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On the cover: The Good Luck Plant. This is a photo of a healthy Iron Cross Shamrock plant. We all need a little good luck after one year of a pandemic that created changes to the way we live. and interact across our country and the world. The cultivated Shamrock plant (*Oxalis regnellii)* has hundreds of variations, and is found in abundance around Saint Patrick's Day. It has clover-shaped leaves that grow in variable shades of green and purple tones. Photo credit: https://i.etsystatic.com/17057214/r/il/f0671c/2711915536/il_794xN.2711915536_em7r.jpg. CZ Grain Unique Plants and Natural Products, Russell, Iowa. https://www.etsy.com/listing/911645818/10-iron-cross-shamrock-bulbs-grow-oxalis?ref=reviews

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Molecular and Ultrastructural Analysis of a *Porocephalus* sp. (Arthropoda: Pentastomida) Removed from a Rattlesnake

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ABSTRACT

Ultrastructural and molecular data are provided from a single adult female pentastomid opportunistically collected from a road-killed rattlesnake in Russell, KS. Ultrastructural data consisted of light and SEM microscopy of the pentastomid and its eggs, while molecular data consisted of partial 18S and 28S ribosomal sequences and a partial cytochrome *c* oxidase subunit 1 sequence from the same specimen used for SEM. Ultrastructural and molecular data support generic identification of the pentastomid as *Porocephalus* sp. These molecular data were also used with previously published pentastomid sequence data for a concatenated phylogenetic analysis, which support the current, morphology-based taxonomic placement of the genus.

Keywords: *Pentastomida; Molecular Identification; Egg Structure; Tongue Worms; Porocephalus crotali*

INTRODUCTION

Pentastomids, sometimes called tongue worms, are highly modified parasitic crustaceans with a vermiform, often annulated abdomen, and a cephalothorax bearing a sucking mouth flanked by hooks (Riley 1986). There are over 140 currently-accepted species of pentastomids, about 90% of which are parasites of reptiles (Riley 1986, Almeida and Christoffersen 1999). Adult and larval stages of pentastomids are highly specialized for endoparasitism (Riley 1986). Classification of pentastomids has been challenging, placing them at various times in the Tardigrada, Annelida, Platyhelminthes, Nematoda, and (lastly) Arthropoda. Most parasitologists now agree they unambiguously belong in the arthropod class Crustacea (Riley et al. 1978, Lavrov et al. 2004). Pentastomids are not commonly encountered and therefore not subjected to detailed ultrastructural, morphological, or molecular analysis. For this reason, an adult female pentastomid and her eggs, removed from a road-killed rattlesnake (species unknown) and believed to be *Porocephalus crotali*, was examined by both light and scanning electron microscopy and subsequently identified molecularly.

MATERIALS AND METHODS

Three pentastomes (parasites) were obtained by the 3rd and 4th authors from a road-killed rattlesnake in Russell, KS, during August 2018 (Figure 1).

One female specimen was placed in 70% ethanol and submitted to Mississippi State University for identification and analysis. Eggs removed from the specimen by cutting open the abdomen were placed on a slide, fixed, stained with Giemsa, and photographed at 50X with a Leica MZ75 dissecting microscope. The specimen was fixed in Karnovsky's fixative in 0.1mM phosphate buffer at 4°C overnight. It was then rinsed in buffer for one hour and post fixed in 2% O_SO_4 in the same buffer for 4 hours on ice. After rinsing, the specimen was dehydrated in a graded series of ethanol, dried, then mounted on a stub, coated with platinum and examined with a JEOL JSM-6500F at 5kV. SEM focused on the head region and eggs removed from the specimen. A small section of the anterior end of the hindbody of the specimen was excised with sterile scalpel blades and genomic DNA was extracted from this section using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). Partial 18S and 28S ribosomal genes and a partial cytochrome c oxidase subunit 1 (*cox*1) mitochondrial gene were amplified as previously described (Woodyard et al. 2019a).

Figure 1. Pentastome specimens removed from a road-killed rattlesnake (Photo by Lawrence Bircham and Petra Jericke).

The phylogenetic relationship of this *Porocephalus* sp. to other pentastomids was inferred using a concatenated phylogenetic analysis using the 18S ribosomal RNA, 28S ribosomal RNA, and cytochrome *c* oxidase subunit 1 (*cox*1) mitochondrial sequence data. Previously published sequences for each region and the sequences from the present study were aligned using PRANK v. 170427 with the +F option (Löytynoja and Goldman 2008). Alignments were checked for area of random similarity using Aliscore (Misof and Misof 2009) with 100 random pair comparisons, gaps treated as 5^{th} characters, and a window size of 6. Alignment columns determined to be randomly similar by Aliscore were removed with Alicut v. 2.3.1 (Kück 2020). Alignments were concatenated with FastGap v.1.12 (Borchsensius 2009) for Bayesian inference in MrBayes v. 3.2.7 (Ronquist and Huelsenbeck 2003) with nucleotide substitution models, as determined by MEGA v. 10, as follows: 18S (K2+G), 28S (K2+G), *cox*1 codon position 1 (T93+I), *cox*1 codon position 2 (HKY+G), *cox*1 codon position 3 (HKY). Using these data (partition schemes and substitution models), Bayesian phylogenetic analyses was performed in MrBayes using Markov chain Monte Carlo searches of 2 simultaneous runs of 4 chains with sampling every 100^{th} tree for 1,000,000 generations to ensure that the standard deviation of split frequencies reached <0.01. Phylogenetic trees generated from these analyses were formatted in FigTree v. 1.4.4 and refined in Adobe Illustrator v. 23.0.4. Sequences generated in the present study for the 18S, 28S, and *cox*1 regions were each searched against other pentastomid sequences available in GenBank nr/nt database to assess conspecificity (Altschul et al. 1990). Up to the 10 closest matching pentastomid sequences, where deposited data availability allowed, were downloaded and aligned with sequences generated from the present study. Uncorrected p-distances between sequences were then calculated for each region in Geneious (Table 1).

Table 1 Percentage similarity between *Porocephalus sp.* sequences and up to 10 most similar sequences in the NCBI nr/nt nucleotide database for 18S, 28S, and cox1 genes.

18S	
	Porocephalus sp. MT387144
Porocephalus crotali (MG559607)	100.00
Porocephalus sp. (KC904946)	99.17
Kiricephalus pattoni (KC904946)	98.90
Sebekia mississippiensis (MK103080)	98.90
Sebekia sp. 3 (KU975376)	98.90
Armillifer moniliformis (HM048870)	98.62
Armillifer armillatus (KJ877184)	98.35
Alofia merki (KU975378)	98.35
Armillfer grandis (KM023155)	98.07
Kiricephalus coarctatus (MG559634)	98.07
28S	
	Porocephalus sp. MT387200
Porocephalus sp. (EF417058)	99.55
Alofia merki (KU975382)	88.74
Sebekia sp. 3 (KU975380)	86.04
Sebekia sp. 2 (KU975379)	85.59
Sebekia mississippiensis (MK103080)	85.14
Sebekia purdieae (KU975381)	84.68
Raillietiella sp. (AY744894)	79.73
Armillifer sp. (MK089920)	48.65
Raillietiella sp. (EU370448)	46.85
***cox*1**	
	Porocephalus sp. MT383967
Porocephalus crotali (MG559655)	97.67
Armillfer grandis (NC_037187)	80.47
Armillfer armillatus (AY456186)	80.00
Kiricephalus coarctatus (MG559658)	79.53
Alofia merki (KU975383)	78.60
Armillfer agkistrodontis (KX686568)	78.14
Waddycephalus sp.	78.14
Waddycephalus sp. 1	78.14
Waddycephalus sp. 2	78.14
Raillietiella orientalis (MG559637)	72.09

RESULTS AND DISCUSSION

The adult female was yellowish in color, 65mm long x 4mm wide, displaying approximately 40 annular rings and a mouth surrounded by fang-like hooks (Figures 1 and 2a-b). Other than that, there were no discernable body regions or appendages, a finding characteristic of these highly adapted parasites. By light microscopy, eggs removed from the abdomen appeared to have spikes or spines surrounded by a gelatinous material (Figure 2c); however, SEM revealed smooth eggs without any sign of spikes or spines (Figure 2d). The protrusions observed on stained and mounted eggs were likely artifacts of dehydration associated with ethanol-preservation, and hence not observed by SEM.

While sequence data from the present study showed a high degree of similarity to previously published data for *Porocephalus* spp. to the genus level, similarity was not high enough to determine species using publicly available sequences from the most genetically similar pentastomid species. Phylogenetic analysis (Figure 3) placed the genera *Sebekia* and *Levisunguis* in distinct clades. *Alofia merki* sat between these clades, with *Porocephalus* being basal to all three groups. While the body length, annuli count, and the presence of dorsal accessory pieces on the posterior hooks are consistent with previous accounts of multiple *Porocephalus* species, the lack of hook measurements in this case preclude more specific identification (Riley and Self 1979).

Sequence data generated in this study for the 18S and 28S ribosomal regions and the *cox* 1 gene did not support conspecificity with any pentastomid for which sequence data are publicly available. Previously reported intraspecific variation for other pentastomid species at 18S has ranged from 0-0.07% while 28S has ranged from 0-0.9% (Barton and Morgan 2016, Woodyard et al. 2019b, Woodyard et al. 2019a). Similarly, *cox* 1 variation has been reported as by 0-1.03% within species (Kelehear et al. 2011, Barton and Morgan 2016, Sakla et al. 2019, Woodyard et al. 2019b, Woodyard et al. 2019a). While the 18S sequence from our analysis is a 100% match to previously published sequence data from *Porocephalus crotali* in GenBank (MG559607), there is limited data available to determine variability at this marker between species. Notably, multiple species from different genera are all >98% similar to the 18S sequence from the present study, indicating that this particular marker has limited utility for identifying pentastomids even to the genus level. Our 28S sequence data was most similar to *Porocephalus* sp. (98.55%: EF417058). This is more divergent than previously reported for conspecific pentastomids at the 28S region and indicates a lack of conspecificity. Furthermore, the *cox* 1 sequence from the present study is only a 97.67% match to *P. crotali* (MG559655). This is outside the range of previously reported intraspecific variability at this region. While together these data do suggest our pentastomid is a member of the genus

Porocephalus, there is insufficient evidence to make a specific diagnosis based on molecular data. Future molecular characterization of pentastomids subjected to rigorous morphological characterization are needed to confirm the specific identity of the present specimen.

Figure 2. *SEM image of pentastome mouth (A) and mouth hooks (B), eggs by light microscopy (C), and eggs by SEM (D).*

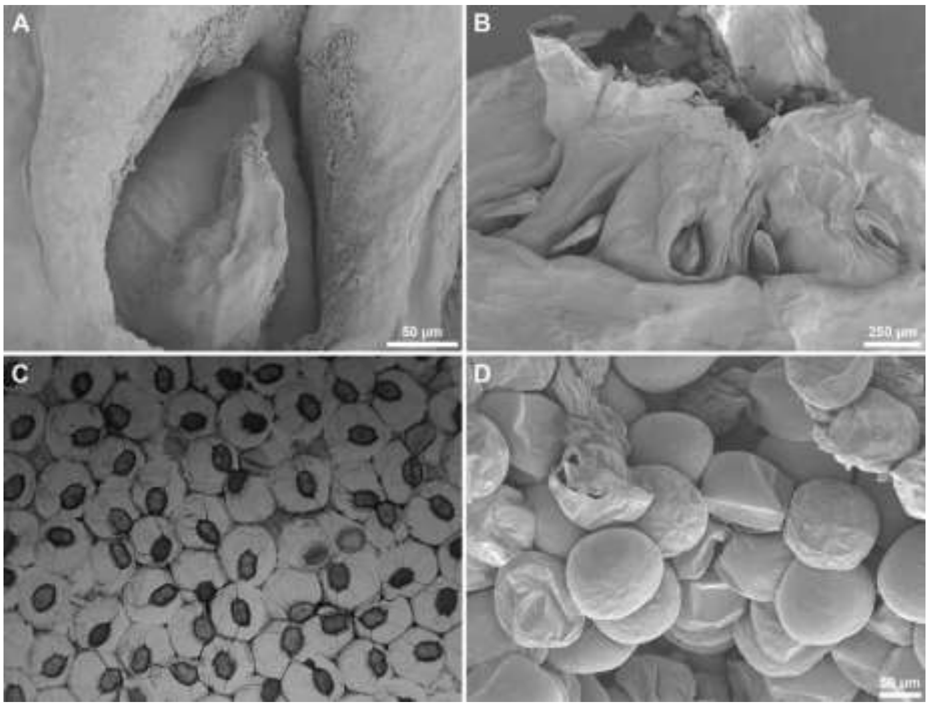
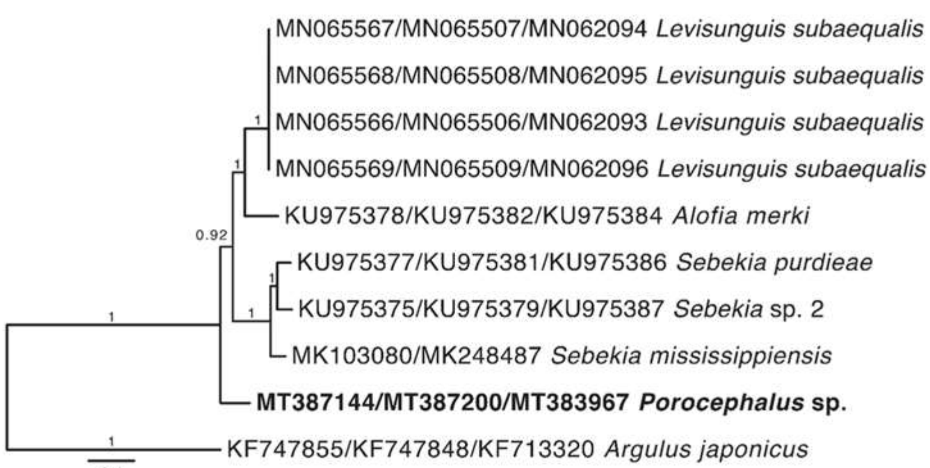


Figure 3. *Phylogenetic tree generated using a concatenated dataset of sequence data from the 18S ribosomal gene, the 28S ribosomal gene, and the cytochrome c oxidase subunit 1 mitochondrial gene (cox1) with available data for each region from GenBank. Numbers above branches indicate Bayesian posterior probabilities. Scale bar indicates number of nucleotide substitutions per site. 18S/28S/cox1 accession numbers precede parasite names. Sequences from the present study are indicated in bold.*



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A Mixed Methods Study of Factors that Enhance Louis Stokes Mississippi Alliance for Minority Participation (LSMAMP) Students Degree Attainment in STEM

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ABSTRACT

The purpose of this study was to advance the literature addressing best practices capable of bridging the retention and completion gap in STEM education for underrepresent minority students. Using a mixed-methods design, this article delineates Louis Stokes Mississippi Alliance for Minority Participation (LSMAMP) program experiences, instructional strategies, institutional practices and students' persistence within the LSMAMP community. Five main themes emerged from the student interviews and survey results: (1) early exposure to STEM and familial support; (2) hands on involvement and academic intervention activities; (3) Peer group support; (4) institutional environment and infrastructural support; and (5) financial incentives. The top choices of faculty about institutional and instructional practices and learning strategies that enhance student learning and degree attainment were faculty mentoring, student opportunities to present research at or attend professional conferences, faculty advising, faculty tutoring/study sessions, peer tutoring, interactive lectures, and student opportunities to connect prior learning to new lecture content.

Keywords: STEM, best practices, LSMAMP student, academic success, minority scholars

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INTRODUCTION

On most institutions of higher education campuses, the persistence and graduation rates of underrepresented minorities (URM) and first-generation students lag behind those of their majority counterparts (Elrod & Kezar, 2015). In a quantitative study conducted by Whalen and Shelley (2010), their analysis revealed that students who are male and non-minority in STEM majors are about 74.6% more likely to be retained and graduate than female or minority STEM students. As a result, there is a growing emphasis on the need to develop sustainable institution-wide models that highlight high impact practices that dramatically improve the graduation rates of URM students in STEM fields (Kuh & O'Donnell, 2013). The Steering Committee for Evaluating Instructional Scholarship for Engineering (2009) is requiring institutions to collect evidence demonstrating their success in undergraduate instruction. Moreover, if the U.S. is to achieve equity among URMs in STEM fields, it is imperative to expose the education literature that addresses effective STEM experiences and teaching methodologies that create the most conducive learning environment capable of bridging the completion gap in STEM education among URM (Espinosa, 2011).

Through funding from Louis Stokes Mississippi Alliance for Minority Participation (LSMAMP), Mississippi's higher education institutions have accomplished laudable strides in ensuring the success of minority students majoring in STEM disciplines. Even though the academic profile for minority students majoring in the STEM field reflect low persistence, participation, and performance (Griffith, 2010), graduation percentages have increased significantly among all nine LSMAMP Mississippi institutions. In the last five years, Mississippi's LSMAMP Alliance reports graduation percentages across it's institutions with an increase of 96%, for the University of Mississippi, 88.4% for University of Southern Mississippi, 82.4% for Delta State University, 91.4% at Mississippi State University, 88.6% at Jackson State University, 72% at Alcorn State University, 52.2% at Mississippi Valley State University, and 48% at Tougaloo College. Most impressively, the number of under-represented minorities who earned doctorates in STEM disciplines at the Mississippi Alliance has an increase of 46.8% from 2015 to 2019 (LSMAMP Database, 2020).

Despite decades of success among the Alliance, continued improvement to enhance success across institutions is warranted, specifically in sciences and engineering fields. From a national perspective, African Americans and Latinos remain underrepresented in all areas of natural sciences and engineering (NSF, 2013). Among the two minority groups, African Americans are disproportionately impacted indicating a decline in bachelor's degree production in physics and engineering from years 2001-2010 in comparison to an incline in bachelor's degrees earned in both physics and engineering fields among Latino students (NSF, 2013). Currently, the Alliance reports variation in persistence and graduation rates across LSAMP institutions in the sciences and engineering disciplines. For example, Jackson State University, a Historically Black College and University (HBCU), has maintained an upward trend in producing science and engineering degrees among LSMAMP students while other HBCUs within the Alliance struggle to achieve the same success. According to NSF (2013) data, HBCUs were the only institution type to show an upward trend in institutional yield ratio among minority Black science and engineering doctorates (NSF, 2013). Many researchers assert that increased minority graduation rates in STEM programs are linked to the cultural connections that minority students experience when attending a minority serving institutions (McGlynn, 2007; Riley, 2015). However, when HBCUs within the Alliance fail to maintain similar science and engineering degree production trends, one must question this notion. Moreover, the University of Mississippi, a Predominately White Institution (PWI) within the Alliance has maintained continuous improvement in degree production among LSMAMP students while other PWIs within the Alliance have struggled to keep pace. Given this variation among institutions and to continue the momentum of the Alliance, it is important to examine student persistence and success across institutions. Through a mixed-methods study, the research questions seek to identify and describe best practices and the relationship between persistence, instructional strategies, program experiences, institutional practices and student success among the LSMAMP community. Results from this study will assist in producing teachable, replicable, and sustainable retention and graduation models that enhance minority student success across LSMAMP institutions throughout the nation, regardless of institution type.

Theoretical Framework / Literature Review

The theoretical foundation for this study is the integration of Kolb's Theory of Experiential Learning and the Diffusions of Innovations Theory. Kolb (1984) described learning as a "process whereby knowledge is created through the transformation of experiences" (p. 41). The Experiential Learning Theory (ELT) explains how the experiences of the learner might be able to predict the importance of influential factors in the learners' ascension into STEM disciplines (Wells & Grabert, 2004). ELT learning is student centered, requiring the learner to insert themselves in the learning experience putting theory into practice by creating a clear understanding or "particular order of practice" (Tennant & Pogson, 2005 p. 155).

No less important, is knowledge that uncovers the effectiveness of the channel of communication when adopting new academic innovations that enhance STEM success. The Diffusion of Innovations Theory, developed by Everett Rogers in 1963 examines the social process that occurs when new innovations, or new ideas are diffused throughout a community, organization, or institution. *An innovation* is an idea, practice, or object perceived as new by an individual or other unit of adoption (Rogers, 1981, p. 35). Diffusion is explained by Rogers as a process by which an innovation is communicated through certain channels over time among the members of a social system. According to Rogers (1981), this form of communication is more effective when individuals "share common meanings, a mutual subcultural language, and are alike in personal and social characteristics; the communication of ideas is likely to have greater effects in terms of knowledge gain, attitude formation in change, and overt behavior change" (p.19).

Instructional activities and experiences that enhance the success of the LSMAMP Alliance have a distinct focus on increasing the social capital of the LSMAMP learning community. A learning community is a group of students who share common values and beliefs and are actively engaged in learning from each other (Learning & Ebbers, 1999). The LSMAMP Alliance fits this pedagogical model as it is designed for targeted groups, such as underrepresented minorities and students with similar academic interest (Learning & Ebbers, 1999). Literature reveals that minorities, specifically enrolled in engineering fields, rely on social capital found in these learning communities for academic success. According to Riley (2015), a pathway to STEM careers and success in engineering fields is achieved through faculty-student interpersonal interactions that focus on adapting to the diverse learning and advising needs of the minority STEM student. The Alliance has created strong pipeline programs that enhance and support minority STEM student success. For example, the Alliance's summer bridge programs expose a cohort of high school graduates to precollege STEM experiences early in their academic career, thereby utilizing the social bonds of a cohort to enhance academic preparation and heighten STEM interest. Additionally, the Scholar's Academy utilized evidence-based retention strategies to include professional development through research opportunities and peer-led tutoring which improves students' critical thinking skills in mathematics, chemistry, and other sciences (Cracolice & Deming, 2001; Quitadamo, Brahler, & Crouch, 2009). Moreover, learning communities build on the development of innovative teaching approaches which Borrego and Henderson (2014) contend, "produces students who are more innovative, flexible and team oriented and able to navigate complexity and ambiguity" (p. 244). It is obvious that improving social capital has influenced the success of the Alliance Programs, but less is understood about the successful ways in which these students are taught and how they experience STEM instruction which directly influences what they learn and the degree to which they persist (Bybee, 2006). Moreover, there is nationally recognized need to reform and improve how math and science are taught in the United States on both the precollege and postsecondary level (NAS, 2011).

Literature reveals that low participation and performance in science, technology, engineering, and mathematics has become an increasingly serious issue for African American and Hispanic students (Griffith, 2010). Many researchers have found that pedagogy, persistence, and institution wide reform are critical factors in improving minority success in STEM disciplines. Lee and Harmon (2013) believed that institution-wide efforts that combine specific academic, social, and research support interventions have resulted in significant improvements in graduation of minority STEM students. Research on STEM postsecondary minority attrition indicates that faculty mentoring, research opportunities, and scholarship support are critical elements in keeping minority STEM students retained in the postsecondary pipeline (Golde, 2005; Depass & Chubin, 2008). Unclear in the literature are the ways in which these high impact practices reform STEM pedagogy and influence persistence and retention among minority STEM students. Addis, Quardokus, Bassham, Becraft, and Boury (2013) believed that increased enrollment and retention in STEM fields requires “top down” change that “occurs when the formal structures of the university and departments are modified to use the skills and knowledge developed in the learning community to guide reform” (p. 24). While understanding how to implement high-impact practices on an institutional and student level may be difficult, it is imperative that institutions recognize that this specialized function is significant to minority STEM student success and has shown evidence in significantly improving the graduation rates of underrepresented minority students (Kuh & O’Donnell, 2013).

In view of critical intervention points, the President’s Council of Advisors on Science and Technology (PCAST) believes there should be a strong focus on the quality of STEM education in the first two years of college to include research on students’ choices, STEM learning processes, and STEM preparation (BHEF, 2013). Additional evidence-based retention strategies documented in the literature directs attention to the connection between STEM students’ success and precollege preparation (Adelman, 2006; Anderson & Kim, 2006; NCES, 2009). Precollege STEM preparation consists of programs and instructional experiences that give students the opportunity to engage in the inquiry process, plan and carry out independent investigations, and seek evidence for their argument (NAS, 2011). Acquiring these skills is highly recommended for undergraduate STEM success. However, access to higher order thinking curricula is resource rich and usually reserved for wealthier students (Gorski, 2009). This might explain why many minority STEM students change their STEM major to less demanding majors or drop out of college altogether after their first or second year (Adelman, 2006). Although literature points to precollege preparation as a significant factor that enhances STEM success, data reveal that many students who abandon STEM majors actually perform well in their introductory courses (BHEF, 2013). To perform well, yet abandon a program, is seemingly contradictory. Hence, it is important to consider the relationship between the students’ perception of progress and the methods in which STEM instruction is taught and the diverse learning needs of the minority STEM student. Currently, there is scant literature that explains why students who engage in precollege STEM experiences still do not receive the knowledge, skills, and content needed to persist beyond introductory courses (Anderson, 2003; Owens, 2009)

Several researchers have examined pedagogical methods that assist STEM instructors with creating instructional environments that help their students acquire the knowledge and skills needed for STEM persistence and success. In view of minorities, Gay (2000) believed that culturally based pedagogy that addresses economics, race, and gender, captivates the imagination, making instruction relatable and motivating to minority students. A National Science Foundation career study seeking to understand learning environments that create engineering pipelines, sought the perception and experiences of 1,400 first-generation undergraduate engineering students. Findings indicate a distinct need to create resource rich environments that adapt to the diverse needs of the minority student by focusing on advising, addressing student learning needs, and building faculty-student relationships (Riley, 2015). Additionally, there is a consensus regarding the success of interactive learning methods in STEM programs to include experiential instruction, peer-based mentoring, and collaborative instruction, noting that science is best learned by doing (Bybee, 2006; Owens, 2009). While it is acknowledged that instructional reform is imperative and precollege experiences and institutional support is critical to minority STEM success, there is still no sound method for determining STEM program effectiveness (Donnelly, 2008) nor the particular powerful combinations of different strategies that enhance minority STEM student success (Borrego & Henderson, 2014).

Higher education institutions cannot escape the reality that “our nation’s universities are not producing graduates in STEM fields in numbers adequate to meet workforce demand” (BHF, 2013 p. 6). More specifically, dismal participation and performance in science and engineering has become an increasingly severe issue for African American and Hispanic students (Griffith, 2010; NSF, 2013). The synthesis of literature regarding the success of minority STEM students in this study is set in the context of understanding the various academic experiences, instructional strategies, and institutional practices that has shown evidence of supporting minority student success in STEM programs. Based on the literature, there is an obvious need for research that examines instructional methods, institutional practices, and precollege experiences that consistently and specifically demonstrate success among minority STEM students across institutional types and throughout science and engineering fields. It is expected that findings will answer the call to understand the knowledge, skills, and content needed to assist more minority students with persisting and completing undergraduate STEM programs (Anderson, 2003; Owens, 2009).

METHODS

This mixed methods study was designed to bridge the gaps between the identification of institutional best practices and the persistence factors that enhanced Louis Stokes Mississippi Alliance for Minority Participation (LSMAMP) student degree attainment in Science, Technology, Engineering and Mathematics (STEM). We selected the mixed methods design in order to gain a better understanding of the phenomenon by incorporating quantitative results and qualitative findings. This design helps to reflect participants' point of view, while examining variations in perceptions among the participations. The mixed methods design enables the participants to provide accounts of their experiences. Data integration principles and practices began with the linkage of the qualitative data collection and the quantitative data collection through sampling. The qualitative database was used to inform the data collection approach of the quantitative design. The datasets were integrated for analysis.

The research explored the following questions; (1) What specific programs and instructional activities and experiences attract students to STEM fields and enhance degree attainment of minority student in LSMAMP program? (2) What precollege and college persistence experiences increase LSMAMP student interest, retention, and bachelor's degree attainment in STEM disciplines? (3) What perceived strategies are successful in ensuring LSMAMP student success from an institutional, instructional, and student persistence perspective? (4) What relationships exist between LSMAMP degree attainment and responses from students and faculty regarding identified institutional best practices and programs, persistence factors and experiences? (5) What differences exist between LSMAMP and non-LSMAMP STEM students' college experiences and the extent to which these experiences influenced STEM interest and persistence on degree attainment?

After obtaining IRB approval, the following qualitative and quantitative methodological approaches were utilized to conduct the study based on the research questions.

Qualitative Methodology

The first methodology employed for this study was a phenomenological approach given that such an approach is appropriate for capturing the subjective understanding of individual participants (Marshall& Rossman, 2016). A phenomenological approach seeks to understand the individuals' *lived experiences* with a particular phenomenon. As such, the researchers endeavored to explore, describe, and analyze the meaning derived from the study participants' experiences. Using a phenomenological approach yielded descriptions that provided the foundation for introspective structural analysis to gain an understanding of the essence of the shared experiences of the study participants.

The researchers assumed that this phenomenological approach would provide insights into the need for understanding institutional best practices and persistence factors that enhance LSMAMP students' degree attainment in STEM using the integration of Kolb's Theory of Experiential Learning and Roger's Diffusions of Innovations Theory. Moreover, it was also assumed that a qualitative research design methodology was best suited for gaining insights into the area being researched. The researchers operated under the assumption that all participants responding to interview questions would respond to their best ability and with honesty.

Site Selection

This study focused on the nine Mississippi LSAMP NSF funded institutions within the current five-years funding circle. The universities and colleges are made up of nine campuses located across multiple communities and municipalities which allowed for an opportunity to observe and understand the institutional practices and the experiences of LSMAMP students and faculty at the various sites.

Population and Sample Selection

This study focused on engaging LSMAMP sophomore, junior, and senior students in STEM as well as coordinators in the programs from the nine sites. The researchers employed a purposive sampling technique in the selection of candidates. Purposive sampling technique, which is sometimes referred to as judgement sampling, is the deliberate selection of a participant due to the traits the participant possesses and how those traits align with the phenomena being studied (Etikan, 2016).

The researchers ensured that there were participants from all colleges and universities identified in the sampling frame. LSMAMP students and five coordinators from the campuses were interviewed. The target population were those sophomore, junior and senior students and program coordinators currently engaged in the LSMAMP program.

Collection of data began after identifying candidates to be interviewed through the researchers' knowledge of individuals currently functioning in the positions previously outlined, the program gate-keepers, and referrals from identified potential candidates. Initial contact with prospective candidates was made via phone to make introductions, provide an overview of the study, and to schedule a Zoom or telephone interview. The initial call, on average, took approximately 10–15 minutes.

After the initial call, prospective candidates were officially invited to participate in the study using a standardized email. The correspondence outlined the purpose of the study, requirements for participation, and the process for securing their privacy as well as the informed consent form for review. A Zoom link with a password was sent to the prospective participants to confirm the date and time of the interview. Candidates were asked to review, sign, and return the informed consent form before the scheduled interview.

As previously indicated, interviews were conducted using a secure Zoom online meeting platform, telephone or electronic survey.

Eighteen students and five coordinators were selected to participate in face to face or telephone interviews and surveys. Due to the Covid-19 pandemic, interviews were conducted using the Zoom online platform and electronic survey. Recruitment was continued until saturation was achieved. Theoretical saturation was reached upon completion of the twenty three interviews. As such, the researchers determined that no further interviews were deemed necessary. This ceasing point is in alignment with Creswell (2013) which suggested that a study group should consist of three to fifteen members who can articulate the lived experiences with the phenomenon being studied. Further, researchers have asserted that qualitative studies usually focus on in-depth, relatively small samples (Patton, 1990) and that small sample sizes are useful in providing rich cultural descriptions (Marshall & Rossman, 2016). The use of interviews, guided by questions grounded in the Experiential Learning and Diffusion of Innovation framework, allowed for deeper insights into the perceived institutional best practices, instructional experiences, and persistence factors that enhanced LSMAMP students' degree attainment in STEM.

Data Collection

Data collection began after approval was granted by Jackson State University Institutional Review Board. All interviews were recorded with consent from the participants which aided in the accuracy of coding responses. Interviews lasted approximately 35 minutes. The interviews were conducted using an interview guide consisting of statements, follow-up and guided questions.

The first set of questions for the student participants focused on demographic information and other relevant background questions. These were followed by specific questions related to the research questions of the study. The participating coordinators questions gauged their perceptions of institutional best practices and instructional strategies relative to student persistence and degree attainment in STEM.

After the interviews, the audio recording was uploaded to Trint, an artificial intelligence (AI) transcription platform. Trint uses AI to transcribe documents from voice to text and holds the ISO 27001certification. This certification was created by the International Standards Organization to provide a global standard for information security management systems (ISMS) and is considered the platinum standard for data security. This platform was used to increase the efficiency of capturing and transcribing data. This method of software transcription has been noted to increase effectiveness while eliminating exorbitant amounts of transcription time (Tessier, 2012).

Journaling and follow-up interviews were used as data collection methods to provide insights on the study. Journaling refers to the practice of keeping a diary or journal that enables the researchers to explore the thoughts, perceptions and attitudes of study participants.

Data Analysis

Data analysis was codified into six phases: organizing data, immersion in the data, generating possible categories and themes, coding the data, interpreting data, and exploring alternative understandings of findings from the data (Marshall & Rossman, 2016). Methods for organizing the data included using an information log that outlined the dates, times, people, as well as initial observations from the interviews. Initially, this information was recorded in Microsoft Office with later reflective memoing captured in ATLAS. ti, a Qualitative Data Analysis (QDA) software program.

Immersion of the data took the form of becoming intimately engaged with the data collected by reading and rereading the material (Marshall & Rossman, 2016). Once the data were transcribed, the researchers read through the transcription in conjunction with the interview recording. During the process, revisions were made to the transcript to accurately capture the responses from the study participants. Pre-coding was used to highlight intriguing quotes or phrases for later consideration. This allowed the researchers to consistently reflect on the people, events, and the phenomenon to help shape the interpretation of the data.

Given the amount of data collected, the researchers engaged in the process of data reduction. This exercise of reducing data provided the researchers with the opportunity for further immersion in the data that led to the recognition of categories and underlying themes. Connections between these categories and themes were applied during the second stage of analysis whereby the researcher's understanding of the participants' perceptions was used to shape the organization of the data for interpretation in the final document.

ATLAS. ti v8 was used for further organization of data and aided in the process of generating categories and themes. In the first stage of analysis, transcripts from each interview were uploaded into the system. Open coding or *in vivo* coding was used to identify core themes and better inform inductive and deductive thinking to analyze the information collected. During this stage of analysis, the researchers were able to define general codes and themes which allowed small segments of data to be considered in detail and compared with one another. Any line or paragraphs of data that could be considered relevant was coded, resulting in 30 initial codes. These codes were merged into similar codes followed by a grouping into larger or "meta" categories.

In the second stage of analysis, axial coding was used to further refine, align, and categorize emergent themes. These themes were aligned with the study framework and research questions. The researchers generated outputs that queried information coded to the research questions typographies.

In the third stage of analysis, eight categories emerged from clustering the coded data. The categories were further merged due to overlapping of categories that formed the basis of the findings.

Biases

Given that it is very difficult for a researcher to divorce himself/herself from the data being analyzed in qualitative research, some strategies were employed to avoid bias and maintain objectivity.

One method identified by Marshall and Rossman (2016) is that of documenting field notes dedicated to self-reflection. They recommended that the researcher allocates time to consider what worked and what did not work and examine own emotions and how those feelings may lead to deeper insights about the phenomenon being studied. With this in mind, after each interview, the researchers took the time to detail overall impressions from the interview and key themes that were heard to channel those emotions into tools that were used to inform the research.

Additionally, to ensure the validity of the findings, the researchers used the following methods: engaged study participants to review the results, identified multiple data sources such as the LSMAMP existing data base to validate findings, and explored alternative explanations of the data findings. By allowing interview participants to review results, the researchers were able to ensure that the interpretations were a true representation of what the participants wanted to convey. Additionally, using multiple data sources provided increased confidence in the research findings while allowing for creative ways to study the proposed phenomena. Finally, exploring other alternative explanations for the data findings allowed the researchers to rule out or account for these alternative explanations resulting in stronger support for the interpretations of the data.

Trustworthiness

Historically, the concept of trustworthiness has been grounded in the natural and experimental sciences, characterized by qualitative criteria such as reliability, validity, objectivity, and generalizability (Marshall & Rossman, 2016). However, alternative constructs have been offered by researchers as a means to uphold the tenants of trustworthiness that is better aligned to qualitative design methods. Researchers Lincoln and Guba established a set of procedures that could be used in qualitative research which includes prolonged engagement, member checks, and peer debriefings (Marshall & Rossman, 2016).

In prolonged engagements, the researcher is present in the setting for long periods. Additionally, the concept of member checks encourages the researcher to share his/her interpretations of the data with the participants interviewed. Peer debriefings involve the researcher triangulating data using multiple sources through multiple methods and discussing emerging themes and trends with other knowledgeable researchers in the field. These procedures align with the validity/credibility concepts in qualitative studies. The option to be present in the setting for long periods was not viable due to campus closures in response to the Covid-19 pandemic. However, the utilization of member checking and peer debriefings served as validation methods.

Quantitative Methodology

This section discusses the researchers' second methodology utilized to collect and analyze the quantitative data. It includes the research questions, population, sample, instrumentation, data collection, and analysis procedures.

Research Questions

The study sought to examine the following research questions:

1. What are students' precollege and college experiences?
2. To what extent do experiences influence students' persistence in STEM and degree attainment?
3. Are there differences in the students' perceptions about their precollege and college experiences and the extent to which certain experiences influenced their persistence in STEM based on:

   a) type of student (LSMAMP student or non LSMAMP student);
   b) student classification;
   c) grade point average

4. Are differences in the students' perceptions about their college experiences and the extent to which certain experiences influenced their persistence in STEM based on type of student (LSMAMP or non LSMAMP student)?

5. What were the faculty members' experiences with STEM programs?

What are faculty members at a LSMAMP institution perception of the influence of these experiences on student learning outcomes?

Population and Study Sample

The study population included all of the nine Mississippi institutions participating in the LSMAMP program for the current NSF funding circle (2018-2023). Louis Stokes Mississippi Alliance for Minority Participation (LSMAMP) consists of a strong collaboration of nine diverse institutions dedicated to the preparation of varied STEM workforce for industry and research in Mississippi and the nation. Out of the average 18,000 STEM students in the partner institutions about 35% are underrepresented minorities (URM) and 255 (4%) of the URM STEM students are covered under the LSMAMP alliance wide and institution-specific activities. Under the leadership of the program manager, Dr. Martha Tchounwou and the institutional coordinators, the participating students have been engaged in strong alliance-wide activities to support them academically and socially for success in college and for professional development for STEM careers. The population universe also included a representative sample of non-LSMAMP STEM majors from the institutions (control group) and five site faculty coordinators from the nine sites.

Purposive sampling was used to select survey participants who met the following criteria; (1) must be a sophomore, junior or senior student in the LSMAMP program, (2) must be a faculty coordinator in the program, and (3) must be a current non LSMAMP student in STEM from any of the nine participating sites. Drawing from the database in the LSMAMP program sites, one hundred and eighty (180) LSMAMP and non LSMAMP students, thirty (30) LSMAMP and non LSMAMP STEM faculty received survey instruments using Qualtrics to gauge their perceptions of institutional and instructional best practices and persistence factors that enhance LSMAMP student degree attainment. Seventy five (75) usable responses were received from students, and fourteen (14) from faculty for data analysis.

Instrumentation

Given the robustness of this study, benchmarks gauging experiences, best practices and persistence factors were collected using two survey instruments validated through content and construct analysis. Below is a detailed summary of each survey instrument:

LSMAMP Student Survey: The Likert Scale Survey sought to understand students' experiences while attaining a degree in a STEM discipline. The survey contained a total of 52 questions and was divided into sections. Section I sought demographic information including gender, student status, classification, parental education, GPA, graduation plans, transfer status, and program of study. Section II used statements in "order of importance" about precollege experiences and the extent to which certain experiences influenced students' interest in STEM. Section III was comprised of statements about participants' experiences with STEM programs as an undergraduate student and the influence of the experiences on learning outcomes. Next were statements about instructional practices and learning strategies that enhance learning. Last were questions about participants' perceptions about institutional practices and the degree to which certain practices influenced persistence, retention, and student outcomes. The Likert Scale choices were: 1= very important; 2 = important; 3 = somewhat important; 4 = not important.

LSMAMP Faculty Survey: The LSMAMP Faculty Survey comprised 37 questions and was used to gather information about the faculty members' experiences with STEM programs as a faculty member at a LSMAMP institution and their perception of the influence of these experiences on student learning outcomes. This four point, Likert Scale STEM faculty survey sought to gauge their understanding and perceptions across a broad spectrum of institutional and instructional practices on student learning outcomes. Faculty were asked to select from the following choices: (1) very important; (2) important; (3) somewhat important; (4) not important. Section I related to their experiences with LSMAMP STEM programs and their perceptions about the influence of the experience on student learning outcomes. Section II related to instructional practices and learning strategies that enhance student learning and degree attainment. Section III was related to institutional practices and the degree to which certain practices influenced student persistence, retention, and program outcomes.

Data Analysis: Since the data were on the ordinal measurement scale, we used non-parametric tests to answer the student participants' research questions about their college experiences and the extent to which certain experiences influenced their interest in STEM. We tested differences in the students' perceptions in STEM based upon: (1) type of student (LSMAMP student or non LSMAMP student), (2) student classification, (3) institution, and (4) GPA. The Mann Whitney U test was computed to examine differences in the students' perceptions about their pre-college and college experiences and the extent to which certain experiences influenced their interest and persistence in STEM based on type of student (LSMAMP student or non LSMAMP student). Next the Kruskal Wallis ANOVA test was computed to examine differences in the students' pre-college and college experiences and the extent to which certain experiences influenced their interest and persistence in STEM based on classification. Researchers next computed the differences based on GPA and institution using the Kruskal Wallis analysis of variance.

To answer the research questions on institutional and instructional best practices from the faculty perspective, descriptive statistics using percentages and mean differences were computed based upon their Likert Scale degree of importance responses to the faculty survey statements.

Qualitative Findings

Description of Participants: Eighteen (18) undergraduate LSMAMP students were interviewed for this study. An additional interview/survey of five (5) LSMAMP site coordinators were also conducted to gauge information on their understanding of best practices and persistence factors that enhance LSMAMP students degree attainment in STEM. All students interviewed were African Americans comprising of seven (7) males and eleven (11) females within the age range of 19 – 21. There were eight (8) seniors and ten (10) juniors. Current GPA ranged from 3.5-3.9 on a scale of 1 to 4.

Table 1 is a demographic description of the representative sample of the students interviewed. Study participants identities were protected through the use of pseudonyms.

Table 1: Demographic Description of Sample

Name (Pseudonym)	Age	Gender	Transfer Yes/No	GPA	Major	Class
Ron	21	Male	Yes	3.7	Chemistry	Senior
Meca	21	Female	Yes	3.7	Biology	Senior
Audora	21	Female	Yes	3.7	Biology	Senior
Deja	20	Female	Yes	3.8	Biology	Junior
Tony	21	Male	Yes	3.9	Engineering	Senior
Gina	20	Female	No	3.9	Biology- Pre Medicine	Junior
Eliza	21	Female	No	3.9	Computer Science	Senior
Wilbur	20	Male	No	3.6	Electrical Engineering	Junior
John	21	Male	No	3.6	Electrical Engineering	Junior
Kristy	20	Female	Yes	3.6	Biology- Pre Medicine	Junior
Yolanda	19	Female	No	3.5	Computer Science	Junior
Stephanie	21	Female	Yes	3.6	Biology- Pre Medicine	Senior
Nina	20	Female	No	3.7	Biology	Junior
Thomas	21	Male	No	3.5	Mathematics	Junior
Stephen	20	Male	No	3.6	Chemical Engineering	Junior
Marlon	21	Male	No	3.5	Chemistry	Junior
Nycole	21	Female	No	3.7	Biochemistry	Senior
Shirley	20	Female	No	3.6	Biology	Senior

Phenomenological Analysis

In this section, five main themes which emerged from the interviews and surveys are summarized: (1) early exposure to STEM and familial support; (2) hands on involvement and academic intervention activities; (3) Peer group support; (4) institutional environment and infrastructural support; and (5) financial incentives.

Theme 1: *Early Exposure to STEM and familial support*

Early exposure to STEM was a commonality among all participants. Most of the participants cited rigorous high school academic preparation as important to their interest and success in STEM. Taking advanced placement (AP) honors classes in high school, pre-college summer bridge or transitional program provided focus and interest for them in STEM disciplines. Most of the participants cited parental and extended family support as instrumental to their interest, persistence and success in STEM. The following are some of the participants' reflections of their experiences.

Ron stated the following about his parents putting him in STEM summer camps each summer; "I feel like that played a pivotal role in us being nurtured and prepping for that [STEM major]". He went on to declare:

Because I think my dad and mom knew that being in summer camps around like-minded individuals around that time of our lives…When you're being honest, a lot of kids, in general, probably wouldn't want to have, or be in summer camps that dealt with scholastic things. They put us in fun camps, where we learned and where we enjoyed it. That helped us to sustain and maintain our interest, and to actually increase our interest in choosing that as a major. I know once I hit sixth grade, literally, the summer going into seventh grade, the summer going into eighth, the summer going into ninth, all the way until, I think, my junior year, they always made it that we always were in one type of STEM related camp every summer. I remember there was one summer where they had me in three camps.

Ron went on to further explain how these camps provided him with opportunities to meet more people with similar STEM interest who were "*striving for*" a similar goal in life and often times did not share the same ethnicity as him. He stated that the rigor of the summer camps pushed him to be greater and think harder. Tony also discussed how he attended math and science camps as a young child and continued to support his math and science aspirations by ensuring that he continued to get consistent exposure to math and science skills during his adolescent years.

Eliza expressed how at the age of 15, she used to help her father take computers apart and how her parents would check out STEM related books from the library which assisted in maintaining her interest in engineering. Wilbur confirmed that his father's love for engineering and consistent visits to his father's work site is what continually inspired him to continue the journey to a STEM degree. Stephen also stated his parents put him in summer engineering, technology and robotics camps as early as elementary age building robots to software development. He acknowledged that this consistent early exposure helped to strengthen his technology and engineering mindset for his future.

Marlon declared that his parents were his main influences for attending college and majoring in STEM, stating, *"They somewhat indoctrinated me with their past experiences. They were the ones that told me I definitely want to pursue higher education, go to college."* He also explained that, "*They enrolled me into the gifted program when I was in elementary, middle, and high school. I attended math camps, science camps, and also they were just very supportive in terms of whatever I wanted to do. Math and science, they just were there to support me"*.

Marlon was an only child and spent a lot of time playing any type of memory or critical thinking games such as Chess, with his parents. He states, *"…they were always there to help me figure things out, by letting me figure it out myself"*.

In addition, study participants spoke of particular friends, siblings, teachers, and guidance counselors who facilitated their preparation to be STEM majors. For example, Nina shared her experiences as such:

My friends and siblings who were already in college, encouraged my interest in STEM as a major. Some *of my teachers and guidance counselor, made sure I was registered in appropriate college preparation classes and mentored me about college options*.

This sentiment was also shared by a number of the participants. As noted by Andrea, "*Being an honor student, with approachable counselors and friends, can make for a smooth transition to being a STEM major*".

