University of Mississippi Medical Center
School of Dentistry

Research Day 2017

February 21, 2017

David A. Felton, DDS, MS
Dean

Jason A. Griggs, PhD, FADM
Associate Dean for Research

School of Dentistry | Office of Research
University of Mississippi Medical Center
2500 North State Street, Room D528-6A
Jackson, MS 39216-4505
Dear Colleagues,

Thank you for joining us on this important day in the future of the School of Dentistry and for your participation in our research day activities. It is indeed a pleasure to welcome you to the 2017 UMMC School of Dentistry Research Day, an annual tradition at the School since 1994! Today our faculty, students, and post-graduate students come together to present their research findings to the members of the School of Dentistry and our Medical Center colleagues. Research is of critical importance to advance the science of dentistry and improve patient care and outcomes. An old adage suggests that “if you’re not doing research, you’re merely teaching the ‘history’ of your specialty.” Consider the advances that we’ve seen in dentistry over the past few decades—new dental materials, dental implant therapies, the development of systematic reviews, digital and CAD/CAM dentistry, advances in adhesive dentistry, advances in pulpal and periodontal research, dental caries assessment and management, and the movement toward minimally invasive dentistry—the list is impressive!

Having our students participate in research is an excellent way to advance the science of dentistry beyond the classroom and clinical environment. In addition, according to the Commission on Dental Accreditation (CODA, Standard 6-3), “Dental education programs must provide opportunities, encourage, and support student participation in research and other scholarly activities mentored by faculty.” Our faculty continue to serve as excellent mentors and role models for our students in the research arena. As you will witness, the quality of the research presented today strongly supports our goal to not only achieve the CODA accreditation standard, but to surpass it.

I am confident that you will enjoy the presentations and that you will witness, first hand, the ever improving quality of the research we’re doing here at the UMMC School of Dentistry. Enjoy the presentations, and celebrate our students’ accomplishments with us!

David A. Felton, DDS, MS
Dean, School of Dentistry
Professor, Department of Care Planning and Restorative Sciences

This has been an exciting year for research in the School of Dentistry. We began collaborations with the new School of Population Health. The president of our local Student Research Group (Kendra Clark) was elected president of the national SRG. The director of our UPSTART program (Dr. Janorkar) was awarded his first NIH R01 grant. Our very own Dr. Roach was awarded UMMC’s bronze medallion for research excellence. We added research study design as a team project in our evidence-based dentistry course. We joined a constellation on nanomaterials in collaboration with the Oxford campus. Finally, I am delighted to report that our aggregate H-index saw a 5% increase!

It is a pleasure to have Dr. Jacques Nör with us as our keynote speaker this year. He is conducting fascinating research on cancer and dental stem cells for which his research group has been continuously funded for 15 years by the NIH. That speaks volumes considering the recent federal budget situation. More importantly, I hear from former students and current colleagues that Dr. Nör has the heart of a teacher, so please welcome him and feel free to ask for his advice.

As usual, the abstracts that we received this year are excellent, and I look forward to hearing our students and faculty present their results and discuss the scientific impact with all of you. Thank you for joining us.

Jason A. Griggs, PhD, FADM
Associate Dean for Research, School of Dentistry
Professor and Chair, Department of Biomedical Materials Science
Program

Lower Amphitheater R153
8:00 – 9:00 am    Keynote Lecture: Dr. Jacques Nör, DDS, MS, PhD
                 “Therapeutic Targeting of Head and Neck Cancer Stem Cells”

Nelson Student Union Conference Rooms A and B
9:15 – 10:00 am    Break
                    Poster preparation
10:00 – 11:30 am   Poster presentations
                    Judging of student posters
                    Biomedical Materials Science lab demonstrations

Nelson Student Union Conference Rooms C and D
11:30 am    Lunch will be served
12:15 pm    Certificates and awards presentation
12:45 pm    Poster removal

Acknowledgements

Faculty Research Mentors
Ronald Caloss, DDS, MD
Associate Professor, Oral-Maxillofacial Surgery & Pathology
Ravi Chandran, DMD, PhD
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Poster Judges
Ahmad Abdelkarim, DDS, MS, PhD, DMD, EdD
Assistant Professor & Chair, Orthodontics
Jennifer Bain, DMD, MSPH, PhD
Assistant Professor & Chair, Periodontics and Preventive Sciences
Kenneth St. John, PhD
Professor Emeritus, Biomedical Materials Science

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Dr. Ron Caloss
Dr. Robin Howard
Dr. Amol Janorkar
Keynote Lecture

“Therapeutic Targeting of Head and Neck Cancer Stem Cells”
Jacques Nör, DDS, MS, PhD
Professor & Chair
Department of Cariology, Restorative Sciences & Endodontics
University of Michigan School of Dentistry, Ann Arbor, MI

Dr. Jacques Nör is the Donald A. Kerr Endowed Professor of Dentistry and Chair of the Department of Cariology, Restorative Sciences and Endodontics at the University of Michigan School of Dentistry. He is also Professor of Otolaryngology in the Medical School and Professor of Biomedical Engineering in the College of Engineering at the University of Michigan. He served as the Co-Director of the University of Michigan Head and Neck SPORE grant funded by the National Cancer Institute (NCI) from 2011-2016, has served as a member of National Institutes of Health (NIH) study sections, and has been continuously funded by the NIH for the last 15 years. Dr. Nör is an elected Fellow of the American Association for the Advancement of Sciences (AAAS) and received the Hatton and the Gies Awards from the International Association for Dental Research (IADR). Dr. Nör received the Distinguished Scientist Award from the IADR in 2012. He recently served as the President of the Michigan Section of the American Association for Dental Research (AADR). Dr. Nör is also the Associate Editor of the Journal of Dental Research and a member of the editorial board of several journals, including the Journal of Endodontics, Operative Dentistry, and Stem Cells in Oral Medicine. His long-term research interests are in tumor angiogenesis and in stem cell biology. Dr. Nör’s current research foci are the study of the pathobiology of stem cells in head and neck tumors, and the study of dental pulp stem cell differentiation in the context of dental pulp tissue engineering.
Mandibular Pathology and Dentition in late 19th and Early 20th Century Mississippi State Insane Asylum Burials: Initial Observations

**K Alford¹, J Griggs², R Didlake¹**

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**Objectives:** In 1855 the Mississippi State Insane Asylum began operations in a then “state of the art” facility on the outskirts of Jackson. The Asylum maintained a large cemetery on this site which is now the last undeveloped space on the present-day campus of the University of Mississippi Medical Center. During recent construction 66 burial sites from this cemetery were exhumed and the remains made available for dental examination.

