

Skeletal, Craniofacial & Oral Biology Symposium 2014



Saturday, May 3, 2014

Cell and Genome Sciences Building

UConn Health

Farmington, CT

UConn | SCHOOL OF
DENTAL MEDICINE

Welcome

Drs. Mina Mina, A. Jon Goldberg and William B. Upholt, Planning Committee for this Symposium, are pleased to welcome all participants and guests to the 2014 Skeletal, Craniofacial and Oral Biology Symposium.

The purpose of the Symposium is to bring together trainees and faculty involved in Skeletal, Craniofacial and Oral Biology training grant activities and related research, and to give them an opportunity to present and discuss their work and interact with the other trainees, mentors and colleagues. Individuals at different levels of research and training are represented at this Symposium; thus, some trainees will present completed work, while others will present work in progress. For all trainees the symposium is an opportunity to network, discuss ongoing research, meet others with common interests and career goals, and enjoy a productive day.

We are very pleased to welcome Linda D. Strausbaugh, PhD, who will present a lecture on “*The fungus among us..... Studies on the mycobiome of the human mouth.*” Dr. Strausbaugh is a professor of Genetics & Genomics in the Department of Molecular and Cell Biology at the University of Connecticut.

Mina Mina, DMD, MSD, PhD

Professor

Chair, Pediatric Dentistry

Director, Skeletal, Craniofacial and Oral Biology Training Grant

A. Jon Goldberg, PhD

Professor

Director, Center for Biomaterials

Co-Director, Skeletal, Craniofacial and Oral Biology Training Grant

William B. Upholt, PhD

Professor Emeritus

Advisor and Previous Co-Director, Skeletal, Craniofacial and Oral Biology Training Grant

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Support

This Symposium is supported by the NIDCR funded Institutional Training Grant, “Skeletal, Craniofacial and Oral Biology Training Grant”, 1 T90 DE021989.

Acknowledgements

This Symposium is the result of much hard work by many people. In particular, the Symposium Planning Committee would like to thank Cynthia C. Smith, Laura Didden, and Lisa Ramsdell for their tireless and excellent work on all aspects of design, registration, and implementation of this Symposium. Without their efforts, the Symposium could not have happened.

This Publication

This publication was designed by Cynthia C. Smith in honor of the future of genomic research. The photographs on the cover show various views of the interior and exterior of the Cell and Genome Sciences Building. She designed it to capture the feel of the dynamic movement of research in this age of technology.

Schedule

Start Time	End Time	Event
7:45am	8:10am	Registration and Continental Breakfast
8:10am	8:15am	Opening Remarks – Edmund and Arlene Grossman Auditorium Jon Goldberg, PhD
8:15am	9:15am	Oral Session 1 – Edmund and Arlene Grossman Auditorium Signaling in Hard Tissue Development Session Chair: Nathaniel Dymnt Karen Sagomonyants Vanessa Piccuillo Thomas Estus Achint Utreja
9:15am	9:35am	Break and Poster Session – CGSB Commons Jason Gibson Patience Meo Burt David Manz Evan Yu Anushree Banerjee Fayekah Assanah
9:35am	10:40am	Guest Speaker – Edmund and Arlene Grossman Auditorium Linda D. Strausbaugh, PhD <i>“The fungus among us…….”</i> Studies on the mycobiome of the human mouth.” <i>Dr. Patricia I. Diaz will introduce the guest speaker.</i>
10:40am	11:00am	Break and Poster Session – CGSB Commons
11:00am	11:45am	Oral Session 2 – Edmund and Arlene Grossman Auditorium miRNA's Session Chair: Thomas Estus Spenser Smith Neha Dole Tiziana Franceschetti
11:45am	12:45pm	Box Lunch and Poster Session – CGSB Commons
12:45pm	1:45pm	Oral Session 3 – Edmund and Arlene Grossman Auditorium Biomechanical Response, Regenerative Engineering Session Chair: Karen Sagomonyants Aja Aravamudhan Eric James Clarke Nelson Nathaniel Dymnt
1:45pm	2:30pm	Alumni Career Panel I-Ping Chen, DDS, PhD Jessica Costa, DMD, PhD Irene K. Reed, PhD



Stage-specific inhibitory effects of FGF2 on dentinogenesis in dental pulp

K. Sagomyants and M. Mina

University of Connecticut Health Center

Abstract:

Introduction: Odontoblast differentiation during physiological and reparative dentinogenesis depends upon multiple signaling molecules. Previous studies demonstrated that fibroblast growth factor 2 (FGF2) exerted both positive and negative effects on odontoblast differentiation. Our recent studies demonstrated that continuous exposure of pulp cells to FGF2 decreased their dentinogenic differentiation, however exposure to FGF2 during the proliferative phase of growth only significantly increased their dentinogenic differentiation. These results suggest that effects of FGF2 on odontoblast differentiation are stage-specific and/or are mediated through distinct signaling pathways.