Theme 2: *Hands-On Involvement and STEM Academic Intervention Activities*

The second theme was the value the participants placed on their involvement in LSMAMP STEM academic intervention activities. The majority of the participants recognized the role of mentoring, engagement in hands-on research, tutoring, workshops, and academic advising as program best practices that enhanced their persistence and degree attainment in STEM.

On mentoring, Deja, a biology major, shared that having a supportive network of LSMAMP students, faculty, and staff mentors were central to her academic success and persistence in STEM. Specifically, she noted:

> *When I have challenges in understanding class assignments or personal issues, my senior cohort mates and Dr. Martha Tchounwou were always available to help me overcome the challenges. My professors were readily available to provide me with guidance on class assignments and time management.*

Participants also stressed how being involved in workshops and seminars that were organized by LSMAMP program offices enhanced their academic activities designed to impact knowledge, refine skills, and build external professional contacts that were instrumental to their persistence and success. Kristy summarized the reflections of the majority of the participants when she said:

> *I have benefited immensely from LSMAMP workshops and seminars where different speakers were brought to campus to share their knowledge on topics like test preparation (GRE), career exploration, study skills, time management skills. Exposure to these workshops and seminars have made me a better student and motivated me for graduate work or career employment when I complete my undergraduate degree.*

Majority of the participants shared their enthusiasm on being exposed to hands-on research by their faculty mentors/tutors and opportunities to attend and present their research at professional meetings through LSMAMP program strongly influenced their persistence and success in STEM. As Shirley shared, "Working in the lab and conducting experiments with my faculty mentor gave me the confidence to complete my research and present my work during a poster session at the Mississippi Academy of Sciences joint conference with LSMAMP program". Nycole shared similar sentiments when she posited:

> *My research experiences attending and presenting my work at STEM conferences as an LSMAMP Scholar has helped to clarify my career plans and enhanced my sense of self-efficacy. Before being selected to this program I was lacking confidence and productive interaction with my fellow students and professors.*

Theme 3: *Peer Group Support*

The participants discussed the importance of having peers with similar aspirations of doing well and supporting each other in their major disciplines. Specifically, they mentioned that group study interactions helped them to comprehend and retain class materials. For example, Deja who is a junior Biology major explained, "I had challenges understanding some of the concepts in class, however, once I met with my study group, some of my classmates helped to explain the ideas in such a way as to make me understand and apply the concepts better". Forming a bond with other LSMAMP scholars was helpful to John because he did not want to disappoint them being dropped from the program. He shared the following:

> *Although we compete as individual students, we also see the competitive advantage for being LSMAMP scholars studying together and learning from each other. I would not have survived Advanced Calculus and other high level engineering classes without the help of my peers.*

Some participants from PWI's stressed the social aspects of studying together and mitigating some of the stressors of being a minority in a majority white

STEM student environment. Eliza put it succinctly, “Without the LSMAMP peer group support and the social and academic relationship I have developed, I would have changed my major to a non-STEM major”. Upon reflection, Yolanda believed that the family atmosphere and comradery from her peers, helped her to stay focused and continue to persist in her pursuit to complete her degree in Computer Science.

Theme 4: *Institutional Environment and Infrastructural Support*

In response to research question # 3, “What perceived strategies are successful in ensuring LSMAMP student success from an institutional, instructional, and student persistence perspective”, interviews and/or surveys were conducted with five out of the nine LSMAMP Site Coordinators.

The Coordinators (identified as coordinator 1 through 5) underscored the positive STEM environment and institutional support as critical components of LSMAMP best practices for student persistence and success. Specifically they identified evidence-based teaching practices, strategic and consistent communication channels, institutional commitment and alignment to existing infrastructure, and participation in a learning community as enhancers of LSMAMP students’ success.

Coordinator 1 shared the following:

The LSMAMP environment encourages evidence-based teaching practices that have been shown to enhance engagement and deep learning by promoting the use of active learning pedagogies, increasing access to experiential learning, field experiences and research for students. We also promote project based assessments to evaluate and validate our students learning.

Coordinator 3 noted as follows:

Our program encourages skill and mastery focus that utilizes performance standards to measure students’ success. In addition, the LSMAMP environment creates opportunities for students to be engaged in field experiences and other service learning opportunities within and outside of the institution.

Coordinators 4 and 5 shared similar sentiments with regard to the instructional practices in their institutional environments. For example, Coordinator 5 posits;

In my institution, we use peer coaching strategy and foundation courses assistance to meet the needs of our students. Monthly meetings and activities designed to solve individual and group academic and social problems of students constitutes parts of our programming environment.

The Coordinators noted the importance of consistent communication channels as a part of their environmental ethos. Coordinator 1 shared the following:

From the state level, Site Coordinators meet monthly to discuss ways we can share activities and resources within the alliance. We also discuss potential supplemental funding opportunities that would provide additional support to the IMAGE scholars. Finally, we discuss ways to build on the success of different aspects of LSMAMP. From the university level, our Student Executive Board meets twice a month to discuss new ideas and methods that would better meet the scholars’ needs. We also discuss community service opportunities and bring in IMAGE alumni for networking and community building experiences. The executive board also discuss ways to collaborate with other organizations and programs (Luck Day, NOBCChE, NSBE, McNair Program, MAPS, etc.) to facilitate research and various learning opportunities. Finally, the executive board share innovative ways to utilize social media (Facebook, Instagram, Twitter, etc.) for advertisement and recruitment purposes. Surveys are sent to scholars after every event to receive feedback on how to improve the activity. At the conclusion of the student’s research experience, questionnaires are also sent to research faculty to gather information on the development of the student throughout the capstone research experience.

Coordinator 3 emphasized the importance of open communication with program students in enhancing their learning and persistence in STEM. Specifically she noted:

We have monthly meetings among our site coordinators to discuss ways to manage and enhance our site programs. Students lead information sessions where challenges and opportunities for student engagement are discussed. This process has helped to encourage students' ownership and sharing of information within the Alliance and among students.

The importance of institutional support for LSMAMP program success was stressed by all the coordinators interviewed. Coordinator 2 surmised as follows:

My institution is very supportive of our LSMAMP program. Our students are provided; (1) dedicated space with technology support and access to a writing center to enhance their skill sets. The coordinator and faculty have access to our Okialert system to monitor students' academic and social support through the Student Success Center. The institution also provides faculty mentors with release-time to conduct research and publications with our students.

Coordinators 1, 3, and 5 shared other institutional support to include; (1) stipends for faculty and student travel and purchase of consumables and supplies needed to support research, (2) dedicated space in the library and program offices for LSMAMP students, and (3) institutional recognition of LSMAMP students and faculty mentors during academic year end banquets.

Coordinator 1 said the following about the importance of her institutions LSMAMP as a learning community:

Participating in the LSMAMP learning community enhances student persistence and success by providing students multiple opportunities to stay active while offering guidance and support. The following activities have been designed to foster professional development, community and leadership building by demystifying the graduate level experience and promoting a well-versed student. These efforts serve as a catalyst in the retention and production of successful students. These activities are: (1) Seminars in personal statements & resume writing, (2) GRE Prep Workshops, (3) Departmental STEM Seminars, (4) Graduate School Workshops, (5) Community Services Projects, (6) Leadership Retreats, and (7) Participation in STEM Organizations.

Coordinator 3 shared similar sentiments about her institutional LSMAMP program when she posited:

Our learning community has provided opportunities for our students to learn as a cohesive group with shared aspirations and goals. They learn team work and engage in group professional development activities as well as personal growth.

Coordinator 4 shared that her institutional LSMAMP program is relatively small with limited students and faculty mentors. However, she aspires her program to be a learning community over time.

Theme 5: *Financial Incentives*

All participants acknowledged the significance of financial support to their persistence and success in STEM. They used one or more forms of assistance (scholarships, grants, loans) to meet their financial obligations. The LSMAMP tuition and stipends were particularly stressed by the participants as critical to their success. For example, Stephen an engineering major shared:

Coming from a low SES background it would have been impossible for me to major in engineering without the financial support from the LSMAMP program. My engineering books and lab accessories are very expensive. I would have changed my major were it not for the financial aid I received as an LSMAMP scholar.

Ron, Andrea, and Yolanda discussed the stressors of working and going to school and their impact on grades and retention. Yolanda shared:

First, I would have dropped out of school as a STEM major without the LSMAMP financial support. Having maintained a full-time job and schooling full-time before I became an LSMAMP student took a toll on me physically and mentally. My grades then were not something to be proud of. The LSMAMP program financial incentives has enabled me to fulfil my dreams and reach my *academic full potential.*

Ron spoke of the assistance from the LSMAMP program office in helping him to navigate through the financial aid process and other scholarship opportunities. He stated "The LSMAMP director provided us with various financial aid opportunities in addition to LSMAMP scholarship and stipends. The workshops on financial aid and financial literacy in addition to other programs scholarship opportunities helped me much". Ron's positive experiences was also shared by the other participants in the study.

Quantitative Findings

Below are preliminary descriptions and findings from the survey results of students and faculty participants.

Student Survey Results

LSMAMP Student Survey was used to gather information from students about their precollege and college experiences and the extent to which certain experiences influenced their persistence in STEM and degree attainment.

We tested differences in the students' perceptions about their precollege and college experiences and the extent to which certain experiences influenced their persistence in STEM based on:

1) type of student (LSAMP student or non LSAMP student);
2) student classification;
3) grade point average

Table 2 is a presentation of the demographic characteristics of the students who were participants in the study. Over 75% of them were LSAMP students, more than one-third of them were sophomores, almost one-third were juniors, and a little more than one-third were seniors. About 85% of them had a grade point average of a 3.5 or better. The largest group of students attended Jackson State University, University of Mississippi, and Tougaloo College. About 24.1% of them were first generation students, and 20.3% of them had parents who had STEM degrees. More than three-quarters of the students intend to attend graduate school.

Table 2: Demographic Characteristics of the Students

	Number	Percent
Are you a LAMP Student?		
Yes	60	75.9
No	15	19.0
Classification		
Senior	25	31.6
Junior	26	32.9
Sophomore	28	35.4
Grade Point Average		
2.5-3.0	8	10.7
3.0-3.5	32	40.5
3.5-4.0	35	44.3
Students' Institutions		
Jackson State University	20	25.3
Mississippi Valley State University	9	11.4
Delta State University	4	5.1
University of Mississippi	11	13.9
Alcorn State University	8	10.1
Tougaloo College	13	16.5
Mississippi State University	5	6.3
Hinds Community College	9	11.4
First Generation College Student		
Yes	19	24.1
No	57	72.2
Career Plans Upon Graduation		
Enter workforce	17	21.5
Attend graduate school	62	78.5
Not sure	5	6.3
Parents Earned STEM Degree		
Yes	16	20.3
No	59	74.7

N=79; missing responses are not included in the tables

Table 3 is a presentation of the program of study of the students. The largest group of students were enrolled in Biology.

The Mann Whitney U test was computed to examine differences in the students' perceptions about their college experiences and the extent to which certain experiences influenced their persistence in STEM based on type of student (LSMAMP or non LSMAMP student). In the category "Seminars w/Subject Matter Experts", there was a significant difference between LSMAMP students and non LSMAMP students ($p < .05$). In the category "Stipends/Incentives", there were significant differences between LSMAMP students and non LSMAMP students ($p < .05$). In the category "Student developed notes/study guides", there was a significant difference between LSMAMP students and non LSMAMP students ($p < .05$) (Table 4). In each case, LSMAMP students had a more positive view than the non LSMAMP students. Only areas where significant differences were detected are listed in the table.

Table 3: Program of Study

	Number	**Percent**
Biochemistry	3	3.8
Biology	23	29.1
Biology Pre-Medicine	1	1.3
Biology pre-Pharmacy	1	1.3
Biology Pre-Pharmacy	1	1.3
Biology, Chemistry, Military Science	1	1.3
Biology/Pre-Physical Therapy	1	1.3
biology/chemistry	1	1.3
Biology/Pre-Med	2	2.5
Biomedical Engineering	2	2.5
Chemical Engineering	3	3.8
chemistry	2	2.5
Chemistry	7	8.9
Computer engineering	1	1.3
Computer Engineering	2	2.5
Computer Engineering/Cyber Security	1	1.3
computer science	2	2.5
Computer science	1	1.3
Computer Science	3	3.8
Electrical engineering	1	1.3
Electrical Engineering	2	2.5
Engineering	1	1.3
First-year student in PhD in Mathematics at University of Alabama	1	1.3
Health physics	1	1.3
M.S. Kinesiology	1	1.3
Mathematics	2	2.5
Mathematics and Computer Science	1	1.3
Mathematics education	1	1.3
Radiology technology	1	1.3
Technology	1	1.3
Total	**79**	**100.0**

Table 4: Mann Whitney U Test – Type of Student by College Experiences and the extent to which Experiences Influenced Persistence in STEM

	Are you a LSAMP Student	N	Mean Rank	Sum of Ranks	Sig.
Seminars with subject matter experts	Yes	56	32.11	1798.00	.050*
	No	11	43.64	480.00	
	Total	67			
Student developed notes/study guides	Yes	56	32.23	1805.00	.036*
	No	11	37.00	407.00	
	Total	67			
Stipends/incentives	Yes	55	31.46	1730.50	.007*
	No	11	43.68	480.50	
	Total	66			

The Kruskal Wallis ANOVA test was computed to examine differences in the students' perceptions about their college experiences and the extent to which certain experiences influenced their interest and persistence in STEM based on classification. In the category "Participating in a Summer Bridge Program", there was a significant difference in the students' perceptions about their college experiences and the extent to which certain experiences influenced their interest and persistence in STEM based on classification ($p < .05$) (Table 5). In this case, seniors supported that statement the most, and juniors had the least positive opinions. Only areas where significant differences were detected are listed in the table.

The Kruskal Wallis ANOVA test was computed to examine differences in the students' perceptions about their college experiences and the extent to which certain experiences influenced their interest and persistence in STEM based on grade point average. There was a significant difference in the perceptions of students with a grade point average of 2.5-3.0 in their perceptions of "How easy is it to obtain the resources that you need from the university library system" ($p < .05$) (Table 6). Students with a grade point average of 2.5-3.0 have the highest mean rank score, indicating that they were least in agreement with the statement than students with a grade point average of 3.1-3.5 and 3.6-4.0. Only areas where significant differences were detected are listed in the table.

Table 5: Kruskal Wallis ANOVA – Classification by College Experiences and the Extent to which Experiences Influenced Interest and Persistence in STEM

	Classification	N	Mean Rank	Sig.
Participating in a Summer Bridge Program	Senior	25	32.14	.007*
	Junior	23	47.50	
	Sophomore	25	32.20	
	Total	73		

Table 6: Kruskal Wallis ANOVA – Grade Point Average by College Experiences and the extent to which Experiences Influenced Interest and Persistence in STEM

	GPA	N	Mean Rank	Sig.
How easy is it to obtain the resources that you need from the university library system	2.5-3.0	7	56.43	.001*
	3.1-3.5	32	41.44	
	3.6-4.0	34	28.82	
	Total	73		

Faculty Survey Results

The faculty provided their perceptions about instructional practices and learning strategies that enhance LSMAMP student learning. They also provided their perceptions about institutional practices and the degree to which instructional practices influenced student persistence, retention, and program outcomes. Table 7 is a presentation of the faculty perceptions about institutional practices that enhance student learning. The top choices by the faculty were the following: (1) Faculty mentoring, (2) student opportunities to present research at or attend professional conferences, (3) faculty advising, (4) faculty tutoring/study sessions, and (5) peer tutoring.

Table 8 is a presentation of the faculty perceptions about instructional practices and learning strategies that enhance LS-MAMP student learning. The top five choices by the faculty were: (1) Hands on lab experiments, (2) Instructor feedback on assignments, quizzes, and tests, (3) Individual projects, (4) Interactive lectures (a mix of instructional methods to include open dialogue), (5) Student opportunities to connect prior learning to new lecture content.

Table 7: Instructional Practices/Learning Strategies that Enhance Learning Outcomes

	Very Important N (%)	Important N (%)	Somewhat Important N (%)	Not Important N (%)
Faculty mentoring	12 (46.2)	1 (3.8)	0 (0.0)	0 (0.0)
Student opportunities to present	11 (42.3)	2 (7.7)	0 (0.0)	0 (0.0)
Faculty advising	10 (38.5)	3 (11.5)	0 (0.0)	0 (0.0)
Faculty tutoring/study sessions	9 (34.6)	3 (11.5)	1 (3.8)	0 (0.0)
Peer tutoring	6 (23.1)	4 (15.4)	3 (11.5)	0 (0.0)

N=26

Table 8: Instructional Practices and Learning Strategies that Enhance Student Learning

Instructional Practices/ Learning Strategies	Very Important N (%)	Important N (%)	Somewhat Important N (%)	Not Important N (%)
Hands on lab experiments	14 (53.8)	0 (0.0)	0 (0.0)	0 (0.0)
Instructor feedback on assignments quizzes and tests	12 (46.2)	2 (7.7)	0 (0.0)	0 (0.0)
Individual projects	11 (42.3)	3 (11.5)	0 (0.0)	0 (0.0)
Student opportunities to connect prior learning to new lecture content	11 (42.3)	3 (11.5)	0 (0.0)	0 (0.0)
Interactive lectures (a mix of instructional methods to include open dialogue)	11 (42.3)	3 (11.5)	0 (0.0)	0 (0.0)

N=26

Table 9 is a presentation of the faculty perceptions regarding the degree to which institutional practices influenced student persistence, retention, and program outcomes. The top five choices by the faculty were: (1) Adequate funding for research lab and learning materials, (2) Internship placements, (3) Student research presentations or publications, (4) Faculty research presentations or conference attendance, (5) Submitting early warning alerts to signal the need for academic interventions, (6) Stipends/faculty incentives, and Convenient scheduling and flexible course formats (online, distance learning, evening classes).

Table 9: Degree to which Institutional Practices Influenced Student Persistence, Retention, and Program Outcomes

Institutional Practices	Very Important N (%)	Important N %)	Somewhat Important N (%)	Not Important N (%)
Adequate funding for research lab and learning materials	13 (50.0)	1 (3.8)	0 (0.0)	0 (0.0)
Internship placement	11 (42.3)	3 (11.5)	0 (0.0)	0 (0.0)
Student research presentations or publications	11 (42.3)	2 (7.7)	1 (3.8)	0 (0.0)
Faculty research presentations or conference attendance	10 (38.5)	2 (7.7)	2 (7.7)	0 (0.0)
Submitting early warning alerts to signal need for academic intervention	9 (34.6)	5 (19.2)	0 (0.0)	0 (0.0)
Faculty networking opportunities outside of the institution	9 (34.6)	4 (15.4)	1 (3.8)	0 (0.0)
Stipends/faculty incentives	9 (34.6)	3 (11.5)	2 (7.7)	0 (0.0)
Convenient scheduling and flexible course formats (online, distance learning, evening classes)	9 (34.6)	5 (19.2)	0 (0.0)	0 (0.0)

N=26

DISCUSSION

In conducting this exploratory mixed-methods study, the authors sought to gain insight and understanding about institutional best practices, instructional experiences, and persistence factors that enhance Mississippi's LSAMP student degree attainment in STEM. This study was guided by interview and survey data on STEM and non-STEM students, STEM faculty, and site coordinators to gauge institutional best practices and persistence factors that support desired student academic outcomes.

Research from the phenomenological analysis yielded five themes that were consistent with the preliminary results from the quantitative data that compared differences in outcomes between LSMAMP and non-LSMAMP participants as well as perceptions of LSMAMP faculty and site coordinators.

First, the results indicate that early exposure to STEM and familial support were important to LSMAMP students' field of choice and preparation for baccalaureate academic experiences and success. This was reinforced by taking advanced placement (AP) honors classes in high school, pre-college summer bridge program provided by LSMAMP as a recruitment strategy provided focus and interest for the students in STEM disciplines. Participants' reflections of familial support were instrumental to their strong interest, persistence, and success in STEM. This is consistent with Furstenberg and Hughes (1995) research positing that strong family support, sometimes extending well beyond the immediate family, equipped students to persist through challenges. LSMAMP practice of creating a familial environment that is supportive of students' social and academic needs served as a pathway for student professional growth and academic enhancement that had a positive impact on their performance and persistence in STEM disciplines. This finding is supported by the research of others (Guifrida, 2005; Slaughter-Defoe et al, 2006; Griffin Toldson, 2012). Another significant result is the value study participants placed on their involvement in LSMAMP STEM academic intervention activities. They recognized the role of mentoring, engagement in hands-on-research, tutoring, workshops, and academic advising as program best practices that enhanced their persistence and degree attainment in STEM. This is consistent with the preliminary survey results from LSMAMP faculty and coordinators agreement with the importance of these factors on the LSMAMP best practices and student success towards STEM degree attainment.

Study results showed the importance of having peers with similar aspirations of doing well and supporting each other in their major disciplines as critically important in LSMAMP students' academic success and persistence. Specifically, the students mentioned that group interactions helped them to comprehend and retain class material. The integration of learning communities, familial type groups within their discipline and open communication provided them with collaborative learning environments and a sense of belongingness among students and faculty mentors. Consistent with this study's diffusion of innovations framework, communication is more effective when individuals "share common meanings, a mutual subcultural language, and are alike in personal and social characteristics, the communication of ideas is likely to have greater effects in terms of knowledge gain, attitude formation in change, and overt behavior change." (Rogers, 1981). Relative to the question of strategies that ensure LSMAMP student success from an institutional, instructional and student persistence perspective, participants underscored the positive STEM environment and institutional support as critical components of LSMAMP best practices for program success. The utilization of evidence-based teaching practices, innovative communication practices within the alliance, shared resources with other institutional STEM programs, faculty and students institutional support were cited by participants as reasons for program success. Study preliminary quantitative results show that LSMAMP students had a more positive view than non-LSMAMP students on these dimensions.

Finally, participants indicated that financial support (stipends, scholarships, grants, loans) enhanced their persistence and success towards their degree attainment. Studies suggest that minority intervention programs in STEM tend to recognize the importance of financial support to students. A report by the U.S. Department of Education (2000) found science and engineering degree completion to be positively related to receiving financial aid from school. National studies have consistently found student aid to be a positive influence on persistence (St. John EP, 1991).

CONCLUSIONS

The results of this study illustrate the importance of monitoring the progress made by STEM programs and the access and opportunity offered to colleges and universities for development of minority STEM students, as educational leaders strive to increase the level of STEM programming. There continues to be a great need for universities to strive to increase the graduation rates of African Americans and other minorities in STEM fields. An increase in the professional development of African American and minority students in the STEM field will position the US to be more competitive in the global economy as students develop to become leaders in the technical workforce.

The funding provided by the NSF LSAMP initiative has enabled Mississippi's higher education institutions to make progress towards closing the gap in the education and training of t minority students ensuring that they can find opportunity for success in the STEM disciplines. Continued attention to the development of African American and minority STEM professionals will ensure that graduates from HBCUs will be in a position to make meaningful contributions to science and other technical areas that will eventually benefit their communities and its constituents.

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Treatment Depth Effects of Combined Magnetic Field Technology using a Commercial Bone Growth Stimulator

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ABSTRACT

Lumbar spinal fusion is one of the more common spinal surgeries, and its use is on the rise. If the bone fails to fuse properly, then a pseudarthrosis or "false joint" develops and results in pain, instability, and disability. Since1974, three types of electrical stimulation technologies have been approved for clinical use to enhance spinal fusions. One such technology is inductive coupling, which includes combined magnetic fields (CMFs). The purpose of this study was to evaluate the effects of a CMF device known as the Donjoy (SpinaLogic®) on MG-63 (ATCC® CRL1427TM) human osteosarcoma cells at treatment depths ranging from 0.5" to 6.0". The cells were grown to confluence on 4-well chamber slides that were kept in a nickel-alloy chamber within an incubator to shield the cells from unwanted environmental electromagnetic fields. During treatment, a specially designed apparatus held both the treatment device and the chamber slide. Briefly, the chamber slide was placed inside an acrylic tube at a specific distance from the transducer housing, and the device was turned on for 30 minutes. The chamber slides were then returned to the incubator to be evaluated at 7, 14, and 21 days post treatment for cell viability and bone nodule formation. Our results showed that compared to control cells, the cells located at 3" from the source had the greatest increase in bone nodule formation 7 days post treatment which is the depth at this consistent with manufacturer recommendations.

Keywords: Combine Magnetic Field Technology, CMG, Electromagnetic Field, Bone growth

INTRODUCTION

According to the American Academy of Orthopedic Surgeons, many Americans are affected by back pain. In fact, 2.4 million Americans will be affected by back pain by the year 2025, and the majority of people will resort to surgery by the age of 60 or 70 years old (Shamie 2011). Most patients with back pain start out with pain management treatments to avoid surgery. Pain medications, physical therapy, and steroid injections are used to control pain. Fifteen percent of these patients will improve without having to resort to surgery. Patients who go on to have spinal fusion surgery still experience back pain even after they recover from the surgery. The procedure for spinal fusion involves joining together two vertebrae to prevent movement in the joint. The fusion is then held together by a bone graft.

Lumbar spinal fusion is one of the more common spinal surgeries, and its use is on the rise. If the bone fails to fuse properly, then a pseudarthrosis or "false joint" develops and results in pain, instability, and disability (Reid 2011). Since 1974, three types of electrical stimulation technologies have been approved for clinical use to enhance spinal fusions. Both invasive and noninvasive electrical bone growth stimulators have been investigated as an adjunct to spinal fusion surgery, to enhance the chances of obtaining a solid spinal fusion. Noninvasive devices have also been investigated to treat a failed fusion.

Electrical and electromagnetic fields can be generated and applied to bones through the following methods: Surgical implantation of a cathode at the fracture site with the production of direct current (DC) electrical stimulation. Invasive devices require surgical implantation of a current generator in an intramuscular or subcutaneous space, with an electrode is implanted within the bone graft. The implantable device typically remains functional for 6 to 9 months after implantation, and provide constant stimulation.

Noninvasive bone stimulators use combined magnetic field technology (CMF) to stimulate bone. CMF is a form of inductive electromagnetic technology that combines a sinusoidal waveform that emits a frequency of 76 Hz against a static electric field. [Behrens et al 2013; Rabjohn, LV 2008] CMF produces a signal that travels through tissue to target bone cell division.

The purpose of this study was to evaluate the distance from the source that provides an increase in bone nodule formation without causing cell injury.

MATERIALS AND METHODS

Osteoblast like cells were exposed to PEMF using a commercial bone growth stimulating device. The Donjoy Bone Growth Stimulator is combined magnetic field (CMF) device that superimposes a time varying magnetic field onto a static magnetic field. The Donjoy device maintains the static magnetic field at 200 milligauss (mG). The dynamic field of the Donjoy device is a sine wave with a frequency of 76.6 Hz and an amplitude of 400 mG peak superimposed in parallel with the static field. This device is used by medical professionals as a tool for healing problem fractures and spinal fusion procedures. The manufacturer suggests that a 30-minute treatment time per day at a distance of 3 inches from the PEMF source is sufficient to stimulate bone growth.

Cell Culture: The MG-63 (ATCC® CRL1427™) human osteosarcoma cell line was obtained from American Type Culture Collection (ATCC, Rockville, MD) and supplemented with Dulbecco's Modified Eagle's Medium (DMEM) + 5% fetal bovine serum + 1% antibiotic/antimycotic solution in T-75 culture flasks where they were grown to confluence under normoxic conditions at 37^{o}C. At confluence, the cells were rinsed once with PBS, trypsinized for three minutes. DMEM-supplemented media was added, and cells were collected and plated in 32 Lab-Tek®II 4-well Chamber Slides^{TM} (Thermo Fisher Scientific, Inc., Waltham, MA) and one 24-well plate at a density of 3 x 10^3 cells/well. When the cells reached confluence the DMEM medium was supplemented with 50 µg ml^{-1} ascorbic acid and 10 mM glycerol 2-phosphate disodium salt hydrate for bone nodule formation. The chamber slides were then placed inside a nickel-alloy chamber within the incubator to shield the cells from unwanted environmental EMFs. Culture media was replaced every three days.

Preliminary Experiment to determine cell harvesting time: Cells were stimulated once for 30 minutes using the Donjoy device and followed over 9 days to compare the doubling time for the cell line in order to determine the appropriate time to analyze cells for changes. Figure 1 shows the growth curve for the cell line. Comparison of the correlation coefficients (z=0.45 one tailed p> 0.3264) indicated no difference between the two treatments. Our results also show a doubling time comparable to 72 hours. Our data is consistent with an article published in Human Cell 2009 Nov; 22(4): 85-93 (Mills et al., 2009) They also showed a 72 hour doubling time for the MG63 cells

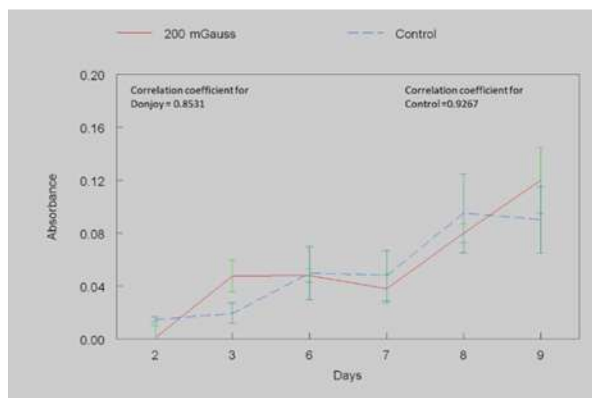

Figure 1. Preliminary study to show growth curve for MG 63 cells after a single 30 minute CMF dose at 3 inches.

Treatment/Exposure: At confluence, each chamber slide was randomly assigned to the CMF SpinaLogic® treatment group or the Control group. All slides in the Control group remained in the nickel-alloy chamber within the incubator for the duration of the experiment except during media renewal or the alamarBlueTM assay. A 30 minute exposure of the chamber slides at distances of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, or 6 inches from the source were performed on day 0. At days, 7, 14, and 21 cells were assessed for viability and nodule formation. The experiment was repeated 3 separate times and the data at each distance were combined.

alamarBlue™ Cell Viability Assay: The alamarBlueTM cell viability assay, which is based on the enzymatic reduction of an indicator dye by viable cells. Briefly, resazurin, the active ingredient of alamarBlue™ reagent, is a non-toxic, cell-permeable compound that is blue in color and virtually non-fluorescent. Upon entering living cells, resazurin is reduced to resorufin, a compound that is red in color and highly fluorescent. Changes in viability can be easily detected using either an absorbance based plate reader. Two hundred microliters of alamarBlue™ reagent were added to each chamber slide and allowed to incubate for four hours after 4 hours the slides were rinsed with sterile PBSS and fresh media was added to the cells. When added to viable cells, alamarBlue™ reagent goes from blue to red in color. This color change can be detected using absorbance 570 and 600 nm.

Bone Nodule formation Alirizin Red Staining: Alizarin Red S (ARS) staining is a technique used to detect calcium-rich deposits in cell culture as a qualitative evaluation of bone mineralization. ARS specifically reacts with calcium cation, forming a chelate, which can then be visualized using phase contrast microscopy to assess fine structure and mineral distribution of the terminated cell culture. ARS staining was performed as described by Gregory et al. (2004). At the appropriate time-points, cell media was removed, and cell culture monolayers were washed with PBS and fixed in 10% formaldehyde for 15 minutes at room temperature. Cells were washed twice with distilled water and 1 mL of 40 mM ARS (pH 4.1, adjusted using 10% ammonium hydroxide) was then added to each well. Plates were incubated at room temperature for 20 minutes with gentle shaking. Unincorporated dye was aspirated, and the cells were washed with distilled water 4 times for 5 minutes of gentle shaking per wash. Plates were positioned at an angle for 2 minutes, after which excess water was removed, and plates were air dried for image capture using phase microscopy.

Statistical Analysis

Descriptive statistics were obtained for each outcome parameter and reported as the mean ± standard deviation (SD). Each data set was tested for normality of distribution using the Shapiro-Wilk and Kolmogorov-Smirnoff tests. For normally distributed (parametric) data, quantitative differences between control and treatment (various distances) groups were analyzed using one-way analysis of variance (ANOVA). The level of significance for all tests was established at $\alpha=0.05$. If a calculated p-value was less than 0.05, post-hoc analysis was performed using Tukey's Multiple Comparison Test to determine between which groups the significant difference exists. For data that was not normally distributed, the equivalent non-parametric test (Kruskal-Wallis) was employed.

RESULTS

Bone cells placed in osteogenic media and exposed to CMF for 30 minutes at different distances from the source had similar viability as control cells maintained inside a nickel-alloy chamber within the incubator to shield the cells from unwanted environmental EMFs Figure 2. Slight differences in the percent reduction in alamar blue were observed from a distance of 0.5 inches to 3.5 inches at 14 and 21 days. However, this difference was not statistically significant.

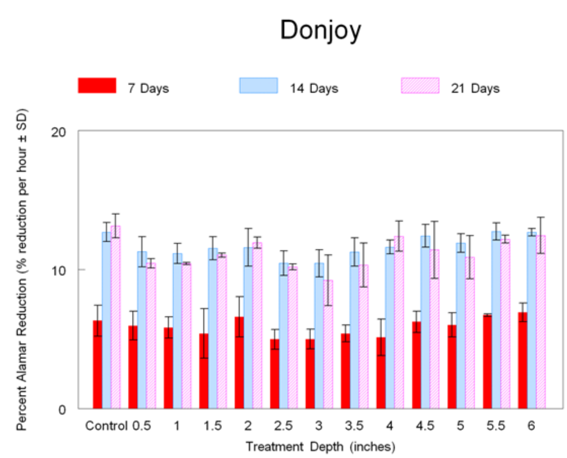

Figure 2. Cell viability using Alamar Blue assay following treatment with CMF at various distances Results are expressed as % reduction of Alamar Blue/hour ± SD

Cellular Morphology: Figures 3 demonstrates cells treated with single exposure of CMF after 7 days. By 14 and 21 days the cells on the chamber slides were confluent and very little differences were observed in cell morphology. At 7 days the cells exposed to CMF at 0.5 inches had fewer cells. The cells exhibited no identifiable cell structure, with dissolution of the chromatin material and cellular necrosis. Whereas, the control cells were clustered and rectangular shaped with large nuclei that contained visible chromatin material. This result suggests that CMF exposure at 0.5 inches was essentially a lethal exposure that resulted in cell necrosis. The cells treated with a single exposure of CMF at 2.5-4.0 inches has large nuclei with visible uniform chromatin patterns. The cells treated with a single exposure of CMF at 4.5-6 appeared like cells that did not receive CMF exposure. Interestingly, cells treated with CMF exhibited an ordered and aligned patterned on the slide (Figure 3) which was evident at all-time points and all distances.

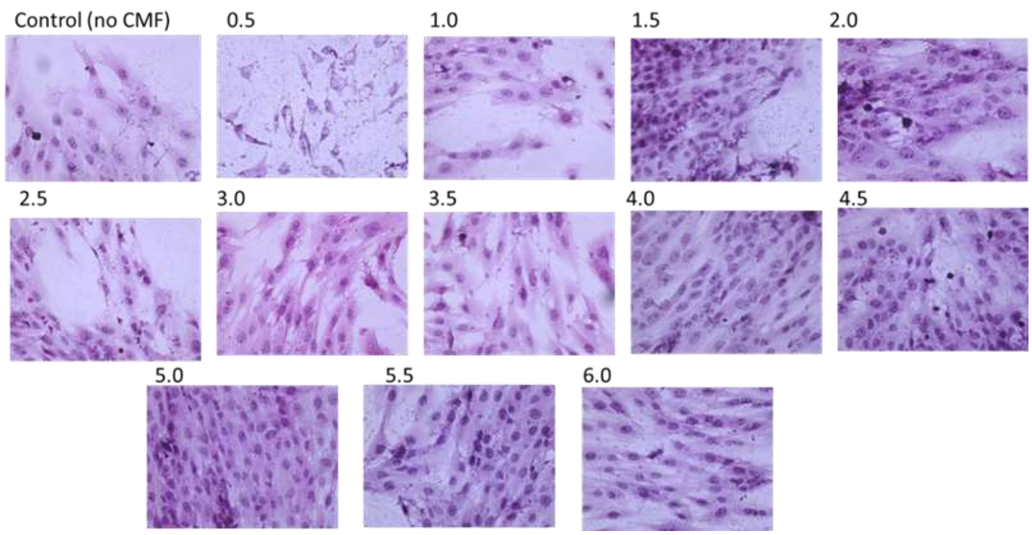

***Figure 3**. Representative Hematoxylin and Eosin stained cells after 7 days post 30 minute single exposure to CMF at distances ranging from 0.5-6 inches and compared with control which were not exposed to show changes in morphology and cellular arrangement.*

Mineralization/Bone Nodule Formation: Figure 4 represents cells exposed after 7 days to show initial mineralization. Qualitative analysis of the slides show an increase in the proportion of cells staining positive with Alizarin red. The Alizarin red stain is widely used to demonstrate calcium deposits in cell culture. There appears to be an increase in calcium deposition in cells treated with a single dose of CMF using DonJoy deveice for 30 minutes at 7 days at distances ranging from 2.5- 4.0 inches when comaprd with control cells which were not exposed to CMF.

When cellular mineralization was measured by selecting a region of interest and counting the number of nodes and measuring the node area between the CMF treated groups and the control groups, there was no significant difference at 21days for any distances after CMF treatment. However, there was a much higher mean cellular mineralization for cells exposed to CMF at 3.0inches compared to the 0.5 inch or 6 inch distances, however, the standard deviation was so large that there was not a significant difference $p< 0.05$. Figure 5 shows representative node formation at 21 days following a single exposure.

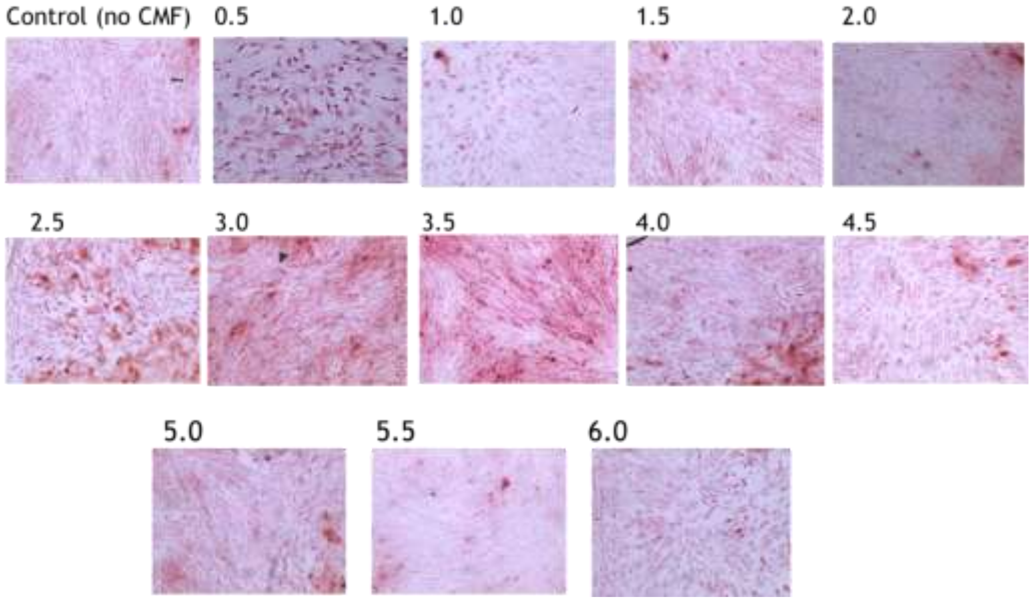

Figure 4. Representative Alizarin red stained cells after 7 days post 30 minute single exposure to CMF at distances ranging from 0.5-6 inches and compared with control which were not exposed to show the induction of bone formation

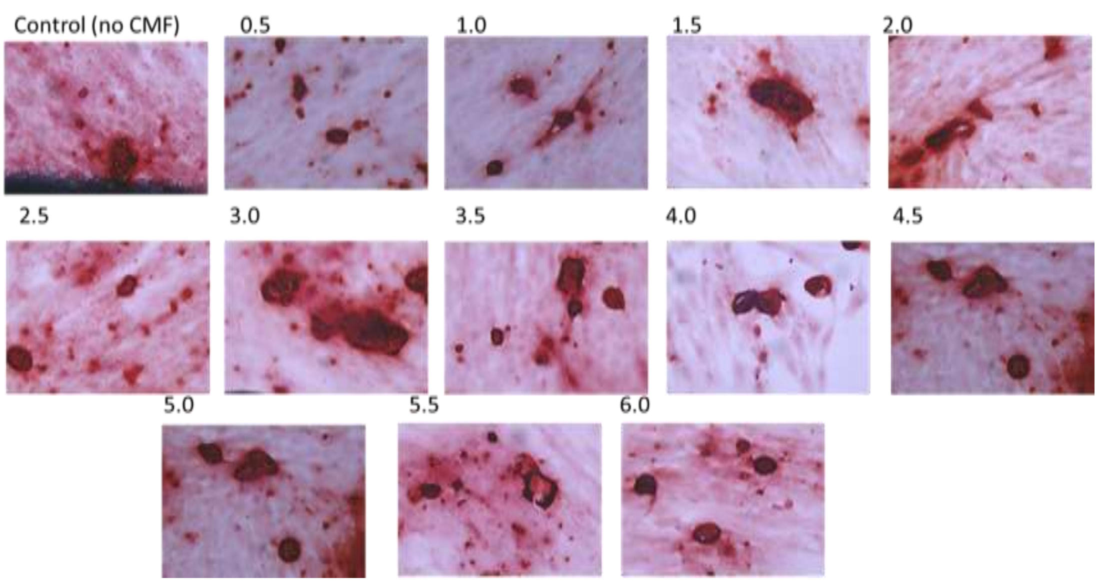

Figure 4. Representative Alizarin red stained cells after 21 days post 30 minute single exposure to CMF at distances ranging from 0.5-6 inches and compared with control which were not exposed to show the increase in bone nodule formation

DISCUSSION

The combined magnetic field (CMF), a unique electromagnetic field that includes dynamic sinusoidal magnetic field and a magnetostatic field, has been used to promote bone healing and spinal fusion clinically by simulating the endogenous production of bone growth factors (Fitzsimmons et al., 1994; Hanft et al., 1998; Linovitz et al., 2002). In clinical studies the CMF stimulation appears to failitate the maturation of newly forming bone, and in addition, CMF greatly increases patient compliance by decreasing the daily treatment time to 30 minutes comparted to the 4 hour pulsed electormagentic fields that are used in clinical settings. Animal studies showed that CMF accelerated the outgrowth of new bone. From week 1 to week 8, CMF therapy promoted osteoid formation. Quicker maturation of the bone was noted after eight weeks of daily exposure to CMF therapy (Quin et al., 1999; Lu et al., 2006; Hu et al., 2014). In our study, qualitiative analysis of the slides showed an appearance of increased mineralization at the 7 day time point in cells treated once with CMF for 30 minutes at distances of 2.5 to 4 inches. Additional studies to quatitatively measure bone markers such as RUNX2 and alkaline phosphastase are needed to follow the peak time needed after stimulation to induce bone formation.

Researchers have also reported that CMF stimulation significantly increased the proliferation of MG63 cells, human normal osteoblast cells, and human derived MSCs as early as 24 hours after stimulation. Kalamolmatyakul et al. (2008) exposed both human normal osteoblast cells and osteoblast-like MG63 cells cultured in standard media to CMF 50Hz at 1.5mV to determine the effect on proliferation. Cell proliferation was determined using an MTT (tetrazolium) colorimetric assay. While both cell groups had a significant increase in cell proliferation compared to the controls, the human normal osteoblast cells showed a 100% increase while the MG63 cells showed a 50% increase. Sun et al. (2009) exposed human bone marrow MSC cells grown in expansion medium and seeded at either 1,000 cells/cm^2 or 3,000 cells/cm^2 to CMF 15 Hz at 1.8mT. Two control groups were used and seeded with either 1,000 or 3,000 cells/cm^2. Cells were detached using trypsin-EDTA and a cell scraper and then counted by a hemocytometer under a microscope to measure cell proliferation. At 24 hours both treated groups had significantly more viable cells when compared to the controls. Tsai et al. (2009) exposed human MSCs cultured in osteogenic medium to CMF 7.5 Hz at 0.13mT, they saw proliferation of the treated cells was not greater than controls at 7 days, which was similar to our study using a single 30 minutes challenge and evaluating after 7 days. Chang et al. (2004) exposed murine osteoblast-like cells cultured in standard medium with CMF at 15 Hz at 0.1mT for 8 hours a day, and found a significant osteoblast proliferation at 3, 5 and 7 days of culture when compared to the controls. In a similar study Barnaba et al. (2013) exposed human MSCs cultured in standard medium to daily CFM 14.9 Hz at 0.4mT and found significant increases in cells numbers at 7 and 10 days when compared to controls. Additional studies are needed to determine the maximum number of exposures that are needed to induce bone formation, and if there is a plateau for bone formation or in excessive exposures induce untoward side effects.

CONCLUSIONS

Combine magnetic field technology an increase in the percentatge of bone mineralization in the treated groups after 21 days, with an optimum treatment depth of 2.5-4 inches. In addition, our results show an earlier induction of mineralization at 7days in cells exposed to the CMF device. It can be postulated that patients can have a less arduous recovery time when using CMF therapy.

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Bold, H.C., C.J. Alexopoulos, and T. Delevoryas. 1980. Morpholgy of plants and fungi, 4th ed. Harper and Row, New York. 819 pp

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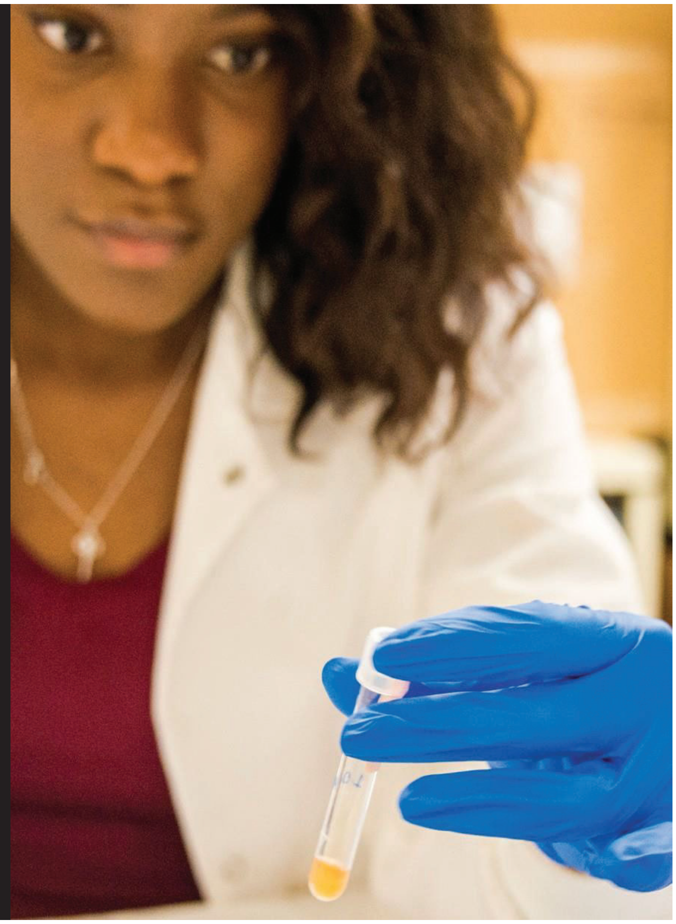

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