**Methods:** Radiographs were collected using a handheld, Nomad Pro 2 radiograph machine and MediaDent software. Images were taken from bucco-lingual and medio-distal perspectives in order to capture the total pulpal anatomy and ensure comprehensive examination. The radiographs and direct physical examination were used to document any abnormalities in the dentition as well as the mandibles and hard palates.

**Results:** Of the 66 bodies that were exhumed, 30 had dentition with several showing complete or portions of the mandible. A few burials had a hard palate present and a full set of adult dentition. Carious lesions were present upon gross and radiographic examination, and alveolar bone loss was noted in the burial with a hard palate. A generalized lack of teeth per burial was also observed.

**Conclusions:** These initial observations indicate that the Nomad Pro 2 is a useful modality for examining archeological remains when teeth are present, but the low yield of oral cavity structures per burial site will require examination of large numbers of specimens in order to make conclusions about oral health in this population.

**Acknowledgments:** Supported by the University of Mississippi Medical Center for Bioethics and Medical Humanities and Mississippi State University.
Utilization of 3D Culture Systems and VEGF to Enhance Endothelial Cell Differentiation

K Clark1, AV Janorkar1

1Department of Biomedical Materials Science, University of Mississippi Medical Center

Objectives: Establishing angiogenesis is a key factor in regenerative medicine and the future of periodontal tissue engineering. Unfortunately, clinical applications of tissue engineering are limited by the lack of adequate blood supply. Endothelial cells are a vital component of the capillaries that provide adequate blood supply and excretion of wastes in tissues. We have previously demonstrated our 3D spheroid model created atop a biopolymer conjugate, elastin-like polypeptide-polyethyleneimine (ELP-PEI), using 3T3-L1 adipocytes. The aim of this study was two-fold: (1) to examine the ability of adipose-derived stem cells (hASCs) to differentiate into endothelial cells in our 3D endothelial spheroid culture system and (2) to determine if the addition of VEGF can enhance endothelial cell differentiation.

Methods: Cell Culture: ELP-PEI (5 mol%) was coated onto 24-well TCPS plates (0.5 mg/cm²). Human adipose-derived stem cells (40,000 cells/cm²) were isolated according our IRB-approved protocol and were cultured on ELP-PEI coated surfaces to form the 3D spheroids and on uncoated TCPS controls to form the 2D monolayer. After 3 days of acclimation in maintenance media (Day 0), cells were exposed to endothelial differentiation medium (EGM-2-MV) containing 2, 20, or 50 ng/mL of VEGF for 7 days. Cells then were collected and assayed for total protein content, DNA content, viability, and endothelial differentiation markers (von Willebrand factor, CD31, Low-density lipoprotein (LDL) uptake). Biochemical Characterization: Total protein content and DNA content were determined using commercially available BCA Total Protein and BioRad Fluorescent DNA quantitation kits. Imaging of cell viability and LDL uptake were performed utilizing Invitrogen viability/cytotoxicity kits and Fisher Dil-acetylated LDL uptake assays. R&D Systems ELISA assays specific to mouse CD31 and vWF proteins were performed.

Results: Fluorescent images showed that all cultures remained viable throughout the experimental time. On Day 7, protein levels in 3D and 2D cultures showed a significant decrease when given 50 ng/mL of VEGF CD31 levels were only detected in 2D and 3D cultures given higher VEGF concentrations (20 ng/mL and 50 ng/mL). vWF levels in 3D cultures increased with the addition of VEGF to reach a value significantly higher than 2D cultures given 50 ng/mL of VEGF. Likewise, fluorescent imaging revealed that 3D cell cultures were able to uptake LDL and exhibit fluorescence, while the 2D cultures were not able to demonstrate this fluorescence.

Conclusions: While culturing hASCs in the presence of endothelial differentiation media supports endothelial cell differentiation, our 3D spheroid model augments this differentiation. Furthermore, the addition of higher concentrations of VEGF further enhanced differentiation to the endothelial cell lineage. These conditions mimic physiological conditions in which neo-angiogenesis can occur. Further studies aim to elucidate how to produce physiological changes such as neo-angiogenesis and sprouting of vessels in our culture systems.

Acknowledgments: Supported by NIH/NIBIB R01 EB020006 and the SOD Intramural Research Support Program.

Surface Modifications of Titanium Alloys Using Anodization

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Objectives: Titanium and titanium alloys are some of the most commonly utilized metals for the manufacture of orthopedic and dental implants due to their great mechanical properties, corrosion resistance, and biocompatibility. In an oxygenated environment, titanium and titanium alloys form a spontaneous amorphous oxide layer that helps protect the metal from corrosion and prevent ion release. This oxide layer has also been reported to contribute to the successful biocompatibility of the implant material. Surface modifications of titanium alloys using anodization have been shown to increase oxide crystallinity and surface energy which may improve its osseointegration properties. The purpose of this study was to compare the oxide crystallinities and surface energies of titanium and titanium alloys modified through anodization.

Methods: The present study was conducted using four biomedical grade titanium alloys: Commercially Pure Titanium (CPTi-4, alpha phase), Titanium 15-Molybdenum (TiMo, beta phase), Titanium 6-Aluminum 7-Niobium (TAN, alpha + beta phase), and Titanium 6-Aluminum 4-Vanadium (TAV, alpha + beta phase). Anodization of these alloys was conducted in two different solutions: Solution A comprised of 3.5 M sulfuric acid, 0.1875 M phosphoric acid, 0.75 M hydrogen peroxide, and 0.25 M oxalic acid; and Solution B comprised of 5.6 M sulfuric acid. The anodization was conducted using a full potentiostatic waveform that increased voltage in 5- to 12-V steps every 10 seconds up to five selected forming voltages ranging from 60 V to 180 V based on previous research. X-ray diffraction (XRD) was utilized to determine the crystalline phases present within each of the anodized layers. Surface energy was calculated for each sample from wetting angle measurements of distilled deionized water and diiodomethane.
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Results: XRD results from CPTi, TAN, and TAV samples anodized in solution A showed only anatase peaks present, and the peak intensities increased with increasing voltage up to 180 V. For the TiMo samples in solution A, anatase formed first, and a combination of anatase/rutile formed at higher forming voltages with rutile becoming more prevalent at higher forming voltages. XRD results from samples anodized in solution B showed that anatase formed at lower forming voltages, and a combination of anatase/rutile formed at higher forming voltages for all samples. The surface energies from samples anodized in solution A showed that the TiMo samples had lower values at approximately 30-40 J/m² compared to 70-75 J/m² for the CPTi-4, TAN, and TAV samples. Surface energies were found to be approximately in the same range at 65-75 J/m² for all of the samples in solution B.

Conclusions: The results showed that the titanium alloy composition and phase do have an impact on the resulting oxide crystallinity and surface energy after anodization. The modifications were also shown to be dependent on the anodization electrolyte. The beta phase alloy (TiMo) was found to have a low surface energy in Solution A, which may lead to poor osteoblast adhesion properties, while the alpha phase (CPTi-4) and alpha + beta phase alloys (TAN and TAV) showed the highest surface energy. This could in turn impact the biocompatibility of the implant.

Upper Airway Volumetric Analysis Utilizing Invivo5

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Objectives: The primary purpose of this study was to assess the accuracy of Anatomage Invivo5’s semi-automatic tool for segmenting airway phantoms scanned with cone beam computed tomography (CBCT). Secondarily, it was to assess the intra- and inter-observer variability among dental professionals of differing experience levels.

Methods: Three phantom airways of differing diameters and of known volumes were fabricated to simulate upper airway and neck anatomy. The actual airway volumes were determined using the equation \( \pi r^2 h \). Phantoms were scanned with an iCAT CBCT. Volumetric analysis of each reconstructed airway was performed with Anatomage Invivo5 (AI) in order to assess accuracy of the software. CBCT digital imaging and communications in medicine (DICOM) data was entered into AI. AI has a semi-automatic tool that allows segmentation and volumetric measurement of the airway structure. Twenty patients were then randomly selected from the University of Mississippi Medical Center, Department of Oral and Maxillofacial Surgery (OMS) database. Patients previously had a CBCT performed for evaluation of a dentofacial deformity. Patient exclusion criteria included obstructive sleep apnea and craniofacial deformities such as cleft lip/palate. Patient DICOM data was entered into AI. Airways were segmented from the level of the hard palate to the superior aspect of the 4th cervical vertebra. Airway segmentation and volumetric measurements were performed in the same fashion as done for the phantoms. Examiners with different levels of experience performed the airway analysis to assess inter-observer variability and at three different time points to assess intra-observer variability. Examiners included a 1st year dental student, a 3rd year dental student, an OMS resident and an OMS attending surgeon. All assessments were performed using the same desktop computer and monitor. Data were compiled on an Excel spreadsheet for analysis. Linear regression analysis was used to assess the precision and accuracy of the volumetric analysis of the phantom airways.

Two-Way Repeated Measures ANOVA (Two Factor Repetition) was used to assess intra- and inter-observer sources of variation.

Results: The examiners’ accuracy and precision fell within a 95% confidence interval of the actual phantom airway volumes. Interestingly, examiners’ estimated airway volume measurements decreased over time. There was no significant inter-observer variability for each data set and time point \((p=0.123)\). Significant intra-observer variability occurred amongst examiners \((6.7\% \text{ variability over time})\). However, the statistical significance does not necessarily equal clinical significance.

Conclusions: AI software was accurate and precise in assessing airway volume in the simulated phantom airways used in this study. Significant intra-observer variability was present. AI measured airways with extreme precision, but only fair accuracy. Future studies might test accuracy of other commercially available software programs as well as more anatomically complex phantom airways so that practitioners may better serve their patients.

Anodized Titanium with Calcium and Phosphorus Surface Enhancements for Dental and Orthopedic Implants

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Objectives: In an ambient environment, titanium naturally forms a thin amorphous oxide layer. This layer may be converted to a crystalline oxide using anodization. Additionally, anodization can incorporate desirable chemical species such as phosphorus \((P)\) from the electrolyte into the oxide layer. Both anatase \((A)\) and rutile \((R)\) crystalline phases of titanium oxide are known to promote bioactivity and antimicrobial effects. The objectives of this research were to
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**Effective Drug Release from Extracellular Matrix Protein Based Composites**

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**Objectives:** Composite hydrogel drug delivery systems have attracted many tissue-engineering researchers from around the world. Controlled drug delivery application is a key factor to reduce the amount of drug necessary to elicit the same therapeutic efficacy as systemic drug delivery. Recently, we prepared elastin-like polypeptides (ELP) – collagen composites with significantly better mechanical properties and equivalent biocompatibility compared to collagen scaffolds [1]. ELP exhibits an inverse phase transition behavior in response to changes in its environment. Previously we showed that ELP-collagen hydrogels exhibit a tunable release of bioactive agents compared to collagen-only hydrogels [2]. The main objective of the study was to evaluate compositional factors (collagen concentration, ELP addition, and chemical crosslinking of collagen) to determine the extent to which we can further achieve sustained drug release characteristics of the collagen-based composites. Specifically, we report the drug release characteristics of composites with carefully selected compositions for the release of model osteogenic protein (Bone Morphogenetic Protein, rhBMP-2) and a commonly used antibiotic in adjunct therapy (doxycycline hyclate).

**Methods:** Hydrogel preparation: Eight different compositions were prepared by varying concentration of collagen, ELP addition, and chemical crosslinking of collagen (EDC/NHS = 1:1). ELP (MW=17 kDa) was produced from genetically engineered E. coli. ELP-collagen hydrogels were formed by incubating ELP and collagen (rat tail tendon) in 1 N NaOH, DI H₂O, and 10X DMEM at 37 °C overnight. The procedure is detailed elsewhere [1,2]. Bioactive Agent Loading and Release: Doxycycline hyclate (0.5% w/w) and rhBMP-2 (0.005% w/w) concentrations were calculated based on the weight of collagen and ELP. Amounts of doxycycline released into 100 μL alpha-MEM cell culture medium at 37 °C were analyzed by measuring absorbance at 345 nm using an xMark microplate spectrophotometer (Bio-Rad). The release of rhBMP-2 into 100 μL alpha-MEM at 37 °C from composite was assayed by ELISA (R&D Biosystems). The release of bioactive agents was followed up to seven days.

**Results:** Doxycycline release showed a bi-phasic release profile with the initial burst release followed by a gradual release. Osteoinductive protein, rhBMP-2, exhibited a nearly linear release profile in all hydrogel compositions. The compositional factors of collagen concentration, ELP addition, and chemical crosslinking did not affect doxycycline release. However, our results indicate that the collagen hydrogel prepared using the higher (6 mg/mL) collagen concentration showed a significantly slower rhBMP-2 release. Similarly, the incorporation of ELP into collagen hydrogels achieved a significantly slower rhBMP-2 release. Interestingly, chemical crosslinking did not dramatically affect rhBMP-2 release. We hypothesize that increasing collagen concentration and ELP addition create denser hydrogels with smaller average pore size that allow a slower release of the higher molecular weight rhBMP-2 but have no effect on the release of the smaller molecular weight doxycycline.

**Conclusions:** Overall, all hydrogels released doxycycline and rhBMP-2 at acceptable levels, with nearly complete doxycycline release in one week, and a slower rhBMP-2 release. These release kinetics are clinically relevant as antibiotic release in one week post-operatively will fend off post-surgical infections, and a slower rhBMP-2 protein release will enhance the osteogenic response of the implanted scaffold. In the future, we will perform drug release against four bacterial strains (Escherichia coli, Pseudomonas aeruginosa, Streptococcus sanguinis, and methicillin-resistant Staphylococcus aureus) that are commonly encountered in clinical settings to further evaluate the effectiveness of doxycycline released from these compositions after seven days.
**Poster Abstracts**

**Acknowledgments:** Supported by the National Institutes of Health/National Institute of Dental and Craniofacial Research (R03 DE024257)


**Saliva Bone Density Biomarkers Analyzed via Clinical Strips and ELISA**

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**Objectives:** Osteoporosis is a condition of decreased bone mineral density (BMD). The ability to quickly and easily identify patients with osteoporosis is a new topic in diagnostic medicine. The association between BMD and low osteocalcin level or high deoxypyridinoline (DPD) level in serum has been known. In attempts to have a saliva test strip that aids as an early detector of bone loss and osteoporosis, this Phase II clinical study analyzed the association between bone density computer tomography (CT) scans and saliva osteocalcin and DPD levels, measured by enzyme-linked immunosorbent assay (ELISA) and clinical salivary test strips.

**Methods:** A total of 159 participants were studied, with 90 classified as having normal bone mineral density (BMD) and 69 with low BMD, according to quantitative computer tomography CT (qCT). Those participants with low BMD were older (p<0.001) and were nonblack (p=0.001). Salivary test strips were used clinically to measure levels of DPD and osteocalcin. Salivary samples were taken from the participants, frozen, and subsequently analyzed by ELISA-based capture assays using the commercial kits MicroVue Osteocalcin and MicroVue DPD.

**Results:** Results for prediction of BMD measured on the continuous scale (linear regression) and two separate dichotomous variables (logistic regression displayed as odds ratios (OR)) were statistically calculated. None of the models fit the data well, and no statistical significance was reached.

**Conclusions:** The link between bone turnover biomarkers and osteoporosis is known, but further research is warranted in order to find a clinical test strip and ELISA commercial kit to test for these biomarkers and correlate with the radiographic BMD readings. Several explanations for the inconclusive results include a commercial ELISA kit that was to test urine but was instead used to analyze the salivary samples and low concentrations of the biomarkers in saliva and insensitive clinical test strips. Further production of clinical test strips and ELISA commercial kits for salivary samples as well as more investigation into the levels of these bone turnover biomarkers in saliva is needed in order to achieve a clinical test strip that can accurately identify patients with low bone turnover and who have the potential for osteoporosis.

**Dental Care — A Largely Unmet Need for Persons Living with HIV/AIDS in Mississippi**

*J White*, *DD Krause*

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**Objectives:** This is a mixed methods study that examines the most critically unmet healthcare need of persons living with HIV/AIDS (PLWHA) in Mississippi. In a preliminary study by Krause et al., face-to-face interviews were conducted with a random sample of 220 PLWHA in Mississippi. It was found in that study that one of the greatest unmet needs of that population is dental care. Many different factors may contribute to this unmet need, such as age, sex, race, education, income, insurance, and facility type. The objective of this study is to examine demographic and behavioral correlates of perceived unmet need for oral health care among PLWHA in Mississippi, both quantitatively and qualitatively, and to provide insight into satisfaction with the dental care received and the quality of care provided by health care facilities in Mississippi.

**Methods:** Participants were asked if they needed a comprehensive list of health care or services in the last twelve months and if they had received each care or service. From the 220 participants, three subgroups were created. These were participants who, 1) had a perceived need for dental care and had that need met, 2) had a perceived need and did not have that need met, and 3) did not have a perceived need for dental care. IBM SPSS was used to run frequencies, cross-tabs, and chi-square analyses. Reasons reported for not obtaining dental care included cost or lack of financial resources; low motivation, apathy, or not a priority; lack of knowledge; logistics, transportation issues, no available provider, or lack quality of service; fear or distrust; and displacement. Data were analyzed based on demographic, enabling, and behavioral characteristics. Qualitative data are reported that include reasons participants had an unmet need, the type of facility that provided dental care, and the quality ratings that participants provided for each facility type.

**Results:** The unmet need of dental care was found to be highly correlated with demographic factors such as sex and ethnicity, income of less than $10,000 per year, unemployment, and lack of insurance. The greatest perceived barrier to dental care was cost or lack of financial resources. Within the facility types, private offices were the most often used facility type (47%). On a quality rating scale of very poor to excellent, the private facility type received the highest ratings of excellent from 64% of participants.
Conclusions: Even though 25% of this sample needing dental care was covered by Medicaid, half of those did not receive dental care. This may be because the current Medicaid health care policy in Mississippi does not include dental care coverage. With the insights revealed through this study, steps could be taken to determine what measures might improve Medicaid health care policy. Private and public facilities can take a closer look at their patient populations and determine methods in which they can make dental care more accessible for PLWHA.

Acknowledgments: The data collection portion of this study was funded by the Ryan White Program provided by the U.S. Health Resources and Services Administration.

Synthesis and Characterization of New High Molecular Weight Elastin-Like Polypeptide (ELP)-Polyethyleneimine (PEI) Bio-Polymers

J Cobb¹, AV Janorkar¹

¹Department of Biomedical Materials Science, University of Mississippi Medical Center

Objectives: Elastin like polypeptide (ELP)-polyethyleneimine (PEI) block copolymers are a class of multifunctional polymers that have been shown to exhibit the inverse phase transition behavior of ELP as well as the polyelectrolyte nature of the PEI. Previous work with ELP-PEI has utilized a low molecular weight block of PEI (<1200 g/mol) which, used in low concentrations in surface coatings, has been shown to elicit cellular aggregations into three-dimensional structures. At higher concentrations, PEI has been shown to have applications in antimicrobial coatings. In this work, we report the synthesis and characterization of a high molecular weight ELP-PEI copolymer (PEI=10,000 g/mol).

Methods: ELP (MW = 17,000 Da) produced from genetically engineered E. coli was reacted to PEI (MW = 800 and 10,000 g/mol) using a carbodiimide reaction reported elsewhere. Reaction conversion was determined by treating the copolymer with formaldehyde, after which the fluorescence was measured and compared to a previously generated standard curve. Dynamic light scattering was used for solution behavior characterization in various solution media. Atomic force microscopy was used to capture surface images of tissue culture polystyrene surfaces (TCPS) coated with ELP/ELP-PEI.

Results: Due to the regular repeat structure (NH₂-[CH²]-) of PEI and the large amount of amide groups present in ELP, it is very difficult to distinguish ELP-PEI 800 and ELP-PEI 10K copolymers with traditional techniques such as FT-IR spectroscopy. Therefore, a model was built around the OPA fluorescence assay to determine the amount of ELP that was conjugated to PEI. This model shows that while the increased amination due to PEI conjugation increases the OPA fluorescence, the PEI fluorescence is somewhat masked by ELP at lower %conjugations. This is an important finding that helps more accurately estimate an unknown %conjugation. DLS measurements on ELP-PEI 10K exhibits a tri-modal transition curve with an onset of 35°C and a radius of hydration (Rh) value of 550 nm. In 0.2 M NaCl, the transition curve shifts to bimodal with an onset of 33 °C and Rh value of 950 nm. In the higher salt concentration of 1 M, the curve exhibits a single transition at 23 °C with the Rh value of 950 nm. AFM results show that both coatings had similar smooth morphologies indicated by the Ra values of 0.087 ± 0.011 nm for the ELP-PEI 10K and 0.088 ± 0.011 nm for ELP-PEI 800.

Conclusions: The OPA assay was found to be a more reliable method to characterize ELP-PEI polymers than the more traditional FT-IR ATR. DLS showed that the larger the PEI block, the more influence it has on the transition temperature and aggregate size of the polymers in solution. Lastly, AFM revealed that the ELP-PEI polymers form smooth and uniform surfaces when used as a coating.

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Adipogenic Differentiation of Human Adipose-Derived Stem Cells Grown as Three-Dimensional In Vitro Model

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Objectives: It is important to study adipose tissue behavior on a cellular level, to more efficiently combat the problem of obesity, as well as to prevent the issues that stem from it. Elastin-like polypeptide-polyethyleneimine (ELP-PEI) copolymer coatings have been tested in the past for several different cell types, in which the positively-charged polyelectrolyte encourages cells to form three-dimensional (3-D) spheroids while tethering the spheroids to the ELP-PEI coating [1,2]. The 3-D spheroids have been shown to be better in vitro model for adipocyte culture compared to the 2-D monolayer [2]. Here, we report on the effect of the molecular weight of the polyelectrolyte (PEI) block on the spheroid organization kinetics and overall spheroid size of human adipose-derived stem cells (hASCs) differentiated along the adipogenic lineage on the ELP-PEI coatings.
Poster Abstracts

**Methods:** ELP-PE Conjugation: ELP-PE conjugates were synthesized by first activating the ε-terminus COOH on ELP using carbodiimide and then conjugating the activated ELP with two different molecular weights (800 and 10,000 Da) of polyethyleneimine (PEI) at 4 °C. To achieve similar concentrations of amine groups across the wells, 0.5 mg/cm² of ELP-PE surface concentration was used with 15 mol% of ELP-PEI 800 and 1.2 mol% ELP-PEI 10,000. TCPS wells were coated by physical adsorption by drying the ELP-PEI solution at 37 °C. Cell Culture: hASCs were isolated according to the IRB-approval protocol and cultured on ELP-PEI coated surfaces (26,000 cells/cm²) to form the 3-D spheroids over three days of acclimation period. Following further three days of exposure to differentiation cocktail, cells were exposed to media containing linoleic acid (LA; C18:2 Ω-6) and a control media containing no fatty acids (DMEM + 10% FBS) for three more days. Spheroid formation kinetics and size were monitored using Olympus IX81 optical microscope equipped with a Hamamatsu digital camera and LiveCell unit. Time-lapse images were recorded throughout the experiment on at least three different positions per well of three replicate wells. Images were analyzed using the ImageJ software.

**Results:** The study investigated the spheroid growth kinetics of hASCs atop the two ELP-PEI surfaces, namely ELP-PEI 800 and ELP-PEI 10,000 at seed time (Day 0), at the end of the spheroid formation period (Day 3), at the end of the differentiation period (Day 6), and during the adipocytic maturation period (Day 9). The spheroid formation kinetics were analyzed using time-lapse microscopy to quantitatively determine the spheroid dimensions. The spheroids formed atop the ELP-PEI 800 remained small (diameter ~ 50 μm) throughout the culture period, while those formed atop the ELP-PEI 10,000 gradually increased in size to reach a maximal spheroid diameter of ~ 200 μm. The spheroid sizes generally reduced in the maturation phase, likely due to most fatty spheroids getting lost during media changes.

**Conclusions:** Overall, our results demonstrated that molecular weight of the polyelectrolyte block in the ELP-PEI conjugate affects spheroid size. Such control in spheroid size formation will further allow us to elucidate the effect of aggregate size on cellular function.

**Acknowledgments:** Supported by NIH/NIBIB R01 EB020006.


**Characterization and Optimization of Composite Scaffolds for Guided Bone Regeneration**

*B Gurumurthy¹, IA Griggs¹, AV Janorkar¹

¹Department of Biomedical Materials Science, University of Mississippi Medical Center

**Objectives:** The success of scaffolds used for guided bone regeneration (GBR) depend on physical characteristics, mechanical properties and biochemical properties. Currently used collagen scaffolds have good biochemical properties, but they fail due to their poor mechanical and physical properties. We have previously shown that elastin-like polypeptide (ELP) infused collagen scaffolds have improved mechanical properties and osteogenic differentiation. The current challenge is to find an optimal composition of the scaffold without compromising on the requirements for each of the properties. The objectives of this study were to perform physical and mechanical characterization, optimize the mechanical properties using response surface methodology (RSM), and identify the composite with optimal properties for future experiments.

**Methods:** 2-7 mg/mL collagen and 0-24 mg/mL ELP were used in 11 different proportions to form the composites. Mechanical properties were determined by uniaxial tensile testing, and composite concentration was optimized with RSM. Physical properties were characterized by swelling ratio, differential scanning calorimetry, scanning electron microscopy (SEM), and FTIR spectroscopy.

**Results:** Mechanical properties varied with composition. 6:18 mg/mL collagen:ELP composite had the maximum strength and modulus, but had lower toughness than many other composites. RSM optimized using just 5 compositions (versus the 11 we had to prepare for mechanical testing) and directed us to 6:12 mg/mL composition that may have high toughness without negatively impacting strength and modulus. Physical characterization of 6:18 mg/mL composite against its control (6:0 mg/mL) revealed that the addition of ELP in composites resulted in ELP-collagen interactions leading to reinforcement of the scaffold. SEM images showed porous fibrillar and dense afibrillar collagenous microstructure and additionally, 6:18 mg/mL showed a more open porous structure with characteristic ELP aggregates.

**Conclusions:** Overall, mechanical and physical properties vary with ELP and collagen concentration through the presence of ELP-collagen interactions. RSM efficiently performed optimization of composition by considering target levels, minimal requirements, and relative importance of the mechanical properties and predicted a new composition for future testing. Taken together, the composites prepared in this research can form good quality, rigid porous structures required for GBR as well as other tissue engineering
**Poster Abstracts**

**Acknowledgments:** Supported by the National Institutes of Health/ National Institute of Dental and Craniofacial Research (R03 DE024257)

**Photocatalytic Bactericidal Effects of Anodized Titanium**

*S Jain¹, S Williamson¹, M Roach¹, M Marquart²

¹Department of Biomedical Materials Science, University of Mississippi Medical Center; ²Department of Microbiology and Immunology, University of Mississippi Medical Center

**Objectives:** Titanium anodization is used to produce roughened surfaces known to enhance the bone-to-implant contact and hence the clinical success of dental implants. Additionally, both anatase (A) and rutile (R) phases of titanium oxide have shown antimicrobial properties when photocatalytically activated using UV light sources. However, it is not clearly understood whether a single phase or a combination oxide produces the best results. Additionally, it is not known whether UVA or UVC treatments produce the greatest effect. The objective of this study was to evaluate the UVA and UVC photocatalytic bactericidal activity of anodized coatings containing combinations of anatase, rutile, and amorphous oxide against Streptococcus sanguinis (strep).

**Methods:** Square one-inch samples were fabricated from commercially pure titanium (CP-Ti) grade 4 sheet and anodized using potentiostatic 12-V, 10-second steps up to 108 V or 180 V in four mixed-acid electrolytes. Non-anodized titanium samples were used for controls. 180-V samples in the four electrolytes were previously shown to produce coatings that were primarily A, primarily R, approximately 50/50 A/R, and primarily amorphous. Phase ratios were confirmed using X-ray diffraction. Duplicate samples for each combination were exposed to UVA or UVC treatments for 10 minutes or no-UV treatment. All samples were seeded with 400 µL of 10⁶ colony-forming units (CFUs)/mL of strep (strain 1736), incubated for 24h in 40 mL of Todd Hewitt broth at 37 °C and 5% CO₂ and assessed for attached bacteria CFUs. A total of three readings were made on duplicate samples (n=6). Each anodization and UV treatment group was compared to the non-UV treated titanium control group. For non-UV treated samples, the electrolyte C 108V and D 180V groups. UVC light activation was shown to significantly reduce bacterial CFUs for oxides containing predominately anatase, rutile, or amorphous phases. However, the approximately equal combination of anatase and rutile phases in the electrolyte C 180V group showed significantly higher bacterial CFUs compared to the non-UV treated titanium control group.

**Results:** For non-UV treated samples, the electrolyte C 108V and the electrolyte B 180V groups showed significantly lower bacterial CFUs compared to the titanium control group. However, the electrolyte C 180V group showed the highest bacterial CFUs in the entire study. A 10 min UVA activation treatment significantly reduced bacterial CFUs on all anodized and titanium control groups except electrolyte C 180V and D 180V groups. UVC light activation was only effective for the electrolyte B 108V group. Therefore, UVA light activation was shown to significantly reduce bacterial CFUs for oxides containing predominately anatase, rutile, or amorphous phases. However, the approximately equal combination of anatase and rutile phases in the electrolyte C 180V group showed significantly higher bacterial CFUs compared to the non-UV treated titanium control group.

**Conclusions:** UVA treatments on anodized coatings containing predominately anatase, rutile, or amorphous single phase oxides, showed the best effect against Streptococcus sanguinis. These treatments show potential to kill contaminating streptococcal species, which are the one of most occurring species in dental infections.

**Fractal Dimension of Ceramic Fixed Dental Prostheses (FDP)**

*K Jodha¹, B Key¹, SM Salazar Marocho¹, Y Duan¹, J Mecholsky², J Griggs²

¹Department of Biomedical Materials Science, University of Mississippi Medical Center; ²Department of Materials Science and Engineering, University of Florida

**Objectives:** The fracture surfaces of ceramic prostheses can be characterized using fractal geometry to provide a wealth of information about the material properties and the conditions that were present at the time of failure. This potentially useful tool is not widely employed because the current methods of fractal analysis are labor intensive, technique sensitive, and statistically biased. Our team has developed a new failure analysis method. It is an automated, unbiased method of rapidly and precisely measuring the fractal dimensional increment (D*) of fracture surfaces. The proposed project aims to validate this tool as an enabling technology to allow (1) determination of the failure origin in multilayered structures and (2) determination of material fracture toughness; both of which are useful in improving material processing.

**Methods:** We have tested two benchmark materials (silica glass and NIST standardized Si₃N₄) and we will test three dental ceramics (Y-TZP, lithium disilicate glass-ceramic, and fluorapatite layered ceramic) with surface crack in flexure (SCF) method in accordance with the ASTM C1421 standard for their fracture toughness. The fractured specimens were then analyzed with scanning electron microscope (SEM) and atomic force microscope (AFM). The D* values of leveled fracture surfaces were calculated using the Minkowski Cover technique in the FRACTALS software. The relation of K₁c and D* for each ceramic was compared with the linear regression model in the literature for the same family of ceramics.
**Poster Abstracts**

**Results:** The Si₃N₄ specimens were on the same trend line of fracture toughness vs. the square root of D* graph, and followed the behavior of polycrystalline materials as observed by Hill et al. using the traditional method. The silica glass specimens followed a trend similar to single crystal quartz.

**Conclusions:** Additional experiments need to be conducted to completely define the relations between KᵦC and D* for all three families of ceramics. In addition, we will test the utility of fractal analysis by verifying our ability to detect which of three commercially available dental ceramics is the failure origin in bilayered specimens (glass-ceramic veneered with porcelain, zirconia veneered with porcelain, and zirconia veneered with glass-ceramic). The resulting protocol may also be applicable to ceramic components used in orthopedic surgery and the automotive, aerospace, and semiconductor industries.

**Acknowledgments:** Supported by grant R01 DE024333 and UMMC Intramural Research Support Program.

**Fracture Toughness and Unbiased Fractal Dimensional Increment in Fracture Surfaces**

*B Key*, SM Salazar Marocho*, J Mecholsky*, J Griggs*

1Mississippi State University; 2Department of Biomedical Materials Science, University of Mississippi Medical Center; 3Department of Materials Science and Engineering, University of Florida

**Objectives:** To determine the regression model relating fracture toughness (KᵦC) to the fractal dimensional increment (D*) of the fracture surface calculated using a new and unbiased technique on a standard reference material (Si₃N₄). Additionally, our accuracy in using the surface crack in flexure (SCF) method to calculate the KᵦC of Si₃N₄ was tested.

**Methods:** The fracture toughness (KᵦC) of rectangular beams of NIST standardized Si₃N₄ (n=10) was evaluated by the SCF method according to ASTM C1421 with a Knoop indentation load of 49 N. Fracture surfaces were analyzed using fractography and fractal analysis. The critical flaw sizes were measured using a scanning electron microscope. Epoxy replicas of the mirror regions of the fracture surfaces were prepared and scanned using atomic force microscopy as follows: 5 μm x 5 μm scanning area with 512 lines at a rate of 0.592 Hz. The height data from the surfaces was imported into a custom MathCAD script, and FRACfALS software was used to determine D* by the Minkowski cover technique.

**Results:** The calculated KᵦC value for Si₃N₄ (4.62 ± 0.14) was similar to the value found in the Standard Reference literature (4.57 ± 0.12) for the same material (Student’s t-test, p = 0.57). Fracture toughness was found to be linearly correlated to square root of D*. A regression equation was found (KᵦC = 12.86 D½*).

**Conclusions:** The calculated KᵦC for Si₃N₄ agrees closely with the standard reference value, and the regression model relating KᵦC and D* was determined. The examination of more materials will enable further validation of the unbiased technique.

**Acknowledgments:** Supported by NIH Grant 1R01 DE024333.

**Finite Element Analysis of a Three-Unit Fixed Dental Prosthesis**

E Theilman*, Y Duan*, J Griggs*

1Department of Biomedical Materials Science, University of Mississippi Medical Center

**Objectives:** To create three-dimensional numerical models of a multilayered three-unit all-ceramic fixed dental prosthesis (FDP) and to investigate the effects of various combinations of veneer and framework materials on the stress distribution in the prosthesis using finite element analysis (FEA).

**Methods:** 3D models were created in Mimics based on micro-CT images. The following materials from the IPS e.max system (Ivoclar Vivadent) were defined using elastic constants found in the literature: Ceram, CAD, and ZirCAD. Three different framework/veneer material combinations were simulated as follows: (1) CAD/Ceram; (2) ZirCAD/Ceram; (3) ZirCAD/CAD. The ZirCAD/CAD model was created with a fusion glass-ceramic layer between veneer and framework to simulate the e.max CAD-on technique. A load of 150 N (axial direction) was applied on the central fossa of the pontic. Models were then exported into FEA software (ABAQUS) for stress analysis.

**Results:** All models had stress concentrations on the occlusal surface surrounding the loading area and at the gingival embrasure of connector in the veneer layer. The ZirCAD/Ceram model had the lowest stress value in the veneer layer at the gingival embrasure area of connector among three models. The CAD/Ceram model had the lowest maximum stress in the framework at the gingival embrasures of the connectors. The ZirCAD/CAD model had the lowest maximum stress in the luting agent layer at the cervical margin area of the premolar abutment.

**Conclusions:** Stress distributions were significantly influenced by different combinations of veneer and framework materials for a three-unit FDP. All three all-ceramic systems were resistant to fracture at the given physiological occlusal loading level.

**Acknowledgement:** Supported by NIH grant 1R01 DE024333 and the SURE Program.
Research Opportunities and Awards at the University of Mississippi School of Dentistry

Honors in Research Program
The Honors in Research Program (HRP) provides an opportunity for eligible dental students to choose advanced study in dental research or basic health science and receive recognition for their accomplishments on their transcripts and at graduation. Honors work consists of hypothesis driven research in some aspect of dental or basic health science. Students conduct laboratory research, clinical research, or population research (e.g., improving current clinical practices, exploring controversies in dentistry, engaging in basic and biomedical materials research) with the guidance and supervision of a UMMC faculty member.

Honors in Research Graduates - 2009-2016
Jennifer Bain, Jason Brown, Reid Lester, Kristin Balus, Curtis Caskey, Lacey Harris, Stacey Ritter, Camille Sandifer, Corey Shook, Phebe Winters

School of Dentistry Intramural Research Support Program (IRSP)
The goal of the Intramural Research Support Program is to provide seed funding for research activities in the School of Dentistry. In addition to faculty, pre-doctoral students and residents who develop a faculty-mentored research project are eligible to apply for small grants to cover materials and supplies. Priority will be given to those research projects which involve School of Dentistry students.

Student Research Group (SRG)
The School of Dentistry Student Research Group is a branch of the American Association for Dental Research (AADR) National Student Research Group (NSRG) and is composed of dental and graduate students committed to research and the advancement of further education. Goals of the organization are to expose dental students to various student research projects, aid in the application process for residencies to dental specialties, and to encourage student participation in dental research. Meetings allow students to share and evaluate on-going research projects within the School of Dentistry.

Student Research Group Officers for 2016-2017
President – Kendra Clark
Vice-President – Bhuvaneswari Gurumurthy
Treasurer – Caleb Hardman
Secretary – Sarah Burnham Kimbrough
Faculty Advisor – Dr. Jennifer Bain

Awards and Honors
2016 ADA/Dentsply Student Clinician Award – Kendra Clark was the ADA/Dentsply Student Clinician Award winner and was presented the award at Student Awards Day 2016.

2016 Hinman Student Research Award – Jiman Nelson received this award and represented UMMC at the Hinman Student Research Symposium, in Memphis, TN, October 28 - 30, 2016 at the historic Peabody Hotel.

52nd Annual Colgate Dental Students’ Conference on Research
Kendra Clark was selected as UMMC’s representative to attend this conference, which introduces outstanding dental students to scientists from the ADA Foundation’s Dr. Anthony Volpe Research Center on the NIST campus in Gaithersburg, MD. The conference was held on October 2-4, 2016.

2016 Quintessence Award for Research Achievement – Brandon Myrick received the School of Dentistry Quintessence Award for Research at the SOD Awards Day on April 19, 2016. Myrick received this honor for his many combined achievements during his time in the DMD program. Myrick was the Thomas P. Hinman Student Research Award winner in 2014 and presented a poster on “Tooth Structure Removed by Hand Instruments, Magnetostrictive and Piezoelectric Ultrasomics” at the American Association for Dental Research Annual Meeting in 2013. Myrick also served as Secretary of the Student Research Group.

2016-17 President-Elect of the National Student Research Group - Kendra Clark was elected 2016-17 president-elect of the National Student Research Group (NSRG). The NSRG is an arm of the American Association for Dental Research (AADR) and is a student-run organization whose main purpose is to foster an environment in every dental school whereby students interested in enriching their dental education through research are encouraged to do so. It is composed of a network of self-governing local student research group (SRG) chapters at each dental school and is led nationally by officers elected through a majority of votes from all members. Clark will consequently serve as president of the NSRG in 2017-18.

2016 Excellence in Research Award - Dr. Michael Roach, associate professor in the Department of Biomedical Materials Science, received the Bronze Medallion for research excellence at the Excellence in Research Awards Ceremony on November 9, 2016. The Excellence in Research Awards Program recognizes UMMC investigators who have attracted significant extramural funding and who have advanced science through their distinctive research programs. Medallion Awards are based on the total amount of extramural funding received by the investigator for his/her original research.
Student Research Opportunities at the University of Mississippi School of Dentistry

Undergraduate and Professional Student Training in Advanced Research Techniques (UPSTART) Program

The UPSTART Program provides an opportunity for eligible dental, pre-dental, pre-graduate, and high school students to be involved and trained in research at the University of Mississippi School of Dentistry. The program is designed to initiate students in research by pairing with research mentors, teaching general laboratory safety, and instilling essential research skills through hands-on learning. The research experience is provided under the mentorship of a dental faculty member who is actively engaged in research throughout the summer. The program promotes learning of the dental students as well as the undergraduate students from the local colleges and universities in design and successful implementation of research projects through a didactic seminar series, hands-on laboratory research, and peer-judged research presentations. The students have the opportunity to present their research findings as an oral seminar in the “UPSTART Symposium” organized at the end of the UPSTART program. Additionally, the students are expected to present the research performed during the UPSTART program and progress since then on the following School of Dentistry Research Day. Since its inception, 93 students (42 dental, 51 other) have benefited from this program.

For information contact:
Dr. Amol V. Janorkar (Email: ajanorkar@umc.edu / Phone: 601-984-6170)
## Faculty Excellence in Research (as of December 31, 2016)

### Cumulative Publications

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