Purpose: To gain insight into 1) effects of exposure of dental pulp cells to FGF2 during the differentiation/mineralization phase of growth, and 2) signaling pathways mediating effects of FGF2 on dentinogenesis.

Methods: Primary dental pulp cultures from P5-7 pups from various transgenic mice, in which GFP expression is under the control of promoters that are active at specific stages of odontoblast differentiation, were prepared and exposed to vehicle (control) and FGF2 (20 ng/ml) during the differentiation/mineralization phase of growth (between days 7-21). The effects of FGF2 on dentinogenesis were examined using a wide variety of assays.

Results: FGF2 significantly decreased mineralization and expression of Dmp1, Dspp, the percentage of the DSPP-Cerulean⁺ odontoblasts and expression of various GFP transgenes at days 14 and 21. These decreases were preceded by transient increases in expression of Dmp1 and Dspp and the percentage of the DSPP-Cerulean⁺ odontoblasts at day 10. Withdrawal of FGF2 almost completely recovered differentiation potential of pulp cells. FACS studies showed that FGF2 exerted biphasic effects on Dmp1 and Dspp in the 2.3-GFP⁺ population (committed progenitors), and continuously increased their expression in the 2.3-GFP⁻ population (undifferentiated cells). Inhibition of FGF/FGFR and MEK/Erk1/2 pathways reversed FGF2-induced decreases in mineralization and expression of markers of mineralization, especially Dspp (about 20- and 100-fold, respectively).

Conclusions: FGF2 affected dentinogenesis by targeting both undifferentiated cells and committed progenitors in the dental pulp, and through activation of the FGFR and MEK/Erk1/2 signaling pathways.

Acknowledgements:

This work was supported by R01-DE016689 & T90-DE022526 grants from National Institute of Health (NIDCR).

Biography:

8th year PhD student – Skeletal, Craniofacial and Oral Biology (SCOB) program

I'm planning to complete my PhD studies this year and start my Periodontology residency at the UConn Health Center. After completing the residency training, I'm planning to pursue the academic career being involved in clinical practice, research and teaching.

I chose UConn Health Center since there were researchers who conducted the studies I was interested in. In addition, I liked the location of the school and its excellent reputation in the dental research field.



Cbl-PI3K interaction is required for coupling osteoblast and osteoclast functions during fracture repair

¹Piccullo, V., ¹Soung, D., ¹Adapala, N.S., ³Morgan, E., ²Hansen, M., ¹Drissi, H., ¹Sanjay, A.

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²Center for Molecular Medicine, UCHC, Farmington, CT.

³Department of Mechanical Engineering, BU, Boston, MA.

Introduction: Studies have previously linked abnormal fracture healing to increased bone resorption and/or decreased bone formation. However, the molecular pathways that govern bone turnover during fracture repair remain elusive. We have previously shown that the E3 ubiquitin ligase and adaptor protein Casitas B-lineage Lymphoma (Cbl), binds the p85 regulatory subunit of PI3K to enhance osteoblast function while decreasing osteoclast activity during normal bone homeostasis^{1,3}. This was evidenced in vivo, using knock-in mice in which Cbl's interaction with PI3K was abrogated following the substitution of the tyrosine 737 with a phenylalanine (YF mice). These initial results indicated that the disruption of the Cbl-PI3K complex uncouples bone formation and bone resorption during normal bone remodeling. Thus, **we hypothesized that the Cbl-PI3K interaction may result in coupling osteoblast and osteoclast functions in fracture healing, and that abrogation of this interaction would enhance fracture healing.** To test this hypothesis, we performed fracture experiments using the YF mice compared to wild type (WT) control mice and temporally characterized the healing process in these animals.

Methods: Briefly, femurs of 7-9 week old female WT and YF mice were fixed with an intramedullary pin and mid-diaphyseal fractures were created via 3-point bending^{2,4,5}. Mice were sacrificed at 7 days post-fracture (d.p.f.) (WT n=10, YF n=7), 14 d.p.f. (WT n=10, YF n=10), 21 d.p.f. (WT n=18, YF n=19), 28 d.p.f. (WT n=5, YF n=9), and 35 d.p.f. (WT n=11, YF n=10) to study fracture healing by micro-computed tomography (μ CT), histology and histomorphometry, laser capture microdissection, serum collection, and/or torsional testing. Prior to sacrifice, all mice were x-rayed at each time point using a Faxitron. μ CT images were generated using a SCANCO machine and total callus volume and bony callus volume were measured excluding original cortical bone. Fractured femurs were formalin fixed and paraffin embedded for sectioning. Slides were stained with Safranin O/ Fast Green (SO/FG) for histomorphometry of callus and matrix areas. Osteoblast Surface/ Bone Surface (ObS/BS) and osteoclast number were measured from TRAP-stained slides with Alcian Blue and Hematoxylin counterstains using OsteoMeasure software. Laser capture was performed using the Arcturus PixCell II machine set on 100mW power. cDNA was prepared using ABI High Capacity cDNA Synthesis kit. qPCR reactions were done using SYBR Green Master Mix and custom designed primers on ABI Step One Plus Real Time PCR System. Serum analysis for P1NP was performed using the Rat/Mouse PINP EIA kit from Immunodiagnostic Systems. All parametric data were analyzed using the student t-test, while non-parametric data were analyzed using the Mann-Whitney U test. Significance was established at $p < 0.05$. This study was approved by the local governmental animal care committee and was conducted in accordance with the national legislation on protection of animals and the NIH Guidelines for the Care and Use of Laboratory Animals.

Results: Radiographic scoring of fractures over the course of healing showed enhanced fracture callus formation in the YF mice compared to controls. Remodeling of the cartilaginous callus was delayed in the YF mice compared to controls. We found that YF fracture calluses were 150% larger ($p < 0.05$) compared to WT (Figure 1), which resulted from 6-fold ($p < 0.05$) increased residual cartilaginous matrix as assessed by imaging and histomorphometric analyses. Prior to resorption of the bony fracture callus, the peak size of the callus and volume of bony matrix were increased in YF mice by at least 2-fold ($p < 0.05$) reflective of increased bone formation compared to WT mice (Figure 1). Further evaluation of the cellular composition of the fracture calluses revealed a significant 2-fold ($p < 0.05$) increase in the number of TRAP⁺ cells in the YF compared to the WT mice during the remodeling and resorption phases (Figure 2).

Molecular analysis of the remodeling phase by laser capture microdissection (LCM) followed by qRT-PCR supported this finding. We observed 1.7-fold ($p < 0.05$) increase in expression of TRAP, as well as 3.2-fold ($p < 0.05$) increase in Cathepsin K, and 1.5-fold ($p < 0.05$) increase in Calcitonin Receptor. Histomorphometric analysis of osteoblast surface over bone surface (ObS/BS) revealed a 37% ($p < 0.05$) increase during the remodeling phase, and a 25% ($p < 0.05$) increase during the resorption phase of YF calluses (Figure 3). As a potential explanation of enhanced bone formation, our molecular analysis also showed 2-fold ($p < 0.05$) increase in Osterix expression during both the initiation and remodeling phases, and 3.5-fold ($p < 0.05$) and 1.6-fold ($p < 0.05$) increase in Alkaline Phosphatase expression during the initiation and remodeling of the callus, respectively. Additionally, immunohistochemistry for Osterix expression in the fracture callus during the remodeling phase was markedly increased in YF callus sections compared to WT. Serum analysis of P1NP from mice in the initiation and remodeling phases of fracture healing showed a 30% (although not statistically significant) increase in YF samples over WT, which also supported the histomorphometric and molecular results. Finally, torsion testing of fractured femurs demonstrated a significantly higher work to failure in remodeling fracture calluses due to the increased bone volume, indicating that lack of Cbl-PI3K interaction results in increased strength of the healing femurs. Together, this body of data supports the notion that osteoclast function is impaired and osteoblast function is enhanced during fracture healing when the Cbl-PI3K interaction is abrogated, leading to enhanced fracture healing despite delayed remodeling.

Discussion: In conclusion, in the absence of Cbl-PI3K interaction, morphological, cellular, and molecular analyses of fracture healing demonstrate a role for this complex in the coupling of osteoblast and osteoclast function. This indicates that PI3K signaling can regulate osteoblast function, and this regulation may be occurring at the transcriptional level.

Significance: About 8 million fractures are reported in the U.S. every year, and approximately 10% of these cases experience impaired fracture healing due to complications that can result in excessive bone resorption, reduced bone formation, or a combination of both. The results of this study indicate that Cbl regulation of PI3K signaling in osteoblasts and osteoclasts is involved in coupling the functions of these cell types, and that abrogation of Cbl-PI3K interaction results in decreased bone resorption and increased bone formation. Therapeutically manipulating the Cbl-PI3K interaction to uncouple osteoblast and osteoclast function may prove to be an effective strategy for treating patients with impaired fracture healing.

Acknowledgements: This work was made possible with the support of members of the New England Musculoskeletal Institute. Funding was provided by the UConn Health Center, the National Institutes of Health grant AR055601, and the NIAMS Skeletal, Craniofacial & Oral Biology Training Grant 5T90DE021989-02.

Biography: Vanessa received two B.S. degrees in Molecular Cell Biology and Diagnostic Genetic Sciences from UConn, Storrs in 2006. She is currently a 5th year graduate student working on her PhD in Biomedical Sciences. Vanessa aspires to direct a diagnostic cytogenetic laboratory where she can help develop a research program to marry her interests in clinical cytogenetics and translational research. She chose UCHC for graduate school because it afforded her a great variety of experts and fields of research from which to choose for her thesis project. The collaborative community is an excellent environment to conduct a wide-range of experiments by utilizing the many core facilities on campus.

References:

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PTH Stimulates Canonical Wnt Signaling via Protein Kinase A in Primary Osteoblasts

T. Estus¹, S. Choudhary¹, and C. Pilbeam¹

¹New England Musculoskeletal Institute, University of Connecticut Health Center

Parathyroid hormone (PTH) acts systemically to modulate calcium levels and regulate bone homeostasis. Intermittently injected PTH is anabolic for bone and is FDA approved as therapy to treat osteoporosis. In contrast, continuous PTH is catabolic. We believe the switch from anabolic to catabolic occurs because continuous PTH *in vivo* induces continuous cyclooxygenase-2 (Cox-2) expression and prostaglandin E₂ (PGE₂) production. PGE₂ then acts on osteoclasts, also increased by PTH, to produce a factor that inhibits the anabolic response to PTH. The inhibitory factor is made *in vitro* by bone marrow macrophages (BMMs) from wild type (WT), but not Cox-2 knockout (KO), mice stimulated with RANKL to become osteoclasts. The inhibitory factor can be transferred in conditioned media (CM) from WT BMM cultures and shown to block PTH-stimulated osteoblast differentiation *in vitro*. The goal of this study is to test the hypothesis that the inhibitory factor blocks the osteogenic effects of PTH *in vitro* by inhibiting PTH-stimulated protein kinase A (PKA) signaling and, as a consequence, the phosphorylation of β -catenin, a critical component of the canonical Wnt signaling pathway. Primary osteoblasts (POBs) were isolated from calvariae of Cox-2 KO mice, cultured with CM from WT or KO BMMs and treated with PTH or vehicle. PTH increased cAMP production in the presence of KO CM but not in the presence of WT CM. PTH stimulated phosphorylation of β -catenin at both s552 and s675 sites in the presence of KO CM, marking β -catenin for nuclear translocation, whereas no difference was observed in the presence of WT CM. Addition of H89, a PKA inhibitor, inhibited the PTH stimulated β -catenin phosphorylation. To rule out effects of protein kinase C (PKC), which is also inhibited by H89, we treated with a specific PKC inhibitor and found no effect on β -catenin phosphorylation. We conclude that the inhibitory factor made by WT BMMs blocks PTH-stimulated β -catenin phosphorylation, and we speculate that some of the effects of this factor on osteoblast differentiation are due to inhibiting the Wnt signaling pathway.

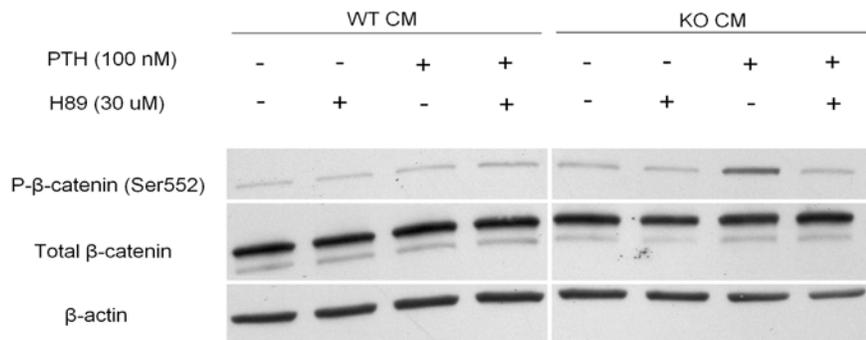


Figure 1. PTH phosphorylates β -catenin at Ser552 in a protein kinase A dependent manner. COX-2 KO POBs were pretreated with either WT or KO CM with the addition of +/- PKA inhibitor, H89 (30 μ M), for 2 hours. Cells were then treated with +/- 10 nM PTH for 15 minutes. Cell lysates were harvested and subject to western blotting.

Acknowledgements: This work was supported by NIH/NIAMS award number NIH-AR060286 and NIH/NIDCR Training Grant 1T90DE021989.

Biography: Thomas is a fourth year PhD student of Biomedical Engineering at the University of Connecticut. He received his bachelor's degree in Bioengineering from California Lutheran University. After receiving his PhD he hopes to become a nuclear propulsion officer aboard a submarine in the United States Navy, to ultimately pursue a career as an astronaut. He came to the University of Connecticut due to the unique, interdisciplinary research that the health center environment provides.



The Temporomandibular Joint as a Dynamic Cellular Structure: Response to Mechanical Loading

Achint Utreja, Xi Jiang, Nathaniel Dymont,

Max Villa, David Rowe

University of Connecticut Health Center

The generation of transgenic mice expressing green fluorescent proteins (GFPs) has greatly aided our understanding of the development of connective tissues such as bone and cartilage. Perturbation of biological systems within their adaptive remodeling capacity is particularly useful in analyzing cellular lineage progression. An analysis of the early fracture response in murine long bones utilizing GFP reporters has previously identified cells at different steps of lineage progression in the formation of the callus.

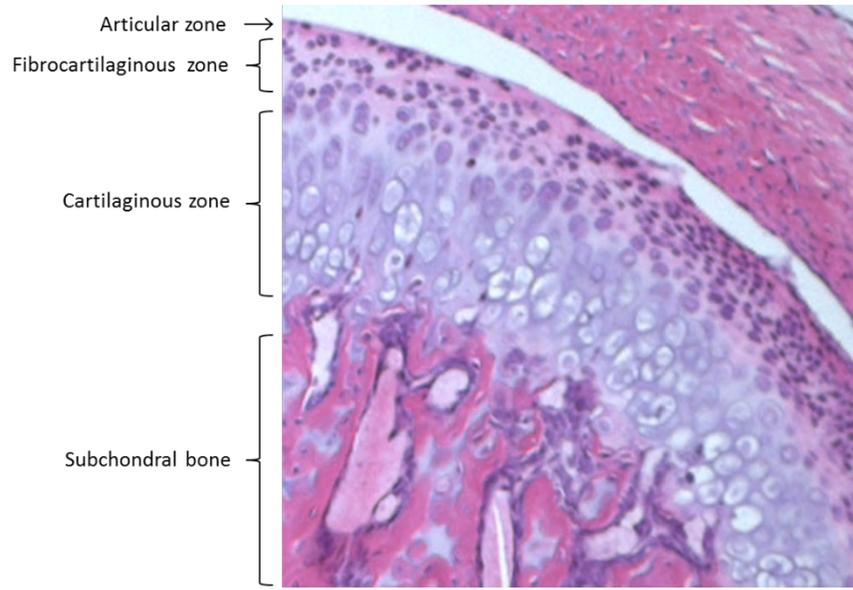
Objectives: To analyze if (i) similar to the early fracture callus, a pathway of lineage progression (Dkk3 → Col1(3.6) → Col2 → Col10) is seen in the temporomandibular joint (TMJ) fibrocartilage, and (ii) loading the joint alters the functional adaptational response of the cellular subpopulations within the condylar cartilage.

Methods: Four-week-old transgenic mice harboring combinations of fluorescent reporters (Dkk3-eGFP, Col1(3.6)-GFPcyan and Col10-RFPcherry) were used to analyze the expression pattern of the transgenes in the mandibular condylar cartilage. To study the effect of TMJ loading, animals in the experimental group were subjected to forced mouth opening with a custom spring exerting 50 grams force for 1 hour/day for 5 days whereas the controls did not receive any force. Alizarin complexone and EdU (a BrdU analog) were injected 24 hours prior to sacrificing the mice and serial sagittal and frontal cryosections of the non-decalcified mandibular condyle were compared to assess the differences between experimental and control groups.

Results: Dkk3 expression was seen in the superficial zone of the mandibular condylar cartilage, followed by Col1(3.6) in the mid-zone and Col10 expression at and below the tidemark. TMJ loading increased the thickness of all three zones and stimulated EdU-labeling within the Dkk3 zone. The thickness of the mineralized cartilage zone increased and Col10 expression extended above the tidemark.

Conclusion: The TMJ responds to loads by adaptive remodeling to form thicker cartilage. This is done by increasing the Dkk3 progenitor zone and expanding the Col10 mineralization zone.

Acknowledgements: This work was supported by NIH grant 1R01-AR052374.



Organization of the mandibular condylar cartilage

Biography:

B.D.S. (Bachelor of Dental Surgery)- Baba Farid University of Health Sciences, India

M.S. in Oral Sciences- University of Illinois at Chicago, Chicago, IL

Combined Ph.D. in Biomedical Science (Skeletal, Craniofacial and Oral Biology) and Orthodontics certificate- University of Connecticut Health Center (5th year)

I would like to find an appointment at an academic institution that allows me to continue basic research and work as an orthodontist. I chose UCHC as it offered a great skeletal biology research program and training experience that would allow me to develop as a researcher. At UCHC, I've been fortunate to learn research skills as well as critical thinking and analysis of the literature.



Wnt5a-mediated Specification of Human Articular Chondrocyte Differentiation into Maturationally-arrested Hyaline-like Chondrocytes

Jason D. Gibson, Rosa M. Guzzo, and Hicham Drissi

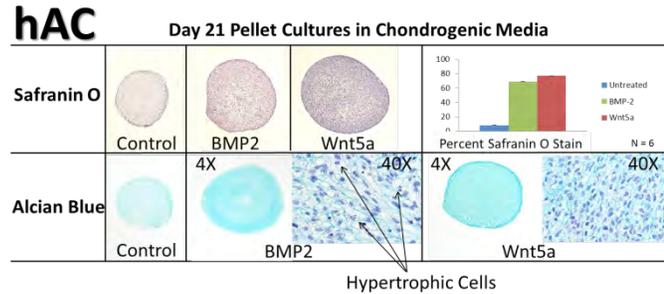
University of Connecticut Health Center, Farmington CT
Department of Orthopaedic Surgery; New England Musculoskeletal Institute

Objectives: The efficient repair of damaged cartilage is hindered by the limited capacity of cartilaginous tissue to self-regenerate. For large cartilage defects sustained from traumatic joint injury, a major treatment modality is autologous cell transplantation utilizing patient-derived articular chondrocytes. However, this procedure most often results in the formation of hypertrophic fibrocartilage rather than articular-like hyaline cartilage. Thus, identifying novel chondrogenic growth factors to control the differentiation of progenitor cells into maturationally-arrested hyaline-like chondrocytes is necessary to devise an effective cell-based strategy for repairing large articular cartilage damage. We and others have demonstrated a role for Wnt5a in the control of cartilage development and maturation through its non-canonical signaling pathway in murine mesenchymal stem cells. We therefore hypothesized that a single growth factor, Wnt5a, can induce the chondrogenic differentiation of progenitor cells while inhibiting their terminal maturation. In this study we investigated the chondrogenic and anti-hypertrophic effects of Wnt5a in the commitment of adult de-differentiated human articular chondrocyte-derived progenitors (hAC) and H9 human embryonic stem cell-derived MSC progenitors (H9-MSC) to a hyaline-like chondrocyte fate.

Methods: Human articular chondrocytes (hAC) were isolated from donated femoral condyle tissue via enzymatic digestion, and then expanded in adherent culture. H9 (WA09) human embryonic stem cells (hESC) were maintained on irradiated mouse embryonic fibroblasts and induced to become mesenchymal stem cell (MSC)-like progenitors via direct plating in defined MSC derivation media. The H9 hESC-derived progenitors (H9-MSC) were characterized by cell-surface expression profiling using flow cytometry, and by their ability to differentiate into the chondrogenic, adipogenic and osteogenic cell lineages. Chondrogenic induction of hAC and hESC-derived MSCs was achieved using high-density pellet cultures in serum-free media containing DMEM (Gibco) supplemented with 1% KSR, 1% ITS+, 50µg/mL ascorbic acid, 40µg/mL L-proline, 10⁻⁷M dexamethasone, 1mM sodium pyruvate, 1% NEAA, 2mM Glutamax, and 1% Pen/Strep. The temporal effects of Wnt5a treatment were compared to that of BMP-2, a widely used chondrogenic growth factor that promotes chondrocyte maturation, but does not limit hypertrophy. Histological assessments of proteoglycan matrix deposition were compared across treatment groups and quantitative RT-PCR custom gene array analyses were performed for comparison of transcriptional profiles.

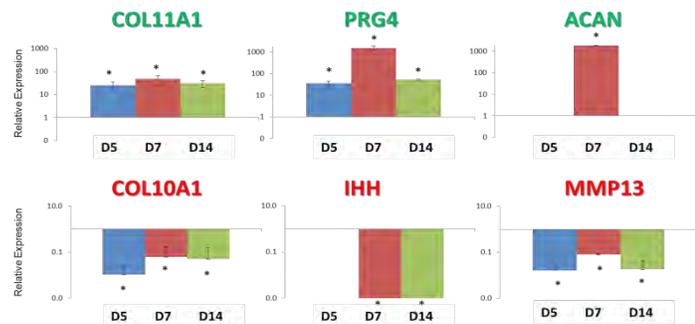
Results: Flow cytometry characterization revealed the H9-MSC progenitors have high similarities in cell-surface expression profiles when compared to that of hAC progenitors derived from healthy donors. These cells furthermore displayed the multipotential to differentiate into cartilage, fat, and bone cell lineages, and are additionally responsive to growth factor induction of chondrogenesis. We found that while the potent chondrogenic factor BMP-2 was able to induce differentiation of both these progenitor cell populations into the chondrogenic lineage it also induced their terminal maturation to hypertrophy. In contrast, Wnt5a treatment induced chondrogenic differentiation of both these progenitor cells while inhibiting their terminal maturation (Fig. 1).

Figure 1: Human Articular Chondrocyte (hAC) Pellet Culture Differentiation. Analysis of hAC progenitors cultured as high-density pellets in serum-free chondrogenic media with or without BMP-2 (100ng/mL), or Wnt5a (50ng/mL). Safranin O and Alcian blue staining showed proteoglycan deposition in both treatment groups (4X). Alcian blue staining with nuclear fast red depicts differences in cellularity across treatment groups (40X), exhibiting larger hypertrophic-like cells in BMP-2 treated pellets, but not in Wnt5a treated samples.



Quantitative RT-PCR results confirmed the temporal progression of the multipotent cell populations towards a chondrogenic phenotype in response to Wnt5a, and we established that Wnt5a not only maintained chondrogenesis while inhibiting chondrocyte hypertrophy, but also induced markers of articular cartilage matrix in both the adult hAC and the embryonic-derived H9-MSC progenitors (Fig. 2).

Figure 2: Quantitative gene expression analyses exhibit the effects of Wnt5a treatment on the chondrogenic differentiation of H9-MSC. Relative expression changes detected via qPCR for transcripts regulated by Wnt5a in hESC-derived MSC-like progenitor cells compared to untreated pellet cultures. Wnt5a has direct effects on the induction of articular chondrocyte markers COL11A1, PRG4 (lubricin), and ACAN, as well as the suppression of hypertrophic chondrocyte markers COL10A1, IHH and MMP13.



Significance: Identifying novel sources of cells and culture conditions that can yield large numbers of articular-like chondrocytes is critical for the development of effective cell-based approaches to regenerate cartilage of the joint. Identification of a single growth factor which can both induce chondrogenesis and inhibit hypertrophy in an unlimited source of progenitor cells will provide a potentially beneficial therapeutic option for large articular cartilage defect repair. Experiments are ongoing to examine the effects of Wnt5a in a translational model of cartilage repair using human osteochondral explants *ex vivo* and a rat chondral defect model *in vivo*.

Acknowledgements: This work is supported by the NIDCR PHS Grant No.5T90DE21989-3 to M. Mina, and the State of Connecticut DPH Stem Cell Grant No.11SCC01 to H. Drissi. The authors would like to thank the Musculoskeletal Transplant Foundation (MTF) for the donation of the human femoral condyle tissue used to isolate the human articular chondrocyte cell populations.

Biography:

Post-doctoral Fellow in Orthopaedic Surgery – 3rd year
 Ph.D. in Genetics and Genomics, University of Connecticut, Storrs, CT
 B.S. in Molecular and Cell Biology, University of Connecticut, Storrs, CT

It is my career objective to promote the advancement of research and education in the fields of molecular and cell biology, genetics and genomics, and translational stem cell biology for improving human health and disease management, and to serve as a mentor and role model for students in higher education to enhance the instruction of rising scientists. I chose a post-doctoral fellowship at UCHC based on our institution's advanced pursuit of medical technologies in the musculoskeletal field, as well as an exciting opportunity to perform translational research using embryonic stem cells to investigate potential cellular therapeutics for the treatment of musculoskeletal disorders. In my time here at UCHC I have learned a variety of techniques for the histological assessment of tissues and the characterization of cell types, and I have been privileged to train with orthopaedic residents to learn surgical models of cartilage defect repair.



Osteoarthritis in Mice Overexpressing High Molecular Isoforms of FGF2

Patience Meo Burt¹, Liping Xiao¹, Caroline Dealy², and Marja Marie Hurley¹

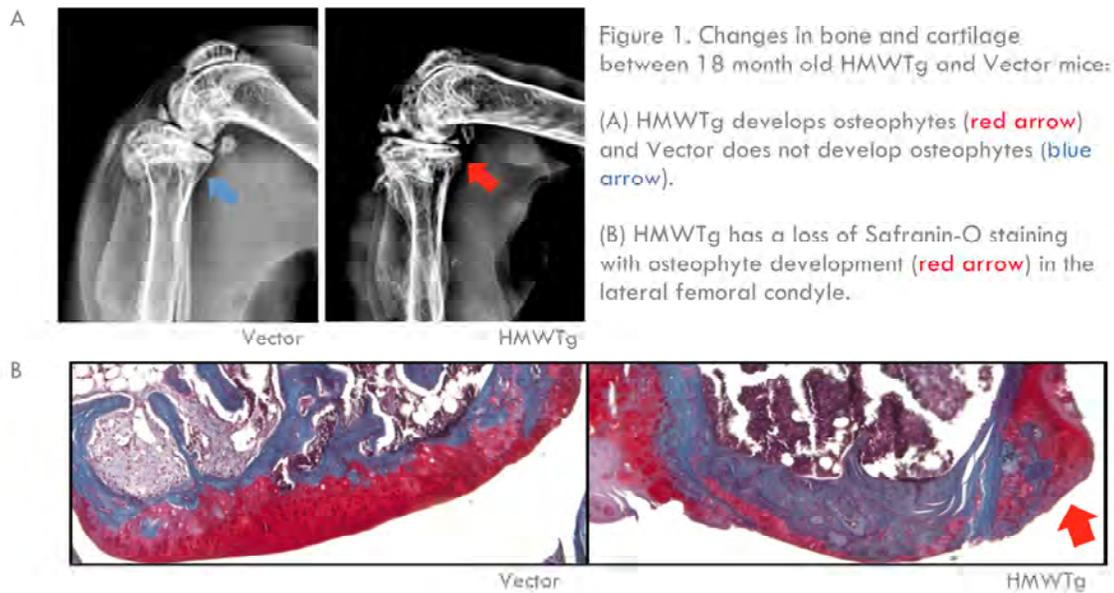
¹ Department of Medicine/Endocrinology, School of Medicine.

²Department of Craniofacial Sciences, School of Dental Medicine

Introduction: Osteoarthritis (OA) is a debilitating joint disease that affects over 27 million adults in the United States, characterized by the loss of articular cartilage and changes in the underlying bone. Currently, there is no permanent treatment for OA and a better understanding of the molecular mechanisms that lead to cartilage degradation will be useful in identifying therapeutic targets to help treat the disease. Patients with X-linked hypophosphatemic rickets (XLH) have an increase in fibroblast growth factor 23 (FGF23) in bone which result in hypophosphatemia, rickets/osteomalacia, as well as, osteoarthropathy, leading to problems associated with degenerative joint disease. Thus, FGF23 may play a role in OA development. We have developed novel transgenic mice overexpressing nuclear high molecular weight (HMW) isoforms of fibroblast growth factor 2 (HMWTg) in pre-osteoblasts. These mice phenocopy XLH since they have rickets/osteomalacia, hypophosphatemia, and increased FGF23 in serum and bone. We therefore hypothesized that mice overexpressing FGF2 HMW isoforms, in which FGF23 is increased, will develop more severe OA signs than Vector control mice at 18 months of age.

Methods/Materials: In order to assess for signs of OA, the knee joints from 18 month old HMWTg and Vector control male mice were collected. Digital x-ray imaging and microCT analysis were performed to visualize changes in bone. To determine articular cartilage integrity, histological analysis of proteoglycan expression by Safranin-O staining was performed. Finally, immunohistochemistry was used to establish the expression of OA markers, such as matrix metalloproteinase 13 (MMP-13), an enzyme which breaks down extracellular matrix components of cartilage and is typically increased during joint degeneration.

Results: Examination of the knees revealed that HMWTg mice had increased osteophyte formation and subchondral thickening, compared to Vector mice, as indicated by x-ray images. MicroCT analysis of HMWTg revealed that trabecular number in the tibia and femur was decreