhoA knockdown on axon regeneration by PgP/siRhoA injection was evaluated by histological analysis. We observed that cAMP level was increased and reduced presence of activated immune cells and apoptotic cells in Rm-PgP treated group. RhoA mRNA expression was significantly reduced in animals receiving PgP/siRhoA nanoparticles compared to the untreated SCI group. We also observed an extensive necrotic cavity and significant astrogliosis in the untreated SCI group, while reduced cavitation /astrogliosis and axonal regeneration into the lesion site in the treated group was observed. Finally, we evaluated the synergistic effect of Rm-PgP and PgP/siRhoA co-administration on functional recovery by Basso-Beattie-Bresnahan (BBB) locomotor rating scale in rat SCI model and functional recovery was significantly improved in nanoparticle treated group than untreated animal group.

LAB-ON-A-CHIP IMMUNOASSAY FOR THERMOELECTRIC QUANTITATION OF TNF-α

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We report thermoelectric lab-on-a-chip immunoassay for quantitation of TNF-α, an inflammatory cytokine that is released by human astrocytes in cell culture media. Since the method is based on detection of the heat released during an enzymatic reaction the thermoelectric immunoassay can be performed using different enzymes. The substrate can be introduced multiple times after the thermoelectric signal returns to baseline level that allows increase the statistical significance of the results. The immunoassay was performed in a microfluidic device with an integrated antimony/bismuth thermopile sensor that has 60 thermocouple pairs. The device had two inlets and single outlet and was fabricated using xurography technique. Anti-TNF-α monoclonal antibody was used to capture the analyte that was followed by detection via glucose oxidase-conjugated secondary antibody. Glucose (100mg dL^{-1}) was injected through a sample loop into the fluid flowing within the microfluidic device. Nanovolt meter connected to the thermoelectric sensor recorded the voltage change caused by the enzymatic reaction. COMSOL simulations were performed to analyze the effect of flow velocity on the diffusion rate of glucose in the microfluidic device. The magnitude of the thermoelectric signal was proportional to the concentration of TNF-α in the biological sample. Standard calibration curve, y= 0.0315 + 2.5296, R^2 0.994, was generated using various concentrations of synthetic TNF-α (0-2000pg mL^{-1}) by plotting the calculated area under the curve of the thermoelectric response versus the concentration of the analyte. TNF-α was quantified using both traditional ELISA protocol (287 pg mL^{-1}) and the microfluidic thermoelectric immunoassay (251 pg ml^{-1}).

Session VII: Radiology and Diagnostics

EFFECT OF PEDICLE-SCREW FIXATION IN LUMBAR SPINE AT L3-L5 LEVEL: A FINITE ELEMENT STUDY

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Deformities in the spine often require pedicle screw insertion, which alters the mechanical environment in the spine resulting in implant loosening and failure. This study examined the alteration of the state of strain at the bone-implant interface in the lumbar spine after pedicle screw insertion. Three-dimensional (3D) geometry of the lumber region (L1-S) and CAD model of pedicle screw was subjected at L3-L5 level to finite element (FE) analysis to examine the strain condition at the 6 selected locations at the bone-implant interface using different screw diameters (5 mm and 6 mm), implant materials (stainless steel and titanium), bone conditions (very strong, strong, normal, weak and very weak) and loading conditions (420 N, 490 N and 588 N). Screw diameter was observed to be the most crucial factor in determining the strain environment. The 6 mm diameter screw did not alter the strain environment significantly ($p<0.05$) as compared to un-implanted bones. Stronger bones, smaller loads and stainless steel did not alter strain environment significantly after the implantation. This preliminary analysis will help in understanding the effect of different physiological and implant parameter on the mechanical environment at the bone-implant interface and will help to design better pedicle screw implants.

EVALUATING A SINGLE NEEDLE HIGH-FREQUENCY IRREVERSIBLE ELECTROPORATION (H-FIRE) PROBE FOR PANCREATIC ABLATION IN VIVO

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INTRODUCTION: Most pancreatic tumors are unresectable due to vascular involvement. Irreversible electroporation (IRE) uses multiple electrodes around the tumor to induce cellular apoptosis while sparing the underlying tissue architecture. High frequency IRE (H-FIRE) is an experimental alternative to commercial IRE that obviates the need for muscle paralysis and cardiac synchronization. We have developed a novel dual electrode-single needle (DESN) probe for H-FIRE delivery to enable rapid

H-FIRE delivery. **METHODS:** A DESN H-FIRE probe placed in the pancreas head or tail in a swine model and, in the absence of paralytics or cardiac synchronization, H-FIRE ablations (2,250V) were performed in 6 animals using a 1-5-1ms, 2-5-2ms, and 5-5-5ms (on-off-on) waveform. **RESULTS:** All animals survived the experimental period with no EKG abnormalities or muscle spasm during H-FIRE delivery. Necropsy demonstrated reproducible pancreatic ablations ($903\pm70mm^3$ vs $935\pm148mm^3$ vs $2498\pm343mm^3$; 1-5-1 vs 2-5-2 vs 5-5-5). Histological analysis revealed extensive cell death within the ablative field in the absence of damage to vascular or ductal structure. Whole tissue staining and immunohistochemistry revealed the area immediately surrounding the probe was characterized by electrically-induced necrosis in the absence of thermal necrosis. Distal to the probe pancreatic tissue stained extensively for caspase 3 activity indicating apoptotic cell death.

CONCLUSION: H-FIRE delivery using the DESN rapidly and reproducibly ablated pancreatic tissue without the need for paralytics or cardiac synchronization. Overcoming the need to place multiple needles, and optimizing pulse delivery settings, raises the possibility of developing H-FIRE for minimally invasive use.

A PREDICTIVE DOSIMETRIC MODEL FOR ESOPHAGITIS INDUCED BY RADIOTHERAPY FOR LUNG CANCER

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Purpose: To establish a predictive model for the incidence of esophagitis for lung cancer patients treated with radiotherapy. **Methods and Materials**: 139 treatment charts of lung cancer patients treated with radiation therapy or combined chemo-radiotherapy from January 01, 2014 to June 30, 2017 at University of Mississippi Radiation Oncology were retrospectively reviewed. Mean esophagus dose (MED) and the endpoints of esophagitis grade 1 and 2 based on Radiation Therapy Oncology Group (RTOG) definitions were derived from the Pinnacle treatment planning system (TPS) and the EPIC electronic medical record (EMR) system, respectively. Binary logistic regression and Probit statistical analysis were used to determine the relationship between the probability of grade 1 and 2 esophagitis with the mean esophagus dose. **Results and Conclusions**: The regression model of the incidence of grade 1 and 2 esophagitis was established. The results suggest that MED is a good predictor of the risk of radiation-induced esophagitis. The mean esophagus doses associated with a 50% incidence probability (TD_{50}) for grade 1 and 2 esophagitis were determined as 1,510 cGy and 4,594 cGy, respectively. The parameters, n, m and TD_{50} as described in the Lyman Kutcher Burman (LKB) model were fitted and compared with other published findings. Our findings may be useful as additional clinical guidelines in treatment planning and plan evaluation, as well as obtaining informed patient consent.

COMPARING RIB CORTICAL THICKNESS MEASUREMENTS FROM COMPUTED TOMOGRAPHY (CT) AND MICRO-CT

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Motor vehicle crashes accounted for 35,000 deaths in the U.S., with many serious injuries occurring in the thorax. Predicting and preventing thoracic injuries can be difficult due to the compositional changes in the thorax with aging. Cortical bone thickness changes were analyzed in anatomical regions and cross-sectional quadrants of the ribs from clinical computed tomography (CT) scans and compared to cortical thicknesses obtained from micro-CT cadaver scans. A cortical thickness estimation algorithm was applied to retrospective CT scans of clinical resolution from 73 males, ages 10-92 years, and compared to thickness measurements from micro-CT scans of six male cadavers. Anterior and lateral regions of the 4^{th}-7^{th} left ribs were analyzed and thicknesses were compared between superior, interior, inferior, and exterior quadrants of rib cross-sections. In both CTs and micro-CTs, ribs were thinner in the anterior compared to lateral regions and interior quadrants were thickest. The average thickness for clinical CTs was 0.79mm for anterior vs 0.94mm for lateral regions (0.15mm difference). Average thickness for micro-CTs was 0.62mm for anterior vs 0.84mm for lateral regions (0.22mm difference). Interior quadrant thickness from clinical CTs was 1.06mm (average of 0.27mm thicker than other quadrants). Interior quadrant thickness from micro-CTs was 1.05mm (average of 0.42mm thicker than other quadrants). This study demonstrates the feasibility of the cortical thickness algorithm for analyzing rib cortical thickness from clinical CT scans. A better understanding of cortical thickness changes with age will lead to more biofidelic thorax models and improved occupant safety for all ages.

VALIDATION OF DETAILED ORGAN MODULARITY IN A SIMPLIFIED HUMAN BODY MODEL

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The significant computational resources required to execute detailed human body finite element models has motivated the development of faster running simplified models (e.g. GHBMC M50-OS). Previous studies have demonstrated the ability to modularly incorporate the validated GHBMC M50-O brain model into the simplified model (GHBMC M50-OS+B), which allowed for localized analysis of the brain at a substantially reduced computational cost. This study expands on this concept through modular incorporation of detailed thoracoabdominal organs into

the simplified model (M50-OS+O). The force-deflection responses between the M50-O, M50-OS, and M50-OS+O were compared through a test matrix of 5 hub-style biomechanics impacts, consisting of a frontal chest hub, oblique hub, lateral plate, and two abdominal bar simulations. Normalized run times for the various models used in this study were 16.8 min/ms for the M50-O, 0.30 min/ms for the M50-OS, and 1.57 min/ms for the M50-OS+O. Response from the abdominal bar simulation shows comparable results between the M50-O, M50-OS+O and the experimental data. The incorporation of the detailed organs into the M50-OS has shown the ability to obtain abdominal force-deflection response comparable to the experimental data and M50-O response, but with a runtime reduction of 90%.

MODULATION OF STEM CELLS WITH ELECTRICAL STIMULATION DELIVERED VIA PENETRATING NANOELECTRODES

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The application of electrical stimulation to stem cells is currently being explored as a method to facilitate their differentiation into various cell lineages. The potential differentiation of adipose-derived stem cells (ADSCs) to cardiac or neural phenotypes is particularly interesting due to the ubiquitous nature of adipose cells throughout the body, their ease of extraction and rapid expansion. In this work, the electrical stimulation is delivered using penetrating nanoelectrodes Penetrating electrodes provide direct access to the cell's interior in a minimally invasive fashion, and with enhanced cell-electrode coupling. Nanoelectrodes (pillars and fins) of controlled height, diameter, and density are fabricated on silicon using nanofabrication techniques. Extensive experiments confirmed the ability of the nanoelectrodes to penetrate cells. ADSC were then modulated by applying electrical stimulus of 500-500 mV/cm for 15 minutes per day for 5 days. The differentiated neural phenotypes were validated using various stem cell surface markers and electrophysiology measurements.

ULTRASOUND-STIMULATED PHASE-CHANGE CONTRAST AGENTS ENHANCE THE PENETRATION OF MACROMOLECULES THROUGH EPITHELIAL MONOLAYERS

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Focused ultrasound (US)-mediated drug delivery is a promising method to transiently permeabilize biological barriers and enhance the delivery of therapeutic molecules in a site-specific and image-guided manner. We have developed a nanometer-scale formulation of phase-change ultrasound contrast agents (PCCAs), which we believe is well suited for applications in US-guided gastrointestinal (GI) drug delivery. PCCAs offer the unique advantage of being appropriately sized (100-200 nm) to navigate the mucus mesh and reach the underlying GI epithelial barrier for permeabilization. As a first step toward this goal, we optimized US parameters (1.0 MHz, 30s exposure, 300-600 kPa, 20-40% duty cycle) necessary to reversibly disrupt Caco-2 monolayers and potentiate transepithelial delivery of macromolecular drug mimics. Caco-2 cells were grown to confluence on permeable Transwell® supports and transferred to a custom water bath for US treatment in the presence of PCCAs and FITC-dextrans (3 or 70 kDa). The receiving compartment was sampled over 72 hours to quantify dextran delivery, and monolayer disruption/recovery kinetics were monitored over the same time frame. We were able to achieve 14-49% transepithelial delivery of 3 kDa dextran and 10-43% delivery of 70 kDa dextran. This is in comparison to the <1% delivery found for control treated cells. In general, disrupted monolayers recovered within 48-72 hours. These data demonstrate that it is feasible to transiently permeabilize epithelial monolayers with US-stimulated PCCAs and suggest that in vivo translation for GI drug delivery applications is warranted.

Session VIII: Biomaterials and Drug Delivery

PRELIMINARY EVALUATION OF ELECTROSPUN POLYDIOXANONE TEMPLATES ELUTING ACTIVE CL-AMIDINE TO INHIBIT HUMAN PAD4

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Neutrophils modulate the microenvironment around tissue regeneration templates through the release of neutrophil extracellular traps by NETosis, a form of regulated cell death dependent on protein deaminase 4 (PAD4) activity. Recently, using human neutrophils, we showed that electrospun polydioxanone (PDO) templates selectively modulate the degree of NETosis, with small diameter (SD) fibers (0.30±0.1 μm) eliciting a higher degree of NETosis than large diameter (LD) fibers (1.9±1.0 μm), which translated to fibrotic encapsulation of SD templates *in vivo*. In this study, Cl-amidine (1-5 mg/mL), an irreversible inhibitor of PAD4, was electrospun with PDO into SD and LD templates, and elution from the templates (n=6) was evaluated over 1 hour using a PAD4 inhibitor assay. At 30 min, SD templates achieved greatest inhibition of PAD4 activity at 99.5±2.1%, which was significantly greater inhibition ($p<0.05$) than LD templates at 52.5±24.7%. This effect may be due to the high surface-area-to-volume ratio of the SD templates, resulting in a significant burst release of Cl-amidine, which may be beneficial for down-regulating PAD4 activity and NETosis, potentially translating to improved tissue regeneration *in vivo*. Future work includes executing an *in vitro* study with human neutrophils to evaluate the ability of the drug to inhibit NETosis.

PRE-CLINICAL INVESTIGATION OF LOCAL LIQUID PACLITAXEL DELIVERY VIA A NOVEL PERFUSION CATHETER

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Non-stent drug delivery platforms have recently emerged as an alternative treatment of peripheral arterial disease. Perfusion catheters have the potential to directly deliver drugs to the medial arterial layer to prevent restenosis. The purpose of this study was to determine the effectiveness of a perfusion catheter to deliver liquid paclitaxel, a proven anti-proliferative agent. A mathematical model was developed to test the impact of pressure on drug penetration into the medial wall. A bench-top model was utilized to determine the varying parameters of a perfusion catheter to maximize liquid paclitaxel delivery using pharmacokinetic evaluation and fluorescent microscopy. In addition, bilateral rabbit iliac arteries were treated with the perfusion catheter and pharmacokinetic evaluation performed at 1 hour to 3 days. The mathematical model demonstrated penetration of drug into the arterial wall is based on treatment chamber pressure. Bench-top testing demonstrated uniform and circumferential penetration of paclitaxel within the treated arteries. The results of the ex vivo test identified two groups with and without an excipient with similar loading conditions. The in vivo pharmacokinetic analysis of these two groups demonstrated the use of contrast agent increased arterial paclitaxel levels and maintained initial paclitaxel dosing up to 3 days (with excipient: 1 hr: 107±62 ng vs. 3 days: 40±23 ng, p = 0.824; no excipient: 1 hr: 247±120 ng vs. 3 days: 2.92±2.9 ng, p=0.009). These results demonstrate the feasibility to deliver liquid paclitaxel directly to the medial layer via a perfusion catheter.

COMPUTATIONAL FLUID DYNAMICS OF AN AORTIC BENCH-TOP MODEL

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In treating diseases within the aorta, minimally invasive endovascular techniques like transcatheter aortic valve replacement (TAVR) are becoming more prevalent. In order to evaluate and optimize the design of catheter devices a robust and durable aortic bench-top model was created for experimentation and testing. In this study, numerous computational fluid dynamic (CFD) simulations were performed on the aortic bench-top model in order to influence hardware settings like effective fluid resistance at the aortic branches and descending aorta. By introducing and manipulating nozzles at the bench-top model outlets, it was found that the model is capable of producing aortic branch fluid resistance values from 1.5 - 2000 mmHg/L/min, which is well within the range of physiological relevance. Once determining the dependence of fluid resistance on nozzle geometry, effective fluid resistance values were applied to the branching arteries and descending aorta to perform pulsatile flow CFD simulations with both blood and water glycerol to verify water glycerol as a blood analog. Water glycerol generated flow profiles and volume flow waveforms comparable to blood on the aortic bench-top model, thus verifying the mixture created in the lab as an effective blood analog.

EFFECTS OF SIMVASTATIN-CONTAINING POLYMERIC PRODRUGS ON BONE FORMATION IN VIVO

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Previous research on simvastatin-containing polymeric prodrugs demonstrated slow degradation in vitro. The objective of the current study was to evaluate in vivo degradation and the bone-forming potential of simvastatin-containing polymeric prodrugs in a rodent model. Poly(ethylene glycol)-*block*-poly(simvastatin) and poly(ethylene glycol)-*block*-poly(simvastatin)-*ran*-glycolide were synthesized by ring opening polymerization and pressed into disks of 6 mm diameter and 2 mm thickness. Poly(lactide-co-glycolide) (PLGA; 75:25) and simvastatin-loaded PLGA of similar dimensions were used as control. Disks were implanted over the calvarium and directly under the periosteum of male Sprague-Dawley rats. Animals were euthanized at predetermined time intervals and the calvaria analyzed for new bone formation by histology and microcomputerized tomography. Woven bone was observed with all samples, and the thickness of the woven bone was increased at the periphery. Bone formation was greatest with poly(ethylene glycol)-*block*-poly(simvastatin), whereas severe skin ulceration was observed at weeks 1 and 2 post-implantation with the faster-degrading poly(ethylene glycol)-*block*-poly(simvastatin)-*ran*-glycolide. Increased swelling, severe skin ulceration, and bone resorption were also observed with simvastatin-loaded PLGA at 4 weeks post-implantation. Of the prodrugs tested, poly(ethylene glycol)-*block*-poly(simvastatin) promoted new bone growth with less inflammation. This study shows that polymeric prodrugs with controlled degradation and sustained release limit the inflammatory responses and promote bone formation.

AN EXPERIMENTAL AND COMPUTATIONAL STUDY OF THE EFFECT OF BIOCERAMIC POROSITY ON DRUG RELEASE KINETICS

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Porous bioceramics are explored as drug carriers in targeted drug delivery applications. The drug release rate, cumulative drug release (CDR) and the duration of release are dependent on the porosity characteristics of the carriers. In this study, Cristobalite disks with different porosities are studied as carriers of the drug Vancomycin. The experimental work

involved preparation of Cristobalite disks followed by a study of the drug binding and release kinetics. Cristobalite disks with different porosity characteristics were loaded with Vancomycin. Drug release kinetics were then studied by immersing the disks in PBS in polystyrene jars. The amount of drug released during various time intervals was measured and CDR was calculated. In addition, SEM analyses of Cris-PEG disk sections were performed to study the pore size distribution of the disks. Computational work was carried out to study the significance of various mechanisms driving drug from the ceramic disks and to aid in optimal delivery systems. Drug release process from the disks involves burst-release and sustained-release phases. These two mechanisms are modeled using the Fickian Theory of Diffusion and the Finite Element Method. Axisymmetric finite element models of the disk and the PBS region were developed and solved using the FEM package ABAQUS and MATLAB. The diffusion and mass-diffusion efficients, essential for these models, were obtained by matching the computational and experimental values of CDR. Relation between the drug release kinetics and the pore size distribution was also studied to identify the pore size categories controlling the release kinetics.

SUSTAINED DELIVERY OF ESTROGEN AS A MODEL FOR REPLACEMENT THERAPY USING OVERIECTOMIZED RODENTS

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Tricalcium phosphate lysine (TCPL) delivery system was used effectively to deliver various organic compounds at sustained levels in many different models. The specific aim of this investigation was to utilize TCPL delivery system as a model for estrogen (E) replacement therapy in post-ovariectomized adult rats mimicking a postmenopausal condition. A total of 24 adult female rats were used in this study. The animals were randomly divided into three different groups: groups 1, and 2 were overiectomized (OVX), and OVX plus E (20 mg loaded TCPL), respectively. Group 3 animals (n=4) served as intact control group. Blood samples were collected biweekly for four weeks. Vaginal smears were taken and screened daily during the entire investigation. The total serum levels of E, P, luteinizing hormone (LH), and follicle stimulating hormone (FSH) were measured by means of radioimmunoassay procedure. Data obtained from this investigation suggest the following: (I) OVX resulted in an increase in total serum levels of LH and FSH within 2 days post-ovariectomy, (II) TCPL were capable of releasing sustained levels of E (15-48 pg/ml) at the end of second day and continued until the four weeks, (III) the sustained level of E was able to suppress the post ovariectomy rise of LH and FSH to almost undetectable levels, (IV) sustained delivery of E resulted in maturation of vaginal epithelium and the smears exhibited the estrus phase throughout the investigational period.

ATOMIC LAYER DEPOSITION OF NANO-COATINGS ON FABRICS FOR ANTIBACTERIAL APPLICATIONS

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About 1.7 million Americans contract hospital-acquired infections every year, resulting in 99,000 inadvertent deaths and an estimated $20 billion in healthcare costs. Here, we investigate the use of atomic layer deposition (ALD) to treat various fabrics with antimicrobial inorganic materials to create unique antibacterial textiles. Our protocols include biological testing of the antimicrobial performance of ALD-treated fabrics against DH5-α, a strain of *E. coli* that is engineered to be suitable for laboratory purposes. Antibacterial performance is tracked as a function of ALD cycle number at a deposition temperature of 90°C. DH5-α is exposed to the fabrics and incubated for 20 hours, after which cultures are diluted, spotted onto Petri dishes, and incubated for another 20 hours. Colony counting is then used to quantify antimicrobial effectiveness. For ZnO ALD coatings of 0, 1, 10, and 100 cycles, we find that only the 100-cycle sample is sufficiently cytotoxic to kill all of the *E. coli* bacteria. Interestingly, for 1 and 10 cycles of ZnO, bacteria grow more rapidly. We attribute this increased growth rate to the Zn^{2+} ions acting as a nutrient for the bacteria. It is known that in order to be an effective antimicrobial agent, ZnO must be "nano-sized" or larger; ZnO, which dissolves into Zn^{2+} in PBS, acts instead as a bacterial metabolite. A more detailed investigation of this transition from nutrient to antimicrobial will be discussed. We will also examine how long these antimicrobial effects last, as well as the stability of ZnO in environments of varying pH.

Session IX: Neuroscience

HIGH-RATE MECHANICAL INSULT CONTRIBUTES TO ALTERATIONS IN BRAIN CELL SIGNALING AND REACTIVITY

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Traumatic brain injury (TBI) is complex pathology with numerous long-term debilitating symptoms associated with damage to the brain parenchyma. It is necessary to better understand TBI at the cellular and molecular level in order to mitigate organ-level dysfunction. In particular, there is a lack in understanding the interplay of injury mechanics and mechanobiological responses in brain cells. This study aimed to analyze such effects from higher-rate injuries, through an *in vitro* injury model of primary mixed brain cells (neurons and astrocytes). Neurons and astrocytes interact in the proper functioning of neural networks and are critical components in the injury response of the central nervous system. The goal of this study was to analyze expression of abundant structural and adhesion molecules expressed by neurons and astrocytes to understand how intra- and intercellular signaling may be compromised as a result of high-rate mechanical insult. Target expression was measured for β-actin, vinculin, β-tubulin, ezrin, connexin-43, and glial fibrillary acidic protein. Transient alterations in both neuron and astrocyte-specific molecules occurred over the time course of 48 hours after insult. This has implications for compromised cell-cell communication and provides potential molecular targets for mechanobiological mechanisms associated with neuron and astrocyte dysfunction following high-rate mechanical insult.

ALLOSTERIC DRUG DESIGNING FOR HORMONE THERAPY RESISTANT BREAST AND PROSTATE CANCERS

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Hormone therapy resistant breast and prostate tumors present a major challenge in drug designing. In about 30% of the cases these tumors, once responded to hormone therapy, are found to lead to progression even in the presence the therapy. In order to address the issue, we identify alternate protein targets by elucidating protein-protein and protein-DNA interfaces of ERα and AR and use them to develop new generation of anti-cancer agents. Using the crystal structures of ERα and AR Ligand binding and DNA binding domains, molecular modeling, molecular dynamics simulations, and bioinformatics we identified the hydrogen-bonding contact motifs that are responsible for dimerization and/or DNA recognition. The crucial amino acids of a motif are then grafted on stable helices (alanine and glutamine) in order to develop peptidic inhibitors. In

ERα, using the dimerization sequence motif LQXXHQXXAQ (497-506) as a template we have developed designer peptides AAHQALAQAAAAAAAAA and AADQADAQAAAAAAAAA which exhibit significant suppression of ER-expression in MCF-7 breast cancer cell lines. The designer peptides inhibit ERα dimerization – an essential process in ER mediated transcription. In AR, protein-protein binding contacts are insignificant. The LCAXRXD motif (578-584) that binds with AR and DNA is being targeted fto develop inhibitor peptide. Author acknowledges financial support from MS-INBRE funded by NCRR/NIH-5P20RR016476-11 and NIGMS/NIH-8P20GM103476-11.

ANTI-INFLAMMATORY CYTOKINE INTERLEUKIN-1 RECEPTOR ANTAGONIST REDUCES LIPOPOLYSACCHARIDE-INDUCED BRAIN HIPPOCAMPAL INJURY AND IMPROVES COGNITIVE IMPAIRMENT IN JUVENILE RATS

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Inflammation in neonatal human and animal models has been shown to be associated with cognitive dysfunction later in life. Our previous studies have shown that administration of interleukin-1 receptor antagonist (IL-1ra) can protect against lipopolysaccharide (LPS)-induced sensorimotor dysfunction and brain inflammation in neonatal rats. The objective of this current study is to further determine whether IL-1ra protects against LPS-induced chronic brain inflammation, hippocampal injury, and cognitive dysfunction in juvenile rats. Intraperitoneal (i.p.) injections of LPS (2 mg/kg) or saline was performed in postnatal day 5 (P5) Sprague-Dawley rat pups, and IL-1ra (100 mg/kg) or vehicle was administered (i.p.) at 5 min, 24, 48, and 72 hours after LPS injection. Neurobehavioral tests were carried out from P14 to P22, and brain injury was examined at P22. Our results showed that neonatal systemic LPS exposure resulted in cognitive deficits and chronic inflammation in juvenile rats which were associated with hippocampal neuronal injury, as indicated by loss of NeuN (neurons) immunoreactivity in the hippocampus of the P22 rat brain. IL-1ra treatment significantly attenuated LPS-induced cognitive deficits and hippocampal injury. IL-1ra administration also significantly attenuated LPS-induced increases in the numbers of Iba1+ cells (microglia) and increases in IL-1β concentration in the hippocampus of the P22 rat brain. These results suggest that IL-1ra provides protection against neonatal LPS exposure-induced chronic inflammation, hippocampal injury, and cognitive deficits in juvenile rats, which may be associated with the blockade of LPS-induced pro-inflammatory cytokine IL-1β.

OXIDATIVE STRESS IS ASSOCIATED WITH DYSFUNCTIONAL NEURODEVELOPMENT IN RAT OFFSPRING EXPOSED TO PLACENTAL INSUFFICIENCY

Norma Ojeda[1], Iman Washington[2], Brenkeevia Langston[2], Oluwatosin Akinyemi[3], Colin Muncie[1], Jonatham Lee[1], Silu Lu[1], Lir-Wan Fan[1]

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Placental Insufficiency is a pregnancy complication compromising the delivery of blood and nutrients to the fetuses. The effects of exposure to this condition on the neurodevelopment of newborn and juvenile offspring are not well understood. We hypothesized that exposure to placental insufficiency is associated with increased oxidative stress status and dysfunctional neurodevelopment in rat offspring. To test our hypothesis, we performed the reduced uterine perfusion (RUP) surgery in pregnant rats at 14 days of gestation to induce placental insufficiency. Motor skills were tested on postnatal day 8 (P8) and postnatal day 21 (P21) along with measurement of oxidative stress markers. Placental insufficiency-exposed offspring showed significant reductions in birthweight and growth pattern when compared to controls ($P<0.05$). Righting reflex and sensorimotor response were significantly delayed at P8 in placental insufficiency-exposed offspring compared to controls ($P<0.05$). Cliff avoidance response and beam walking latency time were significantly delayed in placental insufficiency-exposed offspring compare to controls ($P<0.05$). Markers for oxidative stress at P8 and P21were significantly increased in placental insufficiency-exposed offspring compared to the controls ($P<0.05$). These findings suggest that placental insufficiency is associated with an increase in oxidative stress and dysfunctional neurodevelopment in newborn and juvenile rat offspring

HIGH SENSITIVITY MICROBIOSENSORS FOR DETECTION OF GLUTAMATE AND DOPAMINE IN BRAIN TISSUE

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Enhanced neurochemical microsensors were developed for brain slice recordings where higher sensitivity is required to detect small, dynamic changes in glutamate and dopamine levels. Glutamate microbiosensors were created by drop casting a mixture of 0.1 U/μL glutamate oxidase, 1% BSA and 0.125% glutaraldehyde. After curing, a size-exclusion polymer, m-phenylenediamine, was electrochemically deposited to prevent ascorbic acid, an interferent, from reaching the electrode surface. Dopamine microsensors were created by electrophoretically depositing ~100-nm thick multiwall carbon nanotubes (MWCNT) onto platinum microelectrode arrays and then drop casting 0.2 μL of 5% wt. nafion solution. Murine coronal brain slices were maintained in artificial cerebral spinal fluid. Caudate putamen was used to test dopamine and parietal cortex was used to test glutamate microbiosensors. Current responses to biphasic stimulation, were recorded on a F.A.S.T. 16mkIII system (Quanteon). Responses were compared to standard curves from 1-40 μM of glutamate and 10-400 nm dopamine. The open pores present in the MWCNT film contributed to a significant increase in the electroactive area and adsorption sites for dopamine. *In vitro* calibration studies showed that with nafion coating, the MWCNT modified microelectrode had a 100-fold increase in DA sensitivity (20 nA/μM). The glutamate microbiosensor showed a sensitivity of 25 pA/μM, which is much higher than similar commercial probes (<15 pA/μM) reported in the literature. Future work includes combining these microsensors into a single probe and further refinement of the coatings for chronic, *in vivo* recordings. Funding from NSF EPSCoR RII-2 FEC OIA1632891.

Posters

NEAR-INFRARED CAMERA FOR EARLY DETECTION OF DIABETIC ULCERS

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Peripheral Arterial Occlusive Disease (PAOD), also known as diabetic foot ulceration, is the leading cause of lower extremity amputation. In order to combat this, a near infrared camera prototype has been constructed using a Raspberry Pi system with optical filters. Due to deoxygenated nature of venous blood, blood accumulation can be detected and photographed at near infrared wavelengths. In conjugation with a MATLAB photograph processing program, the device can monitor the progress of ulceration formation by the change in diameter of the blood vessels. Loss of sensation in the peripheral extremities is a common symptom of PAOD, which can allow the formation of ulcers to proceed without the patient's knowledge. In order to detect ulcerations prior to severe complications, regular inspections of the afflicted area are necessary by the physician. This process is costly, time-consuming, and inconvenient for the patient. To improve this process, a handheld, low-cost prototype was developed for patients to self-monitor the progression of their ulceration at home. The current goal of the project is to determine the optimal wavelength, LED arrangement, and imaging conditions to produce the most accurate representation of the vasculature. Multiple images have been captured, processed, and evaluated through the usage of a MATLAB processing code. Further prototype improvements include data sharing between the patient and physician, enhancing user-friendliness, and ensuring universal usage.

THE EFFECT OF GABA RECEPTOR ANTAGONIST IN TRAMADOL AND TRAMADOL/GABAPENTIN MEDIATED ANTINOCICEPTION IN MICE TAIL-FLICK TEST

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Pain prevention as well as treatment is among the major concern for the healthcare authorities across the world. Adequate therapeutic treatment for pain management is still a major clinical target. Our previous studies have demonstrated that gabapentin [a γ-aminobutyric acid (GABA) derivative] potentiates tramadol antinociception and attenuates the tolerance to tramadol in mice tail-flick test. The underlying mechanisms are not clear. It had been acknowledged for that GABA receptors play important role in pain modulation. To induce a change in GABA receptor activity could offer a suitable approach. The present study is focused on whether GABA receptors antagonists induced activity involve in tramadol alone and tramadol in combination with gabapentin mediated antinociceptionin in mice tail-flick test. Experiments were conducted in NIH Swiss male mice (8/treatment group). $GABA_A$ receptor antagonist bicuculline (2 mg/kg), $GABA_B$ receptor antagonist 2-hydroxysaclofen (3 mg/kg) and gabapentin (75mg/kg) were administered to mice 30 min before tramadol (60mg/kg) administration respectively. Mouse tail-flick response latencies to heat stimuli were tested 30 min after tramadol administration for the evaluation of nociceptive reaction. Data were expressed as mean ± standard errors of the mean (SEM). The results showed that GABA receptor antagonist alone did not induce the antinocicptive effect. However, use of tramadol, tramado/gabapentin in combination with bicuculline, the response latency increased from 6.73±0.25, 7.86±0.33 (sec) to 7.63±0.34, 8.66±0.42 (sec) ($p<0.05$). $GABA_A$ receptor involved in the tramadol and tramadol/gabapentin mediated antinociception.

NEONATAL SYSTEMIC EXPOSURE TO LIPOPOLYSACCHARIDE ENHANCES ADULT SUSCEPTIBILITY TO THE NEURODEGENERATIVE DISORDER INDUCED BY PARAQUAT

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We have previously shown that neonatal intracerebral injection of lipopolysaccharide (LPS) increases the risk of rotenone (a commonly used pesticide)-induced dopaminergic damage in adult rats. This study was designed to further test whether neonatal systemic LPS exposure also increases the vulnerability of adult dopaminergic neuron to paraquat, a widely used herbicide. LPS (2 mg/kg) was administered intraperitoneally into postnatal day 5 (P5) rats. On P70, rats were challenged with paraquat through subcutaneous mini-pump infusion at a dose of 0.3 mg/kg per day for 14 days. This paraquat treatment regimen ordinarily does not produce toxic effects on behaviors in normal adult rats. However, LPS pre-exposed rats developed Parkinson's disease-like motor neurobehavioral impairments after paraquat treatment, including bradykinesia (prolongation of the movement time), akinesia (prolongation of the reaction time), and rigidity (increase in muscle tone or magnitude of stretch reflexes). Structural examination of the nigrostriatal pathway revealed that neonatal LPS exposure enhanced paraquat neurotoxicity to cause a significant loss of tyrosine hydroxylase immunoreactive neurons in the substantia nigra, and a decrease in retrogradely labeled nigrostriatal dopaminergic projecting neurons of rats. Our results indicate that perinatal brain inflammation may cause the nigrostriatal system in the adult brain to become more vulnerable to damage by environmental toxins at an ordinarily non-toxic or sub-toxic dose, leading to the development of Parkinson's disease-like motor dysfunction and pathological features.

EFFECT OF A 3D ASSEMBLY TECHNIQUE ON UROTHELIAL TISSUE STRATIFICATION IN VITRO

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The transitional urothelium of the bladder is comprised of layers of umbrella, intermediate, and basal urothelial cells that each serve important physiological functions including; barrier function, elasticity, and signal transduction. The objective of this study was to investigate the effects of a 3D, multilayer assembly technique on urothelial cell differentiation and stratification in vitro. UROtsa cells (urothelial cell line) were coated with fibronectin and gelatin (FN-G) in a layer-by-layer method and seeded at a high density to form multiple layers onto FN-G coated cell culture inserts. Controls were cells seeded without the ECM coating and both groups were cultured in growth media under standard conditions (37 °C, humidified, 95% air, 5% CO_2). After 48 hours, cell morphology and phenotypic marker expression were evaluated via histology and immunofluorescence. The results showed that urothelial cells formed tightly packed multilayers when both cells and the substrate were coated with FN-G. While all groups displayed basal and superficial urothelial cell phenotypic markers, the multilayer structure showed higher expression of umbrella cell markers and tight junction markers at the apical surface. These results indicated that the 3D microenvironment of cultured urothelial cells impacts their phenotype and such 3D constructs may serve as a better urothelial tissue model for testing of pharmacological compounds than conventional monolayer cultures.

IMPACT OF ATRA ON OVALBUMIN AND MOLD-SENSITIZED F344 RATS AND REVERSAL OF HEALTH-RELATED IMPLICATIONS BY CITRAL

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The objective of this study was to evaluate their interaction as a remedy for hypervitaminosis A. This IACUC approved in vivo study used Fischer 344 rats (n = 80 ;229 to 273g), which were randomly assigned to controls as well as ovalbumin and mold-sensitized treatment groups (0.80 mg/kg and 1X109 mold spores combined from 4 strains/100 µl intra-tracheal; all others were dosed by intra-peritoneal injection at days 1 and 7 with 80 mg/kg each of ATRA as well as 20 and 50 mg/kg each of Citrals 1 or 2 individually or in combination to represent all four chemicals and mold spores treatments. Animals were housed in rat cages at the JSU Research Animal Core Facilities and were placed on a 12:12 light–dark cycle. A standard rodent diet and water access were provided ad-libidum. Rat weights were recorded on day 1 and 21, all animals were sacrificed on day 21 and blood was collected and processed for hematological parameters. Results showed that even though C1 and C2 were not toxic individually, their combination at high dosing was lethal. Exposure of ovalbumin-sensitized rats to ATRA showed various levels of weight losses and negative hematological implications that were ameliorated by exposure to Citrals at various combinations with retinoic acid. Taken together, the study showed that there are variable pathophysiological responses from the interaction of ovalbumin, mold spores and retinoic acid and that Citrals were found to be individually effective in reversing health-related pathophysiologies.

PATIENT-SPECIFIC TREATMENT PLANNING FOR IRREVERSIBLE ELECTROPORATION: A NUMERICAL ANALYSIS WITH USING DYNAMIC ELECTRICAL TISSUE PROPERTIES FROM HUMAN PANCREATIC TISSUE

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Irreversible electroporation (IRE) is a minimally invasive focal ablation technique used to nonthermally ablate soft tumor tissue. Due to its nonthermal mechanism of ablation, IRE can be safely implemented in unresectable tumors located near critical vasculature. Although effective, widespread use of IRE is hindered by challenges associated with accurate treatment planning protocols and the total duration of treatment. Our group has established a pre-treatment planning methodology for IRE procedures in locally advanced pancreatic cancer (LAPC). In order to better inform these numerical models, human pancreatic tissue samples from both malignant and healthy tissue were subjected to pulsed electric fields and the resulting voltage and current waveforms were analyzed to determine the changes in tissue impedance as a function of electroporation. This relationship was applied to the numerical model. By simulating Joule Heating effects and heat transfer within biological tissue (Pennes Modified Bioheat Equation), we determined the rise in temperature due to IRE therapy (<12°C) and established that the use of multiple monopolar or bipolar probes are capable of ablating large tumors with minimal rise in temperature. Additionally, by incorporating a dose-dependent thermal damage integral, we calculated the volume of tissue ablated by thermal means, and show this volume to be minimal compared to IRE ablation volume.

DEACTIVATION OF NEMATODE EGGS IN WASTEWATER FOR PARASITIC DISEASE MITIGATION

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The eggs of parasitic helminth worms are incredibly resilient—possessing the ability to survive changing environmental factors and exposure to various chemical treatments—and while conventional sanitation methods are able to inactivate the eggs, they are largely inefficient in doing so. This research reports on the effectiveness of electroporation to dispatch the eggs of Caenorhabditis elegans (C. elegans), a helminth surrogate, and explores applications for wastewater sanitation. This technique which has traditionally used electric pulses to increase cell membrane permeability is used to open pores in non-parasitic nematode eggshells in the current study. This is the first report of such an application, to the best knowledge of the authors. A parametric evaluation of electric field strength and treatment duration of eggs and worms in phosphate buffer solution was performed using a 1-Hz pulse train of 0.01% duty cycle. The extent of pore formation was determined by quantifying the fluorescence intensity of propidium iodide, a fluorescent label that targets C. elegans embryonic DNA. Both in-situ and ex-situ fluorescent microscopic imaging of C. elegans during treatment was performed in custom designed test cells. The results of this research demonstrate that electroporation increases eggshell permeability through potential channel formation within the shell. No obvious change in the geometric size and shape of the eggs was observed. Based on our observations thus far, we discuss current treatment conditions and associated energy consumption requirements for destroying C. elegans eggs, and by extension helminth parasites, in wastewater.

FABRICATION OF 3D ALGINATE HYDROGEL SCAFFOLDS

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Sodium alginate, a natural polymer with low toxicity and proven biocompatibility, has been investigated for cell encapsulation in biotechnology and medical applications like, new drug development, toxicity testing, tissue engineering and controlled release of therapeutic agents. This research focused on fabrication of alginate based hydrogel encapsulates with three different types cells: primary rat hepatocytes, human liver carcinoma cell (HepG2) and human bronchial epithelium (BEAS 2B). Cell encapsulates were made by using electrostatic process. High densities, 25-40 million per ml of cells were successfully encapsulated in the hydrogel microbeads. The encapsulation efficiency was nearly 100 %. Microbeads size was in the range of 300-600 µm, which was determined by using inverted microscopy. Microbeads retained their stable 3D morphology even after 2 weeks of incubation in cell media. LDH assay showed viability of the encapsulated cells at 80% after 24 h incubation. This is also supported by live dead staining of encapsulated cell. These microbeads could be potentially used in different field of biotechnology including tissue engineering and organ specific toxicity testing.

BIODEGRADABLE SIMVASTATIN-CONTAINING POLYMERIC PRODRUGS FOR IMPROVED DRUG RELEASE

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The use of simvastatin for bone regeneration is a promising and growing area of research. Simvastatin can promote osteoblastic activity and inhibit osteoclastic activity, which is important for bone regeneration. Our group previously developed and explored the physical and chemical properties of a simvastatin containing polymeric prodrug [RSC Adv 4: 58287, 2014]. The hydrophobic nature of the prodrug component of the block co-polymer, however, leads to slow release of simvastatin in vitro. In this study, we hypothesized that degradation could be accelerated by chemically modifying the polymer backbone by introducing glycolide and lactide co-monomers. Copolymers were formed by ring-opening polymerization using 5 kDa monomethyl ether poly(ethylene glycol) (mPEG) as the initiator in presence of triazabicyclodecene catalyst. In addition to simvastatin, modified reaction mixtures contained lactide or glycolide. In vitro drug release was evaluated using small prodrug pellets (~20 mg) prepared by solvent casting on Teflon. Discs were incubated in phosphate-buffered saline, pH 7.4, under continuous shaking at 37ºC. Incorporation of the less hydrophobic glycolide monomer in the reaction resulted in a polymer that released more simvastatin compared to incorporation of the hydrophobic lactide monomer. In summary, we modified simvastatin release from the degradable polymeric prodrug by chemically modifying the polymer backbone.

CHARACTERIZATION OF BLUE LIGHT CROSSLINKED POLY(B-AMINO ESTER)S

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Current methods of poly(β-amino ester) (PBAE) crosslinking involve the use of toxic solvents and harmful ultraviolet (UV) irradiation, both of which raise cytotoxicity concerns for *in situ* polymerization. The aim of this study was to develop an aqueous blue light (BL) crosslinking protocol and compare it to UV crosslinking. Poly(ethylene glycol) diacrylate (H), tetra(ethylene glycol) diacrylate (D), and di(ethylene glycol) diacrylate (A) were reacted with isobutylamine (6) using a 1.2:1 molar ratio of acrylate to amine, based on previous research [*Adv Mater* 18:2614, 2006]. Five different macromers were synthesized: H6, DH6 3:1, AH6 3:1, D6, and A6. Degradation and swelling ratios, along with mechanical studies, were used to characterize the two polymerization methods. There were no differences in degradation profiles between the crosslinking methods, but BL crosslinked hydrogels had higher swelling ratios, lower density, and lower modulus when compared to those prepared by UV crosslinking. In summary, BL polymerization imparts differences in mechanical properties, swelling, and density of the PBAEs tested while still retaining the degradation profiles associated with the UV polymerization method

CONCENTRICALLY AND AXIALLY MULTIZONAL HYBRID POLYMERIC SCAFFOLDS

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Spatiotemporal control and patterning of signals is a critical design element in the engineering of scaffolds to mimic and maintain the complex structure of tissues. In the present study, concentrically and axially graded systems were examined to determine the compositional relationship, mass loss, and pattern of porosity development to design application-based scaffolds.

LASER PROBE WITH INTEGRATED COOLING FOR SUBSURFACE TISSUE THERMAL REMODELING

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Introduction: Over 6.5 million women in U.S. suffer from stress urinary incontinence (SUI).Only ~200,000 women choose surgery. There is a role for a non-surgical, minimally invasive procedure that provides thermal shrinkage/remodeling of submucosal collagen in endopelvic fascia. This study describes design, characterization, and preliminary testing of a novel probe with integrated contact cooling for potential use in transvaginal laser treatment of SUI. Methods: Laser energy at 1075 nm was delivered through a 600-micron-core fiber optic patchcord into a 90^0 side-firing probe (19 x 22 mm) with integrated flow cell and sapphire window cooled to -4°C by circulating an alcohol-based solution. An inflatable balloon attached to probe insured contact with vaginal wall. A force sensor and thermocouples monitored pressure and temperature. Thermal lesions were created in three cadavers in a dose escalation study (P = 4.6-6.4 W, Spot = 5.2 mm, Time = 30 s). Results: Thermal lesion areas measured 3.1-4.6 mm^2, while preserving vaginal wall to a depth of 0.8-1.1 mm. Consistent tissue contact and cooling was maintained using force sensors. Conclusions: Preliminary cadaver studies demonstrated subsurface treatment of endopelvic fascia with partial preservation of vaginal wall. Future studies will optimize parameters for thermal remodeling with tissue surface preservation.

FABRICATION OF PLGA/PCL COMPOSITE FIBERS FOR CONTROLLED DRUG RELEASE

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Controlled release of drug with implanted medical devices are useful to avoid high drug dose to limit non-target site toxicities. In traditional drug delivery system, the amount of drug in blood could remain between a maximum and minimum level where the maximum level indicates toxicity to the body and the minimum value implies the ineffectiveness of the drug. However, in controlled drug delivery system, controlled release rate of drug, slow or fast, enables to maintain constant level of drug in blood for the desired period. In our research electrospun composite nano fibrous scaffolds of Poly (d-Lactic-co-glycolic) acid (PLGA) and polycaprolactone (PCL) were designed and fabricated for controlled drug release. Electrospinning is a useful tool in drug delivery as it allows to control diameter, material composition, and geometry of fibers. PLGA and PCL have been chosen because of their long track record to produce various structures and drug delivery devices since they have high profile of biocompatibility and bioresorbability. These polymers can disperse into biologically suitable molecules that can be absorbed and removed from our body through metabolic process. The release of drug can be adjusted by varying polymer weight ratio. We have investigated the nano-fibrous composite scaffold's surface morphology, drug release profile in phosphate-buffered saline (PBS) and mechanical properties for its potential application as a biomedical implant device.

MAGNESIUM OXIDE INCORPORATED ELECTROSPUN NANOFIBER OF NATURAL-SYNTHETIC COMPOSITE POLYMER BLENDS

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The ability to produce composite nanofibers of inorganic particles, natural and synthetic polymers represents a significant advancement in the development of composite materials for potential biomedical

applications because they capitalize on the favorable biological properties of the natural polymer and the ceramic, and superior mechanical properties of the synthetic polymer. However, effective synthesis of well-blended composite fibers remains a great challenge due to the poor miscibility between polymers and ceramic particles at the molecular level. In this study, composite nanofibers of magnesium oxide (MgO), poly(ε-caprolactone) (PCL) and chitosan (CS) with diameters in the range of 0.7–1.3 μm were fabricated by electrospinning their blend solutions in trifluoroethanol and water. To support the potential use of these nanofibrous membranes for biomedical applications their physicochemical properties such as morphology, mechanical strength, and integrity in aqueous medium, were studied. Cellular compatibility was determined using cell viability assays and microscopy imaging, with the results showing that the nanofibrous membranes support 3T3 cell viability and attachments. The new composite nanofibrous membranes developed in this study can mimic the physical structure and function of tissue extracellular matrix (ECM) and thus have potential for many tissue engineering applications.

CHITIN BASED ELECTROSPUN NANOFIBERS AND FILMS FOR APPLICATIONS IN BIOMEDICAL FIELDS

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Tissue Engineered scaffolds should be composed of biocompatible and biodegradable materials, exhibit mechanical properties like those of target tissue as well as have the structural and chemical properties closely mimicking those of the extracellular matrix (ECM). In this research, we explore the possibility of using poly(caprolactone) (PCL) and chitin to prepare nanofibrous mesh and films as suitable devices for biomedical implant applications. Chitin, a natural polymer is nontoxic, biodegradable, biocompatible and has been widely used for wound healing, enzymes immobilization, drug delivery and space-filling implants. PCL, an aliphatic synthetic polymer is biocompatible and has mechanical properties superior to natural polymers. Composite scaffolds of PCL and chitin were fabricated using electrospinning and spin coating technology in the form of nanofibrous mesh and films respectively. Scanning electron microscopy (SEM), Atomic force microscopy (AFM), Fourier transform infrared (FTIR), and mechanical test were done to evaluate the properties of composites in general for biomedical applications.

GENERATION OF RAPID *In Situ* FORMING CHANNELS WITHIN SOFT BIOMATERIALS VIA BIODEGRADABLE FIBER POROGENS

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Generation of continuous predefined channels within soft biomaterials is a challenge facing scaffold design for oriented tissues. Traditional methods of controlled pore generation in biomaterials depend on leaching of rigid sacrificial materials (e.g., sodium chloride, sucrose, and poly(lactic acid)). Such sacrificial casting methods employ harsh solvents and form features prone to damage and collapse during handling. Use of *in situ* degrading porogens circumvents porous material weakness by providing transient stabilization as well as additional drug delivery options. The purpose of this study was to evaluate the viability of rapidly degrading poly(b-amino acid) (PBAE) fibers as continuous *in situ* degrading porogens. PBAEs form versatile hydrogels with mechanical properties appropriate for soft tissue applications and can rapidly degrade via hydrolysis, but have not previously formed fibers. Preliminary thermopolymerization methods demonstrate rapid and consistent discrete fiber production. Encapsulated fibers with exposed ends form channels with defined edges within soft PBAE matrices within 3-5 days of immersion in phosphate-buffered saline. Channels are absent of obstruction and appear to be continuous from end to end. However, completely encapsulated fibers exhibited delayed or incomplete path clearance. Preliminary results indicate that rapidly degrading PBAE fibers may be used to generate continuous paths within soft biomaterials without requiring additional solvent rinses to remove embedded porogens.

CRYOGENIC PRESERVATION OF HEPATOCYTE ENCAPSULATES

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Cryopreserved hepatocyte encapsulates often have reduced viability and metabolic function in comparison with fresh cells and hence are often not suitable for clinical use. The lack of specific methodology for the cryopreservation of engineered cell encapsulates however, poses a challenge in creating a sustained post-thaw model. The aim of this study was to modify the different steps in the standard cryopreservation procedure in an attempt to improve the overall outcome. Controlled rate cryogenics is an attractive option for maximizing cell viability and preserving liver functions. The solution holds numerous potential benefits because primary hepatocytes experience expedited loss of metabolic function ex-vivo. In this work, we designed alginate encapsulated hepatocyte microencapsulates, and subsequently we developed a cryopreservation protocol. Different compound including fructose, alpha lipolic acid, ADP and dexamethasone (DEX) were used in pre-incubation culture to improve encapsulates viability. Encapsulates preincubated in DMEM culture media, DEX media and sugar media showed relatively better viability after thaw and post thaw culture at different time points. It was also found that dynamic post-thaw culture greatly improved sustained viability. Ongoing studies stemming from this research will include varying concentrations of the identified sugars and define application amongst other types of 3D engineered constructs.

OBSERVING TRENDS IN VITAMIN D SUPPLEMENTATION: NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY 2009-2014

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Decades of research have proven the efficacy of vitamin D in health and wellness, as well as, disease prevention. Adequate consumption of vitamin D is not commonly obtained by nutrient consumption in food alone. Therefore vitamin D supplementation is commonly recommended or prescribed to obtained adequate intake of vitamin D. This study analyzed trends in the frequency of Vitamin D supplement consumption for cycle year 2009-2010 through 2013-2014. Supplement information was obtained from National Health and Nutrition Examination Survey (NHANES) responses. Supplements which contained vitamin D were uniquely included in this study. This supplement information was used to observe the trends in vitamin D consumption in the total population, and by gender, race/ethnicity and age groups. The results of this study revealed that vitamin D is consumed mostly through multivitamins. The results also revealed an incline of vitamin D supplement consumption and a decline of those who consume calcium supplements containing vitamin D. This study concludes a positive trend in the consumption of the supplements containing vitamin D, which may be due to years of vitamin D research.

BLUETOOTH ENABLED SMARTPHONE APPLICATION FOR WIRELESS PHOTOPLETHYSMOGRAPHY MONITORING DEVICES

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This article presents the design, prototyping and testing steps of a wireless heart rate monitoring device based on photoplethysmography (PPG) technology. PPG monitoring devices use optical sensors to collect vital signs such as heart rate, blood pressure, oxygen saturation and cardiac output. The collected data must wirelessly be transferred to a smart device in real time for further additional processing. Therefore, wireless connectivity plays an important role in such biomedical instruments. A typical reflection-mode PPG monitoring device is made of an infrared sensor and a photodetector to illuminate the tissue and detect the variations in the light intensity of illuminated tissue, a signal conditioning stage to prepare the captured signals through amplification and filtering, a low-power microcontroller to control and manipulate the analog PPG signals, and a Bluetooth module to transmit the PPG data to a Bluetooth-based smart device such as a tablet. A user-friendly mobile application (App) is then designed and developed using MIT App Inventor 2 in order to acquire and visually display the received analog PPG signals in real-time on the smart device. The aim of this article is to provide a precise step-by-step procedure of designing a PPG monitoring device and detailed procedure of developing a user-friendly app.

IDENTIFICATION OF PATIENTS WITH REACTIVE AIRWAYS AT AMBIENT TEMPERATURES

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This study aimed to characterize respiratory resistance values of reactive and non-reactive airways. Being able to test for reactivity can be beneficial in the medical realm to prescreen patients for respiratory diseases. Reactive airways are airways that narrow due to an external stimulation, which cause a patient to wheeze. The symptoms vary and can be like those of asthma. They are of great interest because patients with reactive airways are exposed to a risk of long-term airway damage. Thirty subjects were asked to breathe ambient temperature room air at 21° Celsius, while breathing through an airflow perturbation device (APD). The APD is a new instrument that rapidly and non-invasively measures respiratory resistance. Respiratory resistance values were examined among subjects at 60 second intervals for a total of 5 measurements. Of 30 subjects between ages 18 to 54 years, 9 (30%) were identified to have reactive airways with statistical significance of $p \leq 0.05$. Testing respiratory resistance using the APD could be a prescreening for reactive airways in exercised-induced asthma in cooler temperatures.

CARDIOVASCULAR RESPONSES FOLLOWING DIFFERENT TYPES OF BREATHING EXERCISES

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Purpose: The purpose of this study was to determine the effect of different types of breathing exercises on blood pressure, heart rate, and respiratory rate. **Subjects:** The study was performed on 45 healthy volunteer subjects ranging from 21-50 years of age. **Methods:** Subjects were randomly divided into three equal groups: control breathing (C, n=15), shallow breathing (SB, n=15) and combined breathing ({diaphragmatic and pursed-lip breaching techniques used together} CB, n=15). Blood pressure (BP), heart rate (HR), and respiratory rate (RR) were recorded before and after the breathing exercises. Each subject was instructed and given a demonstration of their specific breathing exercise. Each subject successfully performed the assigned breathing exercise for fifteen minutes. All data were analyzed using repeated analysis of variance. **Results**: The mean RR in C group before and after breathing exercises were 14.27±2.84 and 14.53±4.61, respectively. The mean RR in SB group before and after breathing exercises were 15.27±3.26 and 17.47±4.07, respectively. The mean RR in CB group were 14.33±3.75 and 12.67±2.50, respectively. SB had significantly increased RR ($P<0.05$) and CB had significantly decreased RR ($P<0.05$). There were no significant changes in blood pressure and heart rate. **Conclusion:** This pilot study indicates that 15 minutes of breathing exercises has no effect on BP and HR, but SB significantly increases respiration and CB significantly decreases RR. **Clinical relevance:** It has been shown that an increase in RR is associated with an increase in stress and a decrease in respiratory efficiency and endurance. Therefore, this pilot study results may suggest that CB breathing technique could be used to decrease RR, to relieve stress and to increase respiratory efficiency. Future study is needed to determine the long term effect of CB breathing on cardiovascular and stress responses, especially for patients with respiratory problems and those patients in stress induced situations.

THE EFFECTIVENESS OF PAP OVER DIFF QUICK (DQ) STAINING METHODS ON THE ASSESSMENT OF ESTRUS CYCLE UPON THE EXPOSURE TO SUSTAINED DELIVERY OF ESTROGEN BENZOATE USING ADULT SD RATS AS A MODEL

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The short length of the estrous cycle in rats (4 days) considered an excellent model to assess the effectiveness of mode of delivery of hormones by means of tricalcium phosphate devices. The advantage would be rapid observations of changes that occur during the reproductive cycle. The aim of the present work was to provide the literature with more insights regarding the distinction between PAP over Diff Quick (DQ) staining methods upon sustained delivery of estrogen benzoate. The target of assessment was to observe the distribution of cornified cells during proestrus, estrus, metestrus and diestrus. Eight female rats (four control and four experimental) were (R1-R8) used in this study. Cyclic activity at 2, 4, 8, 12, 24, 36, 48, 72 hours were determined. Briefly, 0.5 ml Hank's solution was placed within the vaginal canal for few seconds followed by aspiration. This mixture was then smeared onto microscopic slides and stained using a routine PAP and Diff Quick (DQ) staining methods. Data obtained revealed that the PAP stain proved to be a better staining technique than the DQ stain in both nuclear and cytoplasmic details. Histologically, keratinization of the vaginal epithelium appeared to be evident at the estrus phase (day 4) of a 4-day cycle (3 rats out 4). This keratinization process is dependent on the endogenous estradiol secreted between the evening of diestrus 2 (day 2) and that of proestrus (day 3). In the second stage of this experiment, the rats labeled R1-R4 were used as controls, whereas lab rats R5-R8 had estrogen administered (2mg/ml) to them for three days. The results showed a significant increase in the proliferation of degenerative cells in the E treated rats compared to control animals. Inhibition of vaginal keratinization was obvious and this protocol can be used as a rapid and convenient *in vivo* investigational model for screening the effects of agents that have antikeratinizing activity.

THE EFFECTS OF SUSTAINED DELIVERY OF DANAZOL PLUS TESTOSTERONE ON THE FUNCTIONAL ACTIVITY OF KIDNEY USING ADULT RATS AS A MODEL

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Androgens such as danazol (D) therapy has shown to be efficacious in treatment of endometriosis. Several attempts to utilize the native androgen (testosterone (TE)) have shown different physiological responses. The specific objective of this study was to investigate the role of sustained delivery of D alone or in combination with TE on the functional and structural capacity of the kidney using adult female rats as a model. A total of 40 adult female rats were subdivided into five equal groups. Groups I-III were ovariectomized (OVX), rats in groups II and II were implanted with tricalcium phosphate lysine (TCPL) drug delivery system loaded with 40 mg of D or D+TE and rats in group V served as a control (intact) group. At the end of 60 days post treatment the animals were sacrificed and vital organs were collected and analyzed (H&E). The results of this investigation suggest: (i) TCPL delivery system released D and D+TE at a sustained level for 60 days (D= 3-5 ng/ml, TE= 5-7 ng/ml), (ii) the wet weights of kidneys (normalized to body weight) were increased ($p<0.05$) in rats exposed to D compared to control, (iii) no changes in other vital organs (spleen, heart, lungs, and liver), and (iv) animals exposed to sustained delivery of D or D=TE had remarkable kidney tubular epithelial injury. In conclusion, this study suggests that exogenous D or D+TE therapy in female animals could lead to irreversible tubular damage and consequently renal system complication

NANOCRYSTALLINE CERIUM OXIDE CONJUGATED WITH SOD'S ANTIOXIDANT ACTIVITY AFTER HEATING

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After a patient suffers a myocardial infarction, his/her heart has an imbalance between oxygen supply and demand and blood flow must be restored to reduce tissue damage. Oxidative stress, in the form of reactive oxygen species (ROS), plays a large role in this damage. In an effort to reduce the impact of the oxidative stress, superoxide dismutase (SOD) is often used. SOD is an enzyme that catalyzes the transition of superoxide anion into hydrogen peroxide, an inhibitor of SOD. A possible solution for this is conjugating it with nanocrystalline cerium dioxide (nanoceria). Cerium dioxide is known for its antioxidant properties caused by mixed valence states. Since the conjugates need to remain in the body for a long duration, the thermal stability is important. Ceria-SOD conjugates were prepared by mixing the solutions at five different particle ratios including 1:0, 1:5, 1:10, 1:20, and 1:50. The conjugates were heated to 60, 70, 80, and 90 degrees Celsius for 30 minutes. Our unheated sample showed that an increase in nanoceria helped the free radical disproportionation up until 1:10 particle ratio where it hit its maximum. As the heat increased the SOD began to denature and disproportioned fewer free radicals. Increasing the nanoceria in the samples allowed the heated samples to disproportionate more free radicals as compared to the SOD only samples. Results also showed that nanoceria provides an increase in free radical scavenging when conjugated with SOD.