Journal of the Mississippi Academy of Sciences

Volume 61 April 2016 Number 2

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

Research Articles

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A FACILE 3D CONSTRUCT OF GRAPHENE OXIDE EMBEDDED WITH SILVER NANOPARTICLES AND ITS POTENTIAL APPLICATION AS WATER FILTER

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ABSTRACT

Silver nanoparticles (AgNPs) have been widely explored as possible novel antimicrobials. Thus, we developed a facile preparation method for a 3D Graphene Oxide embedded with AgNPs (GO-AgNP) for potential use as a water filter to remove harmful bacteria. GO was synthesized via the modified Hummers' method using graphite. AgNPs were embedded by *in situ* reduction of silver nitrate using sodium borohydride and sodium citrate. AgNPs of two sizes ranges, 6 - 12.5 nm and 36 - 41 nm, were obtained. The GO-AgNP sheets in solution were crosslinked by polyethylene glycol (PEG) in the presence of Na2S to yield GO- AgNP 3D structures. Vacuum filtration was utilized to shape the GO-AgNP as a filter, followed by drying in the oven. The thus prepared filter is stable in water for over 7 months and releases Ag+ at a concentration below 45 nM. Scanning Electron Microscopy shows that the filter is porous with pores ranging from 8–18 μm. Energy-dispersive X-ray spectroscopy shows that AgNPs spread uniformly in the filter. The filter is highly efficient to filter off a very dangerous multidrug-resistant bacterium, *Salmonella typhimurium* DT 104, from water. Spread-plate method indicates that over 99% of the *Salmonella* DT104 are removed.

INTRODUCTION

According to the World Health Organization (WHO) report in 2004 [1], 1.8 million people in developing countries die every year from diarrheal diseases (including cholera), and 90% are children under 5 years old. About 88% of the diarrheal diseases are attributed to unsafe water supply and inadequate sanitation. Therefore, sustainable access to clean and safe drinking water has been identified as a significant challenge in developing countries [2]. For developed countries, sand filtration and chlorine disinfection are used to end waterborne epidemics a century ago [3]. However, the presence of drug-resistant pathogenic bacteria has become the main cause of water contamination recently, causing outbreaks of waterborne diseases at unexpected high levels [4]. Actually, 80% of diseases are due to contaminated drinking water. Even today, unsafe water and lack of sanitation are listed as risk factors associated with increased mortality and morbidity by WHO [5]. Therefore, there is a demand to develop novel technologies to produce clean and safe drinking water.

To address the issue of harmful bacteria in water, antimicrobial materials must be applied. Conventional antibiotics are not optimum since many bacteria have developed resistance to even the 3rd generation of antibiotics according to the 2014 Antimicrobial Resistance Global Report [6]. To solve this problem, silver nanoparticles (AgNPs) have been explored as an antibiotic to overcome drug-resistance. For centuries, silver has been used for wound dressing and antibiotic coating [7, 8]. Recently, AgNPs have been considered as a promising antimicrobial agent [9-11], especially against multidrug resistant bacteria [12]. Owing to this fact, silver has been coated on water purifiers to prevent bacteria and algae from building up in filters. It has been proposed that the catalytic action of silver, in combination with oxygen, acts as a powerful sanitizer that offers an alternative to other disinfectants, like chlorine [13].

There have been research reports to incorporate AgNPs into different materials to construct antimicrobial devices, including polyurethane foam [14, 15], ceramic composite [4,16], polypropylene [17], polyacrylonitrile [18], polyethylene and poly (acrylic acid) [19], cellulose [20, 21], silica beads [22] and other inorganic materials [23, 24]. There are two strategies shown in these studies. One strategy uses solid structures as substrates to have AgNPs coated to its surface to produce filters, like AgNP-modified ceramic or silica filters. Le et al [25] prepared a filter coated with both AgNP and nano-TiO2 as photocatalyst for purification purposes in hospitals. The other strategy uses AgNPs to build with amorphous polymers as composites, which exhibit promising antimicrobial efficacy when applied as water filters [3, 26].

The present study introduces graphene oxide (GO), which has many functional groups including epoxy (-O-),

hydroxyl (-OH) and carboxyl (-COOH) groups [27-29], as a carrier for AgNPs to build the 3D filter [30-32]. The functional groups are binding sites for AgNPs [33]. There have been some reports on combining AgNPs with GO using various reduction methods [34-41], in the form of nanosheet composites [42] or aqueous suspensions [43]. These composites or suspensions have been further used to explore their antimicrobial activity [44-46], their usage as surface enhanced Raman materials [38, 47, 48], as well as their conductive or electrochemical properties [36 37, 45, 49]. Bao et al [42] prepared GO-AgNP composite by *in situ* reduction of adsorbed Ag+ by hydroquinone (HQ) in a citrate buffer solution, followed by vacuum filtration to induce directional flow to yield a AgNP-GO filter paper. The resultant filter paper has effective antibacterial properties against both *E. coli* and *S. aureus*. Zeng et al [50] designed a 3D filter based on AgNPs and GO hydrogel. It consists of controlled porous GO network with well-dispersed AgNPs. It also showed good efficacy against *E. coli* when used as a bactericidal filter.

All these studies tried to combine AgNP and GO and use the composite in water filtration or disinfection system. However, they either lack of control over the 3D structure or did not deal with multidrug resistant (MDR) bacteria. The propagation of MDR bacteria as a worldwide issue is the challenge for water purification [6, 12, 51]. Therefore, this study aims to develop a facile 3D construction method using GO embedded with *in situ* reduced AgNP to be used to filter MDR *Salmonella* DT 104 from water.

MATERIAL AND METHODS

Materials: Graphite, KMnO4, NaNO3, concentrated H2SO4, polyethylene glycol (PEG, MW 2,000), AgNO3, NaBH4, sodium citrate and NaOH were purchased from Fisher Scientific (Houston, TX). Multidrug-resistant *Salmonella typhimurium* DT 104 (ATCC 700408) was from American Type Culture Collection (Manassas, VA). Tryptic soy broth (TSB) and tryptic soy agar (TSA) were purchased from Sigma Aldrich (St. Louis, MO).

Synthesis of GO-AgNP 3D Filter: The procedure is shown in Figure 1. The 2D GO sheets were prepared using the modified Hummers' method [29, 52-54]. Briefly, graphite powder (1.0 g) and NaNO3 (1.0 g) were added to 25 mL of concentrated H2SO4 under 10 ºC in a water bath (cooled with added ice), and the mixture was allowed to stay for 30 min at this temperature. Then KMnO4 (3.0 g) was carefully added into the mixture at <10 ºC and the solution was kept for 30 min with sonication. Then the solution was warmed up to 38 ºC with stirring for 30 min. Afterwards, 100 mL of water was added and was heated to 90 ºC and kept at this temperature for 30 min [51]. After the solution is cooled down to room temperature, 60 mL of water was added and the resultant solution was washed three times by centrifugation at 3000 rpm for 30 min each. The thus obtained GO sheets were re-dispersed in 80 mL of water for further experiments. To prepare GO-AgNP composites, AgNO3 was reduced *in situ* by NaBH4 and sodium citrate [44], where sodium citrate also functions as a coating agent. At room temperature, 5 mL of the above GO solution was diluted to 10 mL and added with 5 mL of 2 mM AgNO3 under sonication for 10 min. NaBH4 (5 mL, 4 mM) and sodium citrate (2 mL of 1%) were added slowly and successively. Sonication was continued for another 30 min and the black GO- AgNP sheets in solution were formed. The GO-AgNP sheets were crosslinked by PEG in the presence of Na2S [44], which was accomplished by adding 200 mg of PEG dissolved in 5 mL of water under continuous sonication for 10 min, followed by addition of 20 mg of Na2S dissolved in 5 mL water. Sonication was continued for 5 min before the resultant solution was moved to heated water bath at 90- 95 ºC for 45 min. During this process, the black solution turned to a dark colloidal solution. After cooling to room temperature, the GO- AgNP colloid was filtered through a cellulose fiber paper (2.5 cm in diameter, with particle retention 5 - 10 µm) to produce the GO-AgNP filter. Vacuum can be applied to accelerate the filtration process. The filter paper was then taken off and the shaped GO-AgNP filter was dried in the oven overnight.

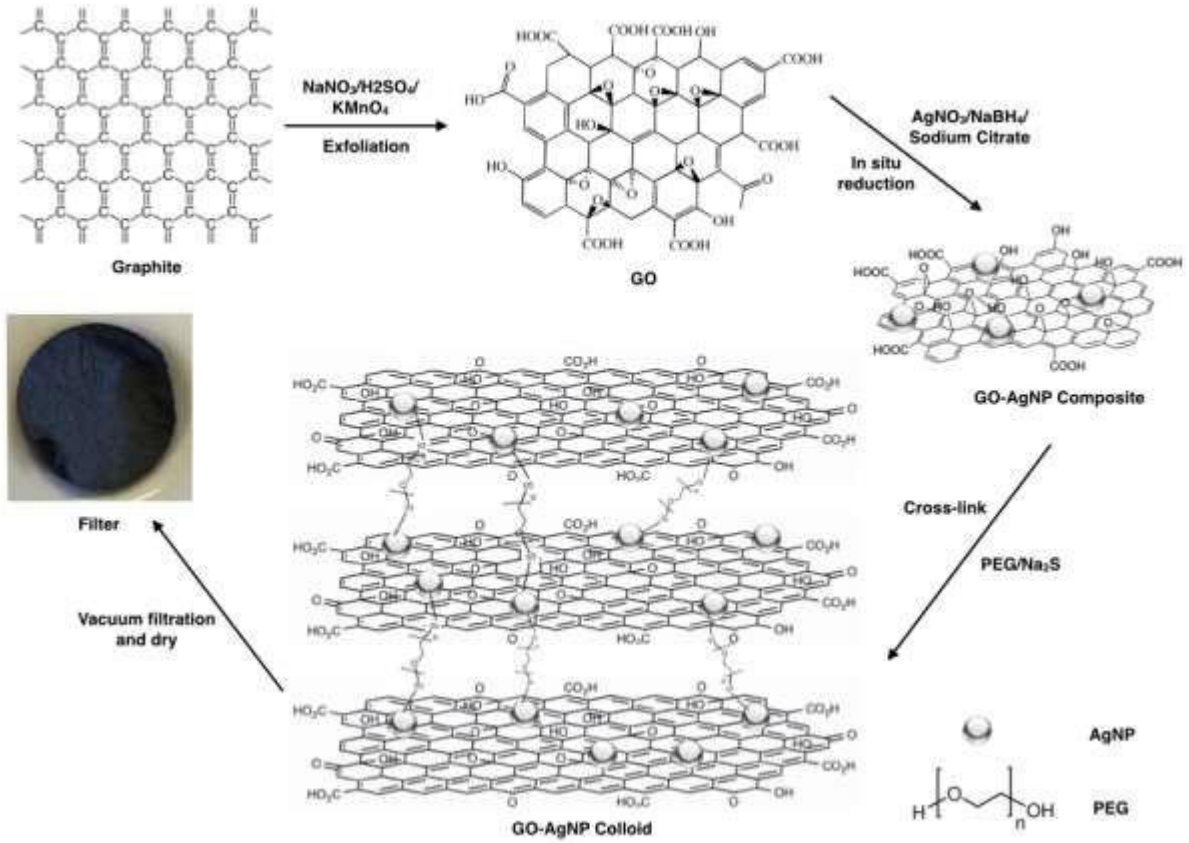

Figure 1. Schematic representation of the synthetic procedure of GO-AgNP filter from graphite and Ag+.

Characterization: The GO and GO-AgNP composites were characterized by JEM-2100F transmission electron microscopy (TEM). The surface of the final GO-AgNP filter was characterized by SEM using a Zeiss Sigma VP FEG-SEM while the element components were analyzed with Thermo System 7 EDS at the University of Southern Mississippi.

Bacterial culture and treatment with GO-AgNP filter: Growth inhibition experiment of *Salmonella typhimurium* DT 104 was carried out as described in our previous report [55]. Briefly, 50 µL of the bacterium was removed from a frozen (-80 ºC) sample and added to 12 mL of TSB, followed by incubation in a C25 class incubator with continuous agitation at 220 rpm for 10 h at 37 ºC. Inoculums of about $1x10^8$ colony forming units per mL (CFU/mL) were achieved [56]. The bacterium solution was further diluted to $1x10^5$ CFU/mL to be used for spread plate counting when after filtration with GO-AgNP filter.

RESULTS AND DISCUSSION

Characterization of GO-AgNP by TEM and SEM: As shown in Figure 2A, the GO sheets prepared are very thin with some as folded structures. After in situ reduction of Ag+, AgNPs are dispersed and attached to GO sheets with mainly two size ranges of 36 - 41 nm (Figure 2B) and 6 - 13 nm (Figure 2C). The amount of 36 - 41 nm sized AgNPs is much smaller than that of the 6 - 13 nm AgNPs. Both NaBH4 and sodium citrate are introduced as reducing agents. We believe that the minor amount AgNPs of 36 - 41 nm are a result of the reduction by sodium citrate while the major amount AgNPs of 6 - 13 nm are the result of reduction by NaBH4. NaBH4 has a stronger reducing ability than that of sodium citrate at room temperature.

SEM is used to characterize the synthesized GO-AgNP filter. As shown in Figure 3, GO-AgNP filter consists of dense porous structure comprised of GO sheets. The pore size ranges from 8 to 18 µm. AgNPs disperse on the surface of GO sheets shown as the white dots in Figure 3C. To further confirm the chemical compositions of the filter, EDS was utilized to analyze the percent of different elements (Ag, C, O, Na and S).

Ag is evenly distributed on or in the chosen surface of the GO-AgNP filter (Figure 4). The same is for C, O, and Na (not shown).

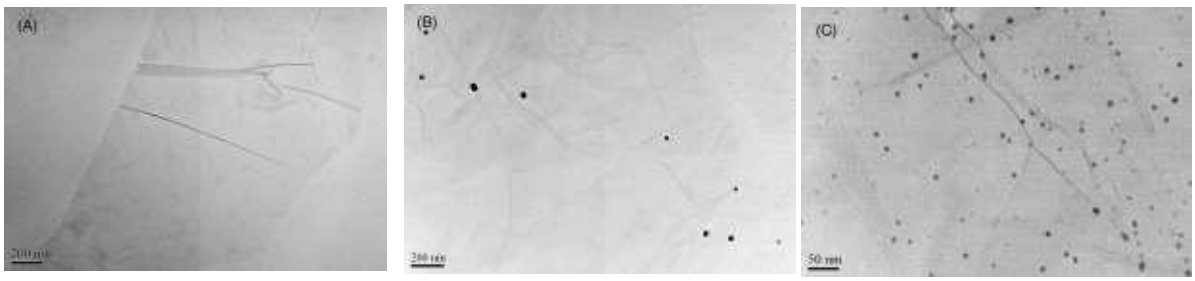

Figure 2. TEM images of GO sheets (A) and GO-AgNP composites (B). Two different sizes of AgNP are observed with smaller sized AgNPs shown in (C) in a greater magnification.

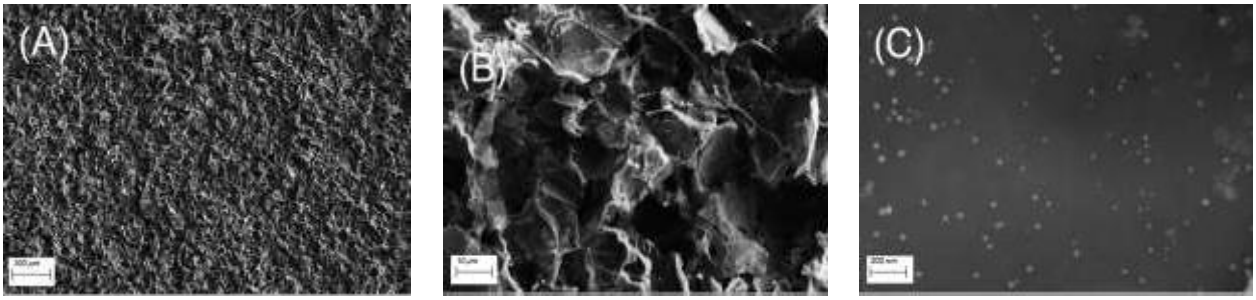

Figure 3. SEM images of the surface of GO-AgNP filter at different magnifications. Scale bars of (A), (B) and (C) are 100 µm, 10 µm and 200 nm.

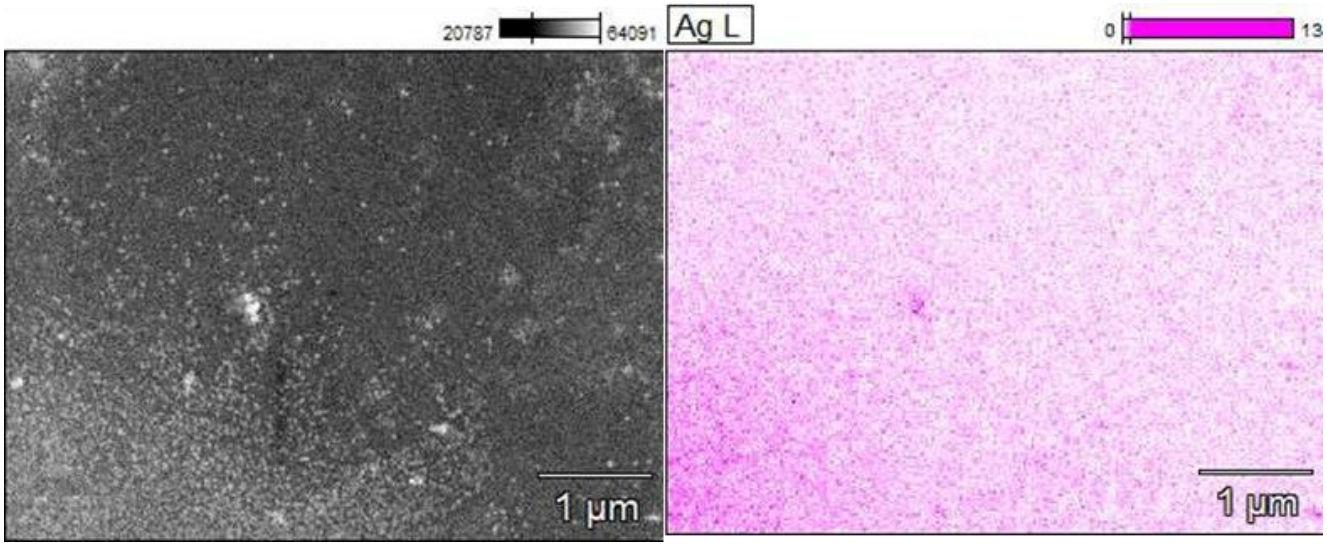

Figure 4. SEM image of a chosen area of the surface of GO-AgNP filter (left) and its EDS image (right) in the form of Ag element composition.

The relative ratio of GO to AgNP is variable. The diameter and the thickness of the produced filter can be controlled by changing the size of the funnel and the initial dosage of GO solution. However, GO must be washed at least 3 times to remove all oxidant species before *in situ* reduction of Ag^+. A slight excess of NaBH4 and sodium citrate converts all Ag^+ to AgNP and stabilizes AgNP at the optimum sizes.

Filtration efficiency and antibacterial activity of the filter against *Salmonella* DT104

Filters with a diameter of 2.5 cm and thickness of 0.3 cm were tested. A 10 mL *Salmonella* solution of $1x10^5$ CFU/mL was filtered. Once the filtration completed, the control, the filtrate and the washings of possible bacteria remained on the filter were tested with the spread plate method. All the colonies were counted after incubation of 12 h. No colonies were found in the filtrate, showing that over 99% of *Salmonella* were filtered off from water. There were no colonies grew from the washings of the filter. This indicates that *Salmonella* bacteria were either completely killed upon the contact with the GO-AgNP filter or are attached to the filter material and cannot be washed off. Therefore, GO-AgNP is highly efficient as a water filter to remove MDR *Salmonella* bacteria.

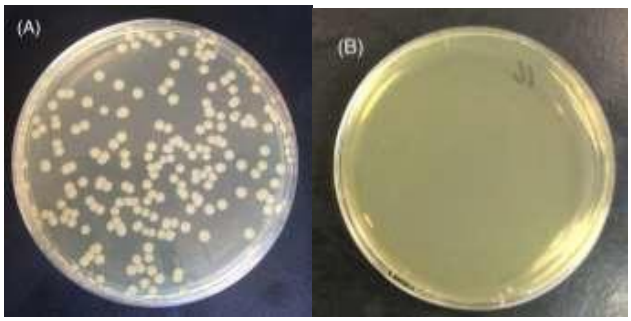

Figure 5. Pictures of colonies of *Salmonella* before (A) and after (B) filtration.

To test the stability of the GO-AgNP filter, it was immersed in water for more than 7 months and it remained with the same shape and morphology. As a negative control, a portion of the cellulose has been introduced into current GO-AgNP filter system. However, due to the lack of chemical binding interaction between cellulose and GO-AgNP, those filters containing cellulose gradually disintegrated and black powder was released when immersed in water for 2 weeks. This confirms that the GO-AgNP filter is crosslinked firmly by chemical bonds and remains stable in water. The aqueous phase of the GO-AgNP was collected for analysis of Ag+ concentration using ICP-MS. The concentration of released Ag+ is less than 45 nM, which is of a safe level for humans [57, 58.]

CONCLUSIONS

A facile 3D GO-AgNP composite is prepared by *in situ* reduction of Ag^+ on GO sheets followed by crosslinking with PEG in the presence of Na2S. Chemical compositions are highly controllable by varying the dosage of Ag^+ and PEG. GO-AgNP is further shaped by filtration with controllable size and thickness of the GO-AgNP filter. The filter has a porous structure with pore sizes ranging from 8 to 18 μm. It exhibits high efficiency to filter off MDR *Salmonella* DT104 from water. The filter is highly stable for 7 months in water, with the released Ag^+ being less than 45 nM.

ACKNOWLEDGEMENT

We thank Jessica Douglas and the School of Polymers and High Performance Materials, The University of Southern Mississippi for surface characterization using SEM and EDS. This research was supported in part by NSF through the Partnership for Research and Education in Materials grant (DMR-0611539). Core research facilities were supported by grants from the NSF (CHE-0840450) and NIH (NCRR 8G12MD007581).

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COMPARATIVE ANTIOXIDANT EFFECTS IN TWO OVARIAN CARCINOMA CELL LINES MEASURING POTENTIAL BIOMARKERS

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ABSTRACT

Cell lines derived from tumors are the most frequently utilized models in cancer research and their use has advanced the understanding of cancer biology tremendously over the past decades. The average five-year survival rates of stage III and IV ovarian cancer patients have been demonstrated as being around 37% and 25%, respectively. This poor survival rate is attributed to diagnosis at advanced stage and resistance to chemotherapy. The present study investigates the measurement of conventional delivery of thymoquinone (TQ) and epigallocatechin-3-gallate (EGCG) when applied to the ovarian cancer cell lines Caov-3 and SK-OV-3. A total of 72 wells were plated with (10^5) Caov-3 and SK-OV-3 cells according to standard lab protocols and subdivided into 4 groups of 6 wells each. Group 1 served as control and groups 2, 3, and 4 were treated with TQ (16 μM), EGCG (3 μg/ml), and TQ + EGCG, respectively. Biomarker evaluations were performed following standard lab techniques. The results of the study revealed: (1) an increase in the SK-OV-3 cell protein levels following treatment with TQ+EGCG which was statistically different ($p<0.05$) at 24 and 48 hours; (2) nitric oxide levels were statistically different ($p<0.05$) following the administration of EGCG and TQ+EGCG at 24 and 48 hours for Caov-3 and TQ at 72 hours for SK-OV-3; and (3) glutathione levels were statistically different ($p<0.05$) following the administration of TQ and EGCG to the SK-OV-3 cell line at 72 hours. These findings demonstrate the necessary and novel approach needed in the development of alternative treatment therapies for ovarian cancer.

INTRODUCTION

Ovarian cancer is a leading cause of mortality among gynecological malignancies worldwide. The excessive mortality rate stems from the broad and generally misinterpreted signs and symptoms associated with the disease (Gong *et al*., 2014). Cell lines derived from tumors are the most frequently utilized models in cancer research and their use has advanced the understanding of cancer biology tremendously over the past decades. The average five-year survival rates of stage III and IV ovarian cancer patients have been demonstrated around 37% and 25%, respectively (Fan *et al.,* 2014). This poor survival rate is attributed to diagnosis at advanced stage and resistance to chemotherapy (Fan *et al.,* 2014). There were an estimated 22,240 new cases with about 14,030 deaths in the United States in 2013 (Fan *et al.,* 2014). Nearly 3% of all gynecological carcinomas include ovarian cancer (Pons *et al*., 2012). Hence, there is a crucial demand to obtain new methodologies and therapies to combat developing drug resistance (Ma *et al*., 2014).

The cause of ovarian cancer is undetermined; however, there are many assumptions including: genetic errors that may occur in the monthly release of an egg and increased hormone levels before and during ovulation which could promote the growth of abnormal cells (Ovarian Cancer, 2015). "The high incidence of ovarian cancer has been attributed to primordial factors that are associated with ovarian senescence, such as oocyte depletion, low levels of steroid production, and increased levels of circulating gonadotropins" (Chuffa *et al*., 2013). The two types of ovarian cancer are epithelial tumors and germ cell carcinoma tumors. These are based on the type of cells from which they are derived (Ovarian Cancer, 2015).

Ovarian cancer is the development of abnormal malignant cells that originates in the ovaries. It has been suggested that malignant cancer cells in the ovaries can metastasize in two ways: directly to other organs in the pelvis and abdomen and through the bloodstream or lymph nodes to other parts of the body (Ovarian Cancer, 2015). Cellular invasion of tumor cells can also occur. This is a cell and tissue-driven process introduced and continued by multiple signaling pathways that control the cytoskeleton dynamic in tumor cells, allowing cells to alter their microenvironment and migrate through tissues (Semprucci *et al*., 2015). There are several modifiable and non-modifiable risk factors for developing ovarian cancer including: genetics, family history, increasing age, reproductive history and infertility, hormone replacement therapy, and obesity. According to prior research, there are several ways to reduce the risk of developing ovarian cancer, including oral contraceptive use, pregnancy, breastfeeding, and removal of ovaries (Ovarian Cancer, 2015).

Recognizing symptoms is crucial for diagnosing ovarian cancer. Any potential change to what is considered to be normal for the patient is significant enough to be followed up with a gynecologist. Some of these symptoms can be bloating, pelvic or abnormal pain, difficulty eating, feeling full quickly, and urgent or frequent urinary

indicators (Ovarian Cancer, 2015).

The present-day treatment for ovarian cancer is carboplatin (Pons *et al.,* 2012). Vast improvements have been made to the survival rate of ovarian cancer with surgical treatment and chemotherapy; however, tumors continue to build resistance to chemotherapeutic treatments (Fu *et al.,* 2015). Primary treatment of ovarian cancer is surgical resection of the disease, followed by the appropriate chemotherapy. However, patients who initially respond to chemotherapeutic drugs soon become resistant and disease reoccurs. The identification of new early biomarkers, more aggressive treatments, and new therapeutic targets for ovarian cancer is required to combat this aggressive cancer (Chen *et al.,* 2015). Some studies have suggested that there needs to be a more profound understanding of the molecular methods associated in the development of ovarian cancer (Cheng *et al*., 2015). These molecular methods could assist in understating this disease's destructive and metastatic capabilities (Tong *et al.,* 2015).

Thymoquinone (TQ) is the main component of the unstable oil of the *Nigella sativa* plant (black seed). TQ has been described as being able to neutralize free oxygen radicals acting as a cell or tissue cleaner by increasing the level of antioxidant enzymes (Sagit *et al*., 2013). Research involving thymoquinone (TQ) verifies its use as an anti-inflammatory, antimicrobial, and antidiabetic agent. It has also been shown to protect many organs from chemotherapy-induced damage (Scheider-Stock *et al.,* 2013). Many studies have established that TQ aids in arresting cell cycle progression and may induce apoptosis in various types of tumor cells (Whoo *et al*., 2011). Molecular methods have expressed the ability of TQ to slow tumor growth and decrease survival (Wilson *et al.,* 2015).

According to the National Cancer Institute, epigallocatechin-3-gallate (EGCG) is only one of more than 1000 different phytochemicals that can act as a cancer defense. EGCG is found almost solely in green tea. EGCG has been found to be the most abundant and the most widely studied catechins and appears to be responsible for most of the beneficial physiological actions associated with green tea consumption (Trudel *et al*., 2012). EGCG has been shown to exhibit an anticancer effect in numerous cancer cell lines, including breast, pancreatic, colorectal, and gastric (Wang *et al*., 2014). Also, EGCG has numerous benefits including its abundance in the environment, decreased expense, and low toxicity (Rao *et al*., 2010). All these factors suggest that EGCG could be developed as a possible anti-cancer therapy (Yan *et al*., 2012).

The cell line Caov-3 is an ovarian adenocarcinoma and was obtained from ATCC. ATCC is the premier global biological materials resource and standards organization whose mission focuses on the acquisition, authentication, production, preservation, development, and distribution of standard reference microorganisms, cell lines, and other materials (ATCC, 2015). It is epithelial-like and was isolated in 1976 and also known as HTB-75. Caov-3 was chosen because it is not in an advanced stage of development as SK-OV-3. In this investigation, we wanted to show a difference in the stages of ovarian cancer. The Caov-3 cell line was harvested from a 54 year old female (Caov-3, 1976). Among the many ovarian cancer cell line studies, Caov-3 have the highest incidence and average metastatic frequency. Caov-3 is an invasive human ovarian papillary carcinoma cell line (Johnson, *et al*., 2010).

The cell line SK-OV-3 is an ovarian adenocarcinoma and was obtained from ATCC. It is derived from the ascites of the mestatic site. It is epithelial-like and was isolated in 1973 and also known as SK-OV-3 is HTB-77. SK-OV-3 cells are resistant to tumor necrosis factor as well as several cytotoxic drugs including diphtheria toxin, cis-platinum, and adriamycin (SK-OV-3, 1973). According to Trudel et al., 2012, EGCG had the greatest inhibitory effect on SK-OV-3, and EGCG induced apoptosis in ovarian cancer cells. Trendowski and colleagues (2015) demonstrated SK-OV-3 cells had a slight, but noticeable resistance to tumor necrosis factor, cisplatin, and doxorubicin.

MATERIAL AND METHODS

A total of 144 wells were plated with 10^5 Caov-3 and SK-OV-3 ovarian cancer cells, respectively. The wells were divided into groups of 72 wells. Each group was subdivided into 4 subgroups of 6 wells. Group 1 served as control and groups 2, 3, and 4 were treated with TQ (16 µM), EGCG (3 µg/ml), and TQ + EGCG, respectively. Each group was terminated at 24, 48, and 72 hours.

BCA Assay: The BCA assay is a biochemical assay for determining the total concentration of protein in a solution following Lowry protein assay. The total protein level measured the metabolic activity of the cells. Serial dilutions for the standard included 100µL of BSA/PBS mixture (2 mg BSA powder plus 1 mL PBS) placed in tube 1 and 2, and then 100µL of PBS was added to tube 2-8. Twenty microliters of standard was placed in each standard line, while 20µL of sample was placed in wells of the 96 well-plate. Then, 200µL of mixed reagent A & B (reagent A; 300µL plus reagent B; 15mL) was added to each standard and sample well. The plate was incubated for 30 minutes. The microtiter plates were read at 492 nm using a Tecan Spectra microtiter plate reader (Garner *et al*., 2014).

Nitric Oxide Assay: Nitrogen dioxide can be assayed spectrophotometrically by measuring the accumulation of its stable degradation products, nitrate, and nitrite. Nitric oxide (NO) plays an important role in neurotransmission, vascular regulation, immune response, and apoptosis. The NO^2 assay was used to measure cell viability. The endpoint colorimetric assay in a 96-well format was used. One-hundred microliters of standard or sample were placed into designated wells, followed by 100µl of mixed Griess reagents, and the reaction was immediately read at 492nm (Kennedy *et al*., 2014).

Glutathione Assay: The glutathione assay measures the glutathione peroxidase used to evaluate cell injury. It is one of the key enzymes in the cellular defense against oxidative stress and the hepatocyte growth factor receptor (Norwood *et al*., 2007). Standard laboratory protocol was used to ascertain the glutathione levels from these cells. Fifty microliters of standard and samples were placed into each well. Afterwards, 100 µL of the reaction mixture was placed into each well. The reaction mixture consisted of 5 mL of DTNB (5,5'-dithio-*bis*-2-nitrobenzoic acid, Ellman's reagent), 5 mL of NADPH (nicotinamide adenine dinucleotide phosphate), 5.75 mL of buffer and glutathione reductase. The plate was placed into the reader and read at 405 nm with a repeat reading after 10 minutes of incubation (Garner *et al*., 2014).

Statistical Methods: The statistical analysis was conducted using SPSS Version 17.0. Analysis of variance (ANOVA) was used to compare the 3 subgroups. ANOVA evaluates the null hypothesis that the means of the group are not statistically different. In its simplest form, ANOVA provides a statistical test of whether or not the means of several groups are all equal, and therefore generalizes student's two-sample *t*-test to more than two groups. Statistically significant effects in ANOVA were often followed up with Tukey's LSD to compare every group mean with every other group mean and typically incorporate a standardized method of controlling for Type I errors. The descriptive statistics of each group were calculated.

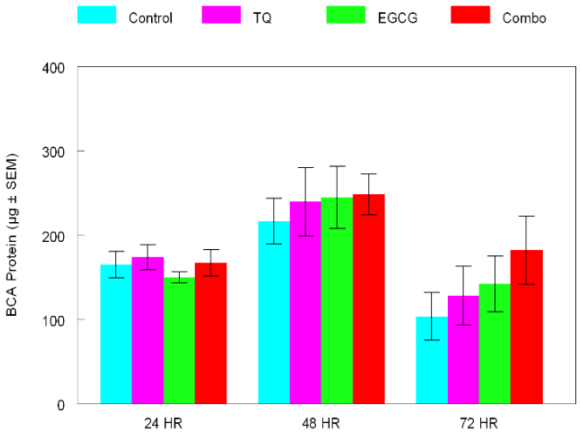

Figure 1: Caov-3 BCA Protein. The effects of conventional delivery of TQ, EGCG, and the combination of TQ and EGCG on Caov-3 ovarian cancer cells at 24, 48, and 72 hours. Using Kruskal-Wallis One Way Analysis of Variance on Ranks $p<0.05$

RESULTS

BCA Assay

Caov-3: Protein levels for Caov-3 cells that were exposed to TQ, EGCG, and the combination of TQ and EGCG at 24, 48, and 72 hours are shown in Figure 1. The differences in the mean values among the treatment groups was not statistically significantly different ($p<0.05$). The data are expressed as average protein concentration (microgram ± SEM). Cellular protein concentrations for the treated groups were not statistically different for the duration of the study. Protein concentrations ranged between 150 to 170 µg in all treatment groups after 24 hours, 210 to 250 µg after 48 hours following treatment, and 100 to 190 µg after 72 following treatment.

SK-OV-3: Protein levels for SK-OV-3 cells that were exposed to TQ, EGCG, and the combination of TQ and EGCG at 24, 48, and 72 hours are shown in Figure 2. Analysis revealed statistically significant difference in the mean values among the treatment groups ($p<0.05$). Following Tukey's LSD, protein levels were unchanged and mean differences were statistically insignificant ($p<0.05$) for TQ and EGCG compared to the control at 24 and 48 hours. In contrast, the combination og TQ and EGCG was statistically significant ($p<0.05$) at 24 and 48 hours. The differences in the mean values among the treatment groups was not statistically different ($p<0.05$) at 72 hours. The data showed combination treatment caused a significant increase in cellular protein concentration at 24 and 48 hours following treatment compared to control and treatment with TQ and EGCG alone. Combination treatment at 72 hours had protein concentrations similar to control, TQ, and EGCG-treated cells.

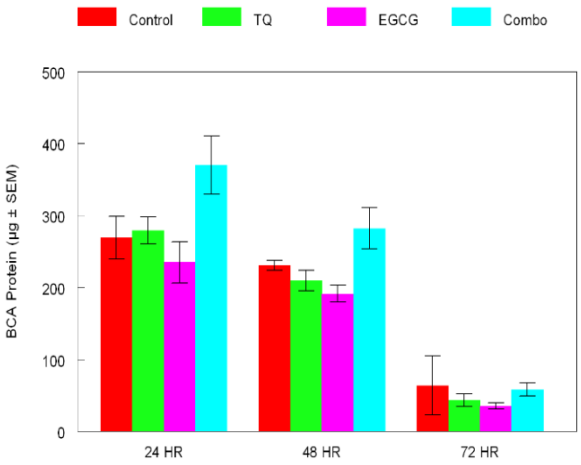

Figure 2: SK-OV-3 BCA Protein. The effects of conventional delivery of TQ, EGCG, and the combination of TQ and EGCG on Caov-3 ovarian cancer cells at 24, 48, and 72 hours. Using Kruskal-Wallis One Way Analysis of Variance on Ranks $p<0.05$

Nitric Oxide Assay

Caov-3: Nitric oxide levels for Caov-3 cells that were exposed to TQ, EGCG, and the combination of TQ and EGCG at 24, 48, and 72 hours are shown in Figure 3. Data revealed the differences in the mean values among the treatment groups are greater than would be expected by chance; therefore, there was a statistically significant difference ($p<0.05$). Following a Tukey pot hoc test, nitric oxide levels were unchanged and statistically insignificant ($p<0.05$) for TQ compared to the control at 24 and 48 hours. In contrast, EGCG and the combination compound were statistically different ($p<0.05$) at 24 and 48 hours. The differences in the mean values among the treatment groups was not statistically significantly different ($p<0.05$) at 72 hours. The data were expressed in micromoles of nitric oxide normalized to cellular protein ± SEM. The results indicate EGCG administration caused a three-fold increase in nitric oxide production at both 24 and 48 hours. Administration of TQ alone did not increase nitric oxide levels; however when given in combination with EGCG, there was a three-fold increase in nitric oxide level at 24 hours and an approximate two-fold increase after 48 hours. Seventy-two hours following a single dose administration of the compounds there was no longer a difference between any groups compared to control untreated cells.

SK-OV-3: Nitric oxide levels for SK-OV-3 cells that were exposed to TQ, EGCG, and the combination of TQ and EGCG at 24, 48, and 72 hours are shown in Figure 4. Data revealed the differences in the mean values among the treatment groups were significantly different ($p<0.05$). Following post-hoc Tukey's, nitric oxide levels were unchanged ($p<0.05$) for TQ compared to the control at 24 and 72 hours. In contrast, EGCG and the combination compound were significantly different ($p<0.05$) at 24 and 72 hours. The differences in the mean values among the treatment groups was not statistically significantly different ($p<0.05$) at 48 hours. The results showed an increase in nitric oxide following the administration of EGCG alone and in combination with TQ compared with TQ alone or untreated control cells. The difference was approximately three-fold higher, and this difference was statistically significant ($p<0.05$). By 48 hours, the nitric oxide levels were still elevated in EGCG and combination groups but the levels were not statistically different ($p<0.05$) from control or TQ treatment alone. By 72 hours, there were no differences in nitric oxide levels between the groups.

Glutathione Assay

Caov-3: Glutathione levels for Caov-3 cells that were exposed to TQ, EGCG, and the combination of TQ and EGCG at 24, 48, and 72 hours are shown in Figure 5. The differences in the mean values among the treatment groups was not statistically different ($p<0.05$). Intracellular oxidative damage was assessed by determining the intracellular glutathione concentration and was expressed as µM glutathione normalized to cellular protein concentration and displayed as average ± SEM. The dosage of TQ, EGCG, and a combination of TQ and EGCG did not induce intracellular oxidative stress over a 72 hour period. The data shows intracellular GSH levels to be maintained between 3 to 10 µM GSH / mg protein for the duration of the study.

SK-OV-3: Glutathione levels for SK-OV-3 cells that were exposed to TQ, EGCG, and the combination of TQ and EGCG at 24, 48, and 72 hours are shown in Figure 6. The differences in the mean values among the treatment groups was not significantly different ($p<0.05$) at 24 and 48 hours. Analysis did reveal the differences in the mean values among the treatment groups were greater than would be expected by chance ($p<0.05$). Following a post hoc Tukey test, glutathione levels were unchanged and statistically insignificant ($p<0.05$) for the combination compound compared to the control at 72 hours. In contrast, TQ and EGCG were statistically different ($p<0.05$) at 72 hours. Intracellular oxidative damage was assessed by determining the intracellular glutathione concentration and the data were expressed as µM glutathione normalized to cellular protein concentration and displayed as average ± SEM. The dosage of TQ, EGCG, and combination of TQ and EGCG did not cause changes in intracellular GS concentration for 24 or 48 hours. By 72 hours, there was a significant two-fold increase in intracellular GSH concentration in cells treated with TQ and three-fold

increase in GSH concentration in cells treated with EGCG. The combination treatment was similar to control values.

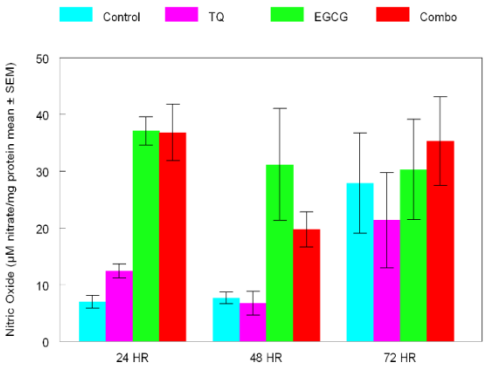

Figure 3: Caov-3 Nitric Oxide. The effects of conventional delivery of TQ, EGCG, and the combination of TQ and EGCG on Caov-3 ovarian cancer cells at 24, 48, and 72 hours. Using Kruskal-Wallis One Way Analysis of Variance on Ranks, there were a statistically significant differences at 24 and 48 hours ($p<0.05$). At 72 hours, there was no statistically significant differences ($p<0.05$).

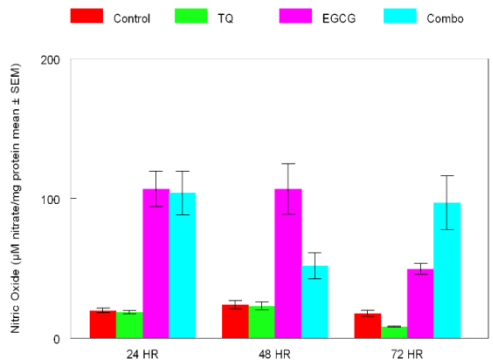

Figure 4: SK-OV-3 Nitric Oxide. The effects of conventional delivery of TQ, EGCG, and the combination of TQ and EGCG on Caov-3 ovarian cancer cells at 24, 48, and 72 hours. Using Kruskal-Wallis One Way Analysis of Variance on Ranks, there was a statistically significant differences at 24 and 48 hours ($p<0.05$).

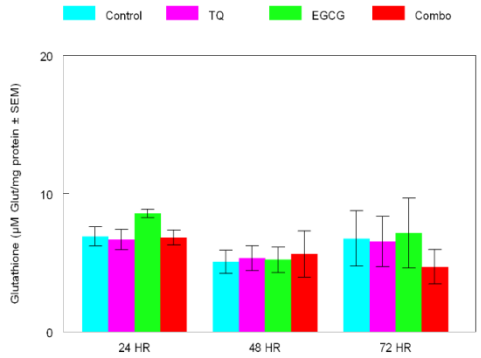

Figure 5: Caov-3 Glutathione. The effects of conventional delivery of TQ, EGCG, and the combination of TQ and EGCG on Caov-3 ovarian cancer cells at 24, 48, and 72 hours. Using Kruskal-Wallis One Way Analysis of Variance on Ranks, there were a statistically significant differences at 24 and 48 hours ($p<0.05$). At 72 hours, there was no statistically significant differences ($p<0.05$).

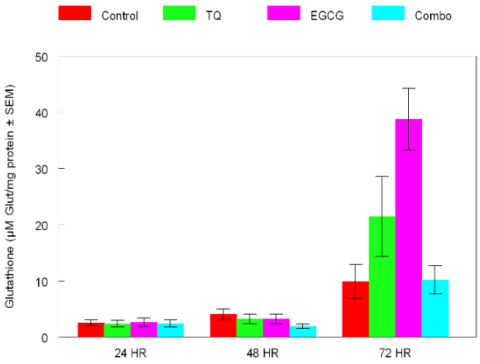

Figure 6: SK-OV-3 Glutathione. The effects of conventional delivery of TQ, EGCG, and the combination of TQ and EGCG on Caov-3 ovarian cancer cells at 24, 48, and 72 hours. Using Kruskal-Wallis One Way Analysis of Variance on Ranks, there were a statistically significant differences at 24 and 48 hours ($p<0.05$). At 72 hours, there was no statistically significant differences ($p<0.05$).

DISCUSSION

Investigation was needed to verify the measurement of conventional delivery of TQ and EGCG when applied to the ovarian cancer cell lines Caov-3 and SK-OV-3. The aim of this investigation was to compare the antioxidant effects in two ovarian carcinoma cell lines measuring potential biomarkers. There have been many other studies documenting the success of TQ and EGCG against various types of cancers. The administration of TQ 10 microM was the most effective in preventing the attachment of Streptococcus pyogens to pre-treated and post-treated epithelial cells in vitro (Hull Vance *et al*., 2010). TQ in combination with selenium was shown to be an effective treatment option against human osteosarcoma cells (Barron *et al*, 2008). EGCG has been shown to induce changes in cellular morphology of pancreatic tumor (PANC-1) cell line, which is a characteristic of apoptosis (Hodges *et al*., 2015). TQ and EGCG in combination has been shown to be a more potent agent than TQ and EGCG alone in affecting change in Caov-3 cancer cells (Harpole *et al*.,2014). Tardy and colleagues (2009) revealed that TQ and EGCG may protect the functional and structural capacity of fibroblast cells in culture.

Caov-3 ovarian cancer cells were exposed to a single dose of either 16 µM TQ, 3µg/ml EGCG, or 16 µM TQ + 3 µg/ml EGCG. The doses of TQ and EGCG were ineffective in reducing cellular protein concentrations after 24, 48 and 72 hours. Nitric oxide levels were increased when EGCG was administered to the cells at 24 and 72 hours. Vasodilation increases blood flow to the cells which may either result in aiding the cancer cells to adapt and survive by increasing blood flow or by acting as a free-radical inducing cellular damage. The nitric oxide levels were also increased when EGCG and the combination treatment TQ+EGCG was administered at 24, 48, and 72 hours. This suggests that EGCG was responsible for the increase in nitric oxide.

SK-OV-3 ovarian cancer cells were exposed to 16 µM TQ, 3 µg/ml EGCG, and 16 µM TQ + 3 µg/ml EGCG, respectively. TQ caused a slight increase in the protein level at 24 hours which suggests that it is ineffective in reducing cellular protein. At 48 and 72 hours, there was a slight decrease in the protein level which suggests a longer incubation time could cause TQ to be more effective in reducing cellular protein concentration and altering cellular viability. Similarly after a single dose of EGCG, the protein levels trended lower than the control protein values at 24, 48, and 72 hours. This suggested that EGCG may also reduce cellular protein concentration over a longer duration.

Similar findings were seen with SK-OV-3 ovarian cancer cells when exposed to 16 µM TQ, 3 µg/ml EGCG, and 16 µM TQ + 3 µg/ml EGCG, respectively. Nitric oxide levels were the same as the control levels when TQ was administered to the cells at 24, 48, and 72 hours. This shows that the antioxidant was neither effective nor ineffective in increasing nitric oxide. The nitric oxide levels were increased when EGCG was administered at 24, 48, and 72 hours. This is in contrast to the reports by others in the literature for nitric oxide production by cancer cell, but similar to those findings for cell types related to endothelial cells.

TQ is known to induce glutathione and is thought to protect cells by inducing the activity of glutathione transferase which aids in cellular detoxification. In this study, TQ did not increase total cellular glutathione content when used alone or in combination with EGCG. Glutathione is a sensitive marker of cell stress. Increases in glutathione content reflect the cells ability to detoxify while decreases in glutathione indicate cell death.

CONCLUSIONS

The outcome of this study has focused on new results which could be considered innovative in the investigation of new therapies used to treat cancer. The data of this study suggest that EGCG alone and the combination therapy with antioxidants is an effective means of generating physiological responses, including cell growth and metabolism suppression, decreasing protein levels, and cell cycle disruption. Future studies are needed to investigate the possibilities of antioxidant usage involving ovarian cancer.

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HUMAN ADENOCARCINOMA ALVEOLAR BASAL EPITHELIAL CELL LINE RESPONSE UPON EXPOSURE OF INTERLEUKINS IN CULTURE

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ABSTRACT

The A549 human adenocarcinoma cell line has type II alveolar epithelial functionality and has shown good suitability as a model for respiratory studies. It is theorized that the alveolar cells, in response to inflammatory cytokines, produce chemokines that control the recruitment and activation of leukocytes to the site of inflammation in obstructive lung diseases such as asthma.**Objective:** The objective of this study was to evaluate a response of A549 cells after exposure to cytokines, such as interleukin 1β (IL-1β) and interleukin 4 (IL-4) in preparation of future exposure to menhaden fish oil, a source rich in omega-3 fatty acids and hydrocortisone. **Methods:** To establish an environment of an inflammatory response, confluent A549 cells were stimulated separately with IL1-β and IL-4 in concentration variation at ten-fold serial dilution. Standard laboratory protocols and sterile techniques were followed throughout the experimentation. The cells were also treated independently and allowed to incubate for 24 and 48 hours. Cellular metabolic activity was measured by alamarBlue®, which uses the natural reducing power of living cells to convert resazurin to resorufin. **Results:** Cellular response was shown after exposure, indicated by increased levels in resazurin reduction when compared to a negative control. ANOVA indicated significant differences among concentration groups of IL-1β with p-value of 8.61E-08 at 24 hours and 2.86E-05 at 48 hours. A post hoc Tukey's test showed 10 ng/mL and 100 ng/mL differed significantly at $p<0.05$ when compared to lower concentration. IL-4 stimulation indicated a significant difference among concentration groups with a p-value of 1.5E-08. A post hoc Tukey's test indicated only 100 ng/mL was significantly different than all lower concentrations, including the negative control at 24 and 48 hours. **Conclusions:** The results from this study established that A549 cells have increased cell viability when stimulated by IL-1β and IL-4. In addition, parameters of treatment concentration were established to prepare an inflammatory model for future investigation. Forthcoming studies will explore the response of eotaxin, a family of chemokines with chemotaxis properties, after the treatment with omega-3 fatty acid and hydrocortisone.

INTRODUCTION

Asthma is a chronic inflammatory disease that affects nearly 300 million people worldwide [1]. The prevalence is global with over 25 million people in the United States experiencing some level of asthma ranging in various levels of severity [2].

The disease is marked with recurrent inflammatory episodes of respiratory distress. These events are a result of variable airflow limitation that is partly or completely reversible either spontaneously or with intervention. This bronchoconstriction of the airways is a consequence of mediators released from blood cells and part of the inflammatory cascade [3]. In addition, the changes in the epithelium airway can undergo specific changes that have more long term tissue damage. This remodeling is a pathological condition that can lead to irreversible damage [4].

According to the literature, the exact mechanism of asthmatic episodes is still unclear. Recent research identified a mediator called eotaxin and revealed its essential role in obstructive lung disease. Eotaxin is a member of a very large superfamily of homologous small peptides called chemokines. It is a chemoattractant that has been found to be elevated in volume during an asthmatic bronchospasm and may have a significant role in triggering the activation and degranulation of eosinophils [5, 6].

The specific aim of this preliminary work is to explore the A549 cell line as a model to mimic the epithelial lining of the airways during an inflammatory episode. A549 cell line was created with adenocarcinomic human alveolar basal cells, including type II alveolar cells [7, 8]. It has been used successfully utilized in other research focused on the respiratory epithelium. To complete the tissue model, a component of the inflammatory activation needs to be present to initiate an environment that is of an inflammatory state. The chosen pro-inflammatory components for this research model were interleukin 1-beta (IL-1β) and interleukin 4 (IL-4). In meeting this objective, parameters can be established to complete an *in vitro* model of the respiratory epithelium that would lead to assessing the effects of eotaxin levels when exposed to potential anti-inflammatory agents, such as omega-3 fatty acids in menhaden fish oil and hydrocortisone

MATERIALS AND METHODS

Cell Cultures: A549 cells were acquired from the American Type Culture Collection (ATCC, Rockfield, MD) and grown in Ham's F12K media supplemented with 10% fetal bovine serum (Sigma-Aldrich) and 1%

antibiotic/antimycotic solution (Corning). Cells were transferred to a 75 cm^2 flask (Corning) and grown to confluency in a humidified 5% CO_2 incubator at 37°C. At confluency, cells were trypsinized (Hyclone EDTA-Trypsin), removed from the flask and counted by the use of a Neubauer hemocytometer. Each segment of this study had cells added to wells of a 96- well plate according to a specific design for each component to be tested.

Plate designs: Plate design 1 was set up to test growth of A549 in cell culture. A two-fold serial dilution was made from 100 µL of cell stock that had a count of 2.28E06 cells/mL. The separate dilutions were placed in the first column of the 96-well plate and replicated six times. The last row of the plate was reserved for 100µL of media only, containing no cells.

Plate design 2 was arranged to test both interleukins in separate experiments. A ten-fold serial dilution was made from a stock solution of 100 ng/mL (1:1). The serial dilution was carried out to 0.001 ng/mL concentration (1:100,000). Each dilution (100µL) was placed in the first column of the 96-well plate and replicated six times. The last row of the plate was reserved for 100µL of media only, containing no cells.

Cellular viability: AlamarBlue® cell viability reagent functions as a cell health indicator by using the natural reducing power of living cells to convert resazurin to the fluorescent molecule, resorufin, as a cell viability indicator. The converted molecule's fluorescence is directly proportional to the number of living cells and corresponds to the cell's metabolic activity [9].

After cells had incubated for 24 hours, the media was removed and replaced with 100 µL of fresh media. The cells were incubated for an additional 30 minutes to allow for acclimation. Ten percent of alamarBlue solution (10 µL) was added to each well. The plate was placed in the incubator and removed at one hour. A 50 µL aliquot of media + alamarBlue was transferred to a clean plate for reading in a spectrophotometer. All wells were read at 570 nm first, and second at a reference point of 620 nm. The percent reduction of resazurin to resorufin was calculated in comparison to a negative control which consists of media + alamarBlue with no cells.

Statistical Analysis: Statistical analysis was performed using SPSS Statistical software. Analysis of variance (ANOVA) was used to determine differences among experimental groups compare to each other and the negative control group. Pairwise comparisons were achieved with a post hoc analysis of Tukey's test and $\alpha = 0.5$ on all data. Visualization of data was done by Microsoft Excel.

RESULTS AND DISCUSSION

The resulting absorbance of alamarBlue reagent was read on a spectrophotometer and results were analyzed by plotting absorbance versus compound concentration. For plate design 1, cellular viability was assessed by plotting percent reduction of alamarBlue® to initial cell number in log of 10. All six replicates were analyzed with only the averages of replicates plotted for 24, 48, and 72 hours (Figure 1).

Plate design 2 for IL-1β at 24 hours yielded a significant variation among groups, with a p-value of 8.61E-08 at 24 hours and 2.86E-05 at 48 hours. A post hoc Tukey test showed the 100 ng/mL and 10ng/mL concentration differed significantly at $p<0.05$ when comparing the lowest five concentration groups, including the negative control at 24 hours, but only differed significantly with $p<0.05$ when comparing the lowest three concentration groups (0.0, 0.001, 0.01 ng/mL) including the negative control, at 48 hours. The 100 ng/mL and 10ng/mL concentration did not differ from one another significantly. Visualization of the average results is represented by plotting percent reduction of AlamarBlue in contrast to IL-1β concentration (Figure 2).

Plate design 2 for IL-4 at 24 hours yielded a significant variation among groups, with a p-value of 1.5E-08 at 24 hours and 7.76E-05 at 48 hours. A post hoc Tukey's test showed the 100 ng/mL at $p <0.05$ when comparing the lowest five concentration groups, including the negative control, at 24 and 48 hours. The concentration of 10ng/mL concentration only differed significantly with the negative control at 24 hours. The remaining groups did not show significant reduction of AlamarBlue values at both time increments (Figure 3).

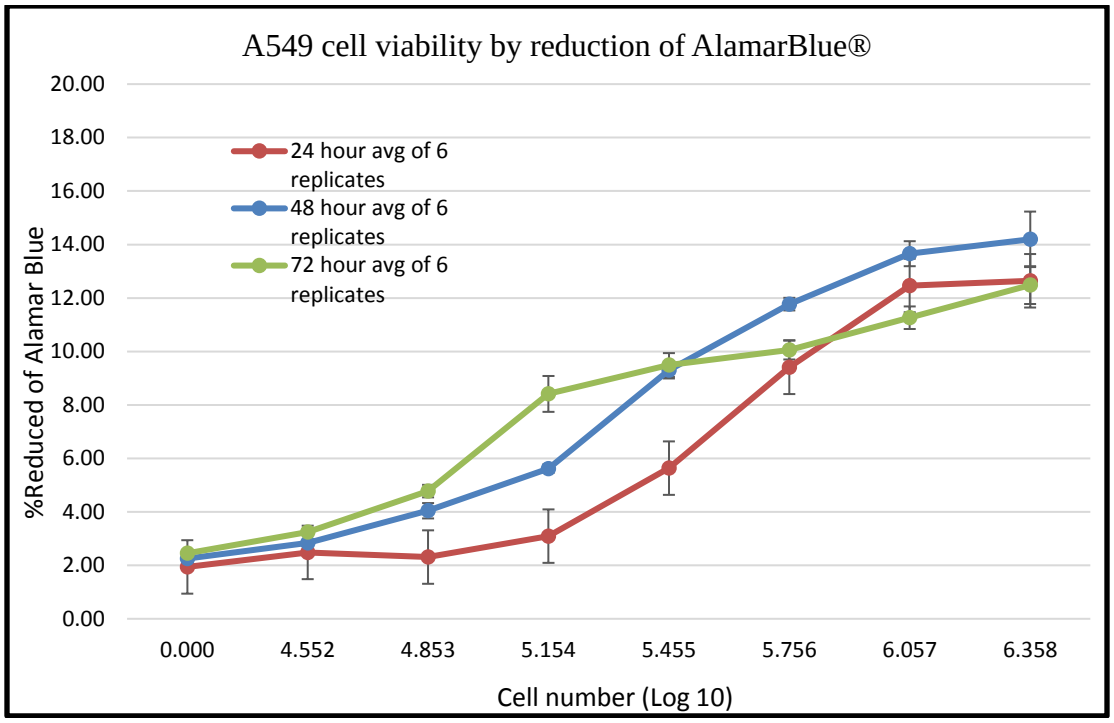

Figure 1. A549 cell viability by reduction of AlamarBlue at 24, 48, and 72 hours of incubation. Values are expressed as the mean ± SE of all replicates. The control group is Ham's F12K media only.

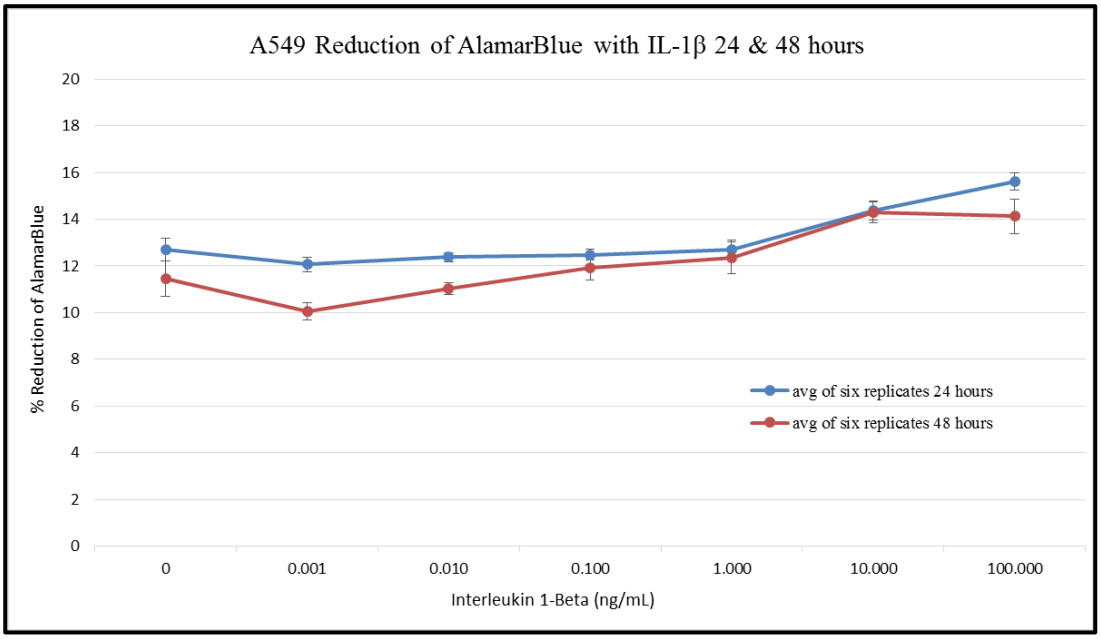

Figure 3. A549 cell viability by reduction of AlamarBlue with addition of IL-1 at 24 and 48 hours of incubation. Values are expressed as the mean ± SE of all replicates. The control group is Ham's F12K media and cells only with no IL-4 addition.

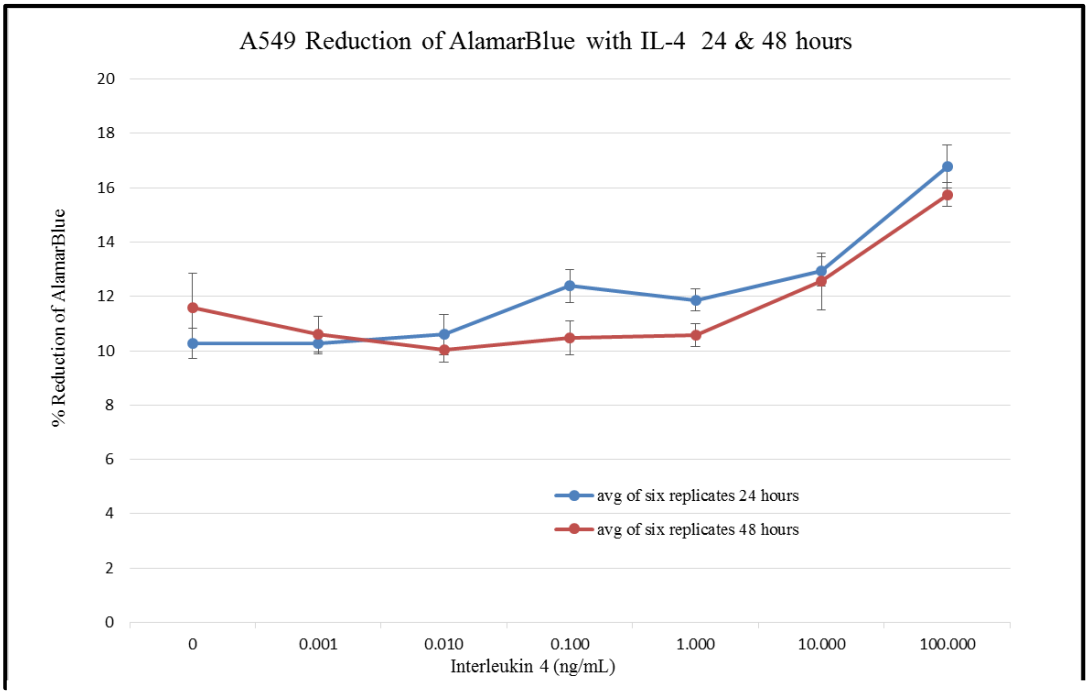

Figure 3. A549 cell viability by reduction of AlamarBlue with addition of IL-4 at 24 and 48 hours of incubation. Values are expressed as the mean ± SE of all replicates. The control group is Ham's F12K media and cells only with no IL-4 addition.

Results of this study indicated that A549 epithelial like cells do respond to the addition of either IL-1B or IL-4. According to the replicates tested, the response is shown by an increase in cell viability when compared to a negative control and measured by conversion of resazurin to resorufin. When cells are alive they maintain a reducing environment within the cytosol of the cell. Resazurin, the active ingredient of alamarBlue reagent, is a cell permeable compound that is blue in color and non-fluorescent. Viable cells continuously convert resazurin to resorufin, increasing the overall fluorescence and color of the media surrounding the cells which can be measured efficiently.

From the results of plate design 1, it is evident that 24 and 48 hours of growth of A549 is still an environment that is conducive to a good working model. The cells are proliferating and have not reached a suppressive state of complete confluency. However, at 72 hours, the smaller well of a 96-well plate becomes too confluent and the integrity of the model is compromised. The desire is to test all future experiments in a target zone of log 5.154 to log 5.455 (1.425E05 to 2.85E05 cells/mL) for seeding in reference to cell number. With this initial cell count, the growth will occur in a linear growth range.

Due to wide variation in the literature concerning the concentration levels of IL-1β and IL-4 in cell culture models, a variation of concentrations was used to help establish adequate parameters for all future experimentation. Statistical analysis indicates that a significant difference does exist in higher concentrations of the interleukins when compared to a negative control.

CONCLUSIONS

The results from this study support the theory that A549 cell line will respond to IL-1β and IL-4 in cell culture. Because this cell line has the morphologic and biochemical feature of alveolar type II cells it will be a suitable model to assess the level of inflammatory mediators such as eotaxin. Future studies will be maintained with concentration levels developed from this preliminary investigation in order to assess if anti-inflammatory agents, such as omega-3 fatty acid and hydrocortisone, have any effect on the eotaxin levels in epithelial cell culture.

ACKNOWLEDGEMENTS

This project was supported by the University of Mississippi Medical Center School of Health Related Professions Development Fund/Private Practice Plan, Jackson, MS.

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Mississippi Academy of Sciences 80th Annual Meeting Division Reports, 2016

Division of Agriculture and Plant Sciences

The meeting was chaired by Dr. Girish K.S. Panicker, Associate Professor and Director of Conservation Research Center, Alcorn State University, and Vice-chaired by Dr. Victor Njiti, Associate Professor and interim chair of the department of Agriculture of Alcorn State University. Papers in agriculture and Plant Sciences were presented by Alcorn State University, Mississippi State University, University of Mississippi, Jackson State University, Alabama A&M, West Virginia State University, and USDA/ARS.

This division had a record number of 35 papers for presentation, including orals and posters. Student competition sessions were well-attended and remarkable. Out of the 35 papers, 25 of them were presented by undergraduate and graduate students at their oral and poster competition sessions. The winners will be given certificates and cash awards. The event was a great success.

Dr. Anand Nanjundaswamy, assistant professor and Dr. Victor Njiti, associate professor of Alcorn, Dr. Te-Ming Pau Tseng, assistant professor and Dr.Raja Reddy, professor of Mississippi State University were the judges.

During the business meeting, Dr. Victor Njiti of Alcorn and Dr. Raja Reddy, professor of Agronomy, Mississippi State University were elected chair and vice chair for the year 2016-17, respectively.

Winners of Poster Competition:

Undergraduate session:

1. Jazmarkey Wiggins, Alcorn State University. First place
2. Jessica Gilbert, Jackson State University. Second place
3. Krystin Cook, Jackson State Universoty. Third place

Winners of Poster competition:

Graduate session:

1. Ahmed Chaloob Saddam, Mississippi State University. First place
2. Firas Alsajri, Mississippi State University. Second place
3. Saroj Sah, Mississippi State University, Third place

Winners of Oral Competition:

Undergraduate session:

1. Margeria Smith, Alcorn State University. First place
2. Willie Mims, Alcorn State University. Second place

Graduate session:

1. Anushobha Regmi, University of Southern Mississippi. First place
2. Ashley Williams, Alcorn State Universiy. Second place
3. David Henderson, Alcorn State University. Third place
4. Melinda Miller-Butler, University of Southern Mississippi. Third place

(David Henderson and Melinda Miller-Butler were in tie for third place and hence, the third place was shared)

Cellular, Molecular, and Developmental Biology Division

- Graduate Oral Presentations
  - First Place: Rebekah Bullard, University of Southern Mississippi
  - Second Place: Gary Crispell, University of Southern Mississippi
  - Third Place: Justin Batte, University of Southern Mississippi
  - Honorable Mention: Mariah Lloyd, Alcorn State University
- Undergraduate Oral Presentations
  - First Place: Kellie Mitchell, Mississippi State University
  - Second Place: Pieter Both, Mississippi State University
- Graduate Poster Presentations
  - First Place: Jian Jiang, Mississippi State University
  - Second Place: Shanti Pandey, University of Southern Mississippi
  - Third Place: Jason Cooper, Mississippi College
- Undergraduate Poster Presentations
  - First Place (tie): LaToyia Downs, University of Southern Mississippi
  - First Place (tie): Cheri Voth, University of Southern Mississippi
  - Second Place: Britton Strickland, University of Southern Mississippi
  - Third Place: Brittany Trunell, University of Southern Mississippi

Chemistry Division

Graduate Oral:

1st place Tamanna Shanta, University of Southern Mississippi

2nd place Freserich McFarland, University of Southern Mississippi

3rd place Suhash Chavva, Jackson State University

Graduate Poster:

1st place Kai Guo, Jackson State University

2nd place Syed Haque, Jackson State University

3rd place Hua Deng, Jackson State University

Undergraduate Poster:

1st place Amanda Kaminski, Millsaps College

2nd place Charles McCormich, University of Southern Mississippi USM

3rd place Stephanie Njemanze Jackson State University

Undergraduate Oral:

1st place Ardith Bravenec, Millsaps College

Health Sciences Division Report

The Health Sciences Division of the Mississippi Academy of Sciences is proud to report another outstanding year at the annual meeting. We presented a two full days of educational and enlightening programs which offered State of the Art Presentations in Population Health, Clinical/Science Technology and Basic/Clinical Science Research. The meeting was highlighted with two Symposia, one on **"Organ Transplantation and Organ Allocation and Recovery"** in the morning, February 18th and the other on Population Health entitled **"Where Science Meets Population Health"**, in the afternoon. The sessions roared through a wide range of Science, Technology, Genomics, Population Health Disparities/Elimination and HIV Prevention. The distinguished panel of speakers included Dr. Christopher Anderson, James D. Hardy Professor, Chair of Department of Surgery, Chief of the Division of Transplant and Hepatobiliary Surgery, at UMMC; Kevin Stump, Chief Executive Officer at Mississippi Organ Recovery Agency (MORA); Dr. James Wilson, Professor of Internal Medicine, Rheumatology and Physiology at UMMC; Dr. Marino A. Bruce, Professor of Sociology and Criminal Justice at Jackson State University (JSU) and an affiliate faculty in Internal Medicine at the UMMC; and Dr. Thomas E. Dobbs, State Epidemiologist, Mississippi State Department of Health. Please read a detail presentation about the symposia speakers in *the abstract edition of the journal (Vol 61, No 1, 2016).*

A total of 98 abstracts were submitted to the Health Sciences Division this year. After evaluation by the chairs and vice-chairs, 92 were accepted for oral/poster presentations in basic/clinical sciences, population health and technology sessions. Abstracts were submitted by the Faculty and students in multi-categories from all Mississippi Universities and Colleges, in addition from institutions and organizations from Mississippi and other states. Parallel Oral presentation sessions were held both on Thursday and Friday. Eighteen oral presentations were given by Faculty, graduate students and undergraduate students. The remainder were posters presented in two poster sessions.

Another highlight of the program was a "hands-on population fun workshop", in which the participants assessed their cardio-pulmonary output, including blood pressure, before and after jump-rope, BMI and maximal lung volume. Dr. George Moll, Professor of Pediatrics, Dr. Eric George, Assistant Professor of Physiology and Biophysics, Olga McDaniel and Jana Bagwell, Co-Chairs of the division facilitated the workshop. Cheshil Dixit (M4) and Teresa Moll (M4) at UMMC, Benita Williams, undergraduate student at UMC and Montianah Roseburgh, undergraduate at JSU assisted the event.

Drs. Jennifer Harpole and Raymond Grill were identified as the possible nominees for Vice –Chairs in 2016-2017. An email survey will be sent out to confirm the nomination.

Many thanks to the invited speakers of the Symposia, to all of our presenters and to our judges who offered their time and expertise. We would also like to offer a special thank you to Dr. George Moll, President of Sigma Xi at UMMC and Dr. William Dottie Jackson, Surgeon at Premier Plastic in Jackson Mississippi for their support of the Academy and for promoting student research by generously donating funds for student awards. This year's student award winners were:

Basic/Clinical/Population Health Science Graduate Students

1st Place Nasir Butt
Targeted combination strategy for prostate cancer using prostate specific PTEN knockout mice.

2nd Place Kaylon Touchstone
Starvation induces polycystic ovarian syndrome (PCOS)-like disease in Drosophila Melanogaster.

3rd Place Xiao Zhang
Novel signatures of microbiome in chronic alcohol consumption monkeys.

Basic/Clinical/Population Health Science Undergraduate Students

1st Place Haley Thomas
Plant rhizoshere bacteria with antagonistic activity against drug resistant pathogens.

2nd Place Lizhuo Ai
Ribosomal frameshifting of Trichomonas vaginalis virus 2, 3 and 4 isolates.

3rd Place **Kiana Moore**
Evaluation of sex education among Mississippi Students

Base Pair

1st Place **Aylin Memili**
Illuminating molecular signatures of antibiotic resistance determinants in bacteria using digital biology.

2nd Place **Ashley Szabo-Johnson**
Effects of ETA blockade on renal injury associated with obesity.

3rd Place **Viviek Patel**
Minocycline reduces systemic lipopolysaccharide-induced white matter injury and neurobehavioral deficits.

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PLEASE READ ALL INSTRUCTIONS BEFORE YOU SUBMIT YOUR ABSTRACT ON-LINE

- Your paper may be presented orally or as a poster. Oral presentations are generally 15 minutes. The speaker should limit the presentation to 10-12 minutes to allow time for discussion; longer presentations should be limited accordingly. Instructions for poster presentations are linked here.
- Enclose a personal check, money order, institutional check, or purchase order for $25 publication charge for each abstract to be published, payable to the Mississippi Academy of Sciences. The publication charge will be refunded if the abstract is not accepted.
- The presenting author must be a member of the Academy at the time the paper/poster is presented. Payment for membership of one author must be sent for the abstract to be accepted.
- Attendance and participation at all sessions requires payment of registration.
- Note that three separate fees are associated with submitting and presenting a paper at the annual meeting of the Mississippi Academy of Sciences.
    1. An abstract fee is assessed to defray the cost of publishing abstracts and
    2. A membership fee is assessed to defray the costs of running the Academy.
    3. Membership/Preregistration payment ($130 regular; $50 student) may accompany the abstract, or you may elect to pay this fee before January 17, or pay full late membership/registration fees at the meeting ($170 regular, $60 student).
- Abstracts may **only** be submitted on line via a link through the MAS website.The appropriate abstract fees can be paid via Paypal or sent via mail to Barbara Holmes at the Academy address.
- **Late abstracts will be accepted with a $10 late fee during November and increased to $25 after that. Late abstracts will be accepted only if there is room in the appropriate division. They will be published in the April issue of the MAS JOURNAL.**
- Submit your appropriate fees NO LATER THAN January 17, 2017.

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Post Office Box 55907
Jackson, MS 39296-5907

GUIDELINES FOR POSTER PRESENTATIONS

- The Academy provides poster backboards. Each backboard is 34" high by 5' wide. Mount the poster on the board assigned to you by your Division Chairperson. Please do not draw, write, or use adhesive material on the boards. You must provide your own thumb tacks.
- Lettering for your poster title should be at least 1" high and follow the format for your abstract. Lettering for your poster text should be at least 3/8" high.
- Posters should be on display during the entire day during which their divisional poster session is scheduled. They must be removed at the end of that day.
- Authors must be present with their poster to discuss their work at the time indicated in the program.

Author Guidelines

Editorial Policy. The Editorial Board publishes articles on all aspects of science that are of general interest to the scientific community. General articles include short reviews of general interest, reports of recent advances in a particular area of science, current events of interest to researchers and science educators, etc. Research papers of sufficiently broad scope to be of interest to most Academy members are also considered. Articles of particular interest in Mississippi are especially encouraged.

Research papers are reports of original research. Submission of a manuscript implies that the paper has not been published and is currently at the time of submission being considered for publication elsewhere. At least one of the authors must be a member of the Academy, and all authors are encouraged to join.

Manuscripts. Submit the manuscript electronically to the Mississippi Academy of Sciences under your profile in the member location of the website. Please also provide a cover letter to the Editor of the Journal. The cover letter should authorize publication: give the full names, contact information, for all authors; and indicate to whom the proofs and correspondence should be sent. Please notify the Editor on any changes prior to publication.

Manuscripts must adhere to the following format:

- One inch margins on 8.5 x 11 inch paper;
- Text should be left-justified using twelve point type;
- Double spaced throughout, including the title and abstract;
- Arabic numerals should be used in preference to words when the number designates anything that can be counted or measured (7 samples, 43 species) with 2 exceptions:
- To begin a sentence (Twenty-one species were found in…)
- When 2 numeric expressions are adjacent in a sentence. The number easiest to express in words should be spelled out and the other left in numeric form (The sections were divided into eight 4-acre plots.).
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- Avoid personal pronouns.

Format

Abstract. In 250 or fewer words summarize any new methods or procedures critical to the results of the study and state the results and conclusions.

Introduction. Describe the knowledge and literature that gave rise to the question examined by, or the hypothesis posed for the research.

Materials and methods. This section should describe the research design, the methods and materials used in the research (subjects, their selection, equipment, laboratory or field procedures), and how the findings were analyzed.

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Black DA, Lindley S, Tucci M, Lawyer T, Benghuzzi H. A new model for the repair of the Achilles tendon in the rat. J Invest Surg. 2011; 24(5): 217-221.

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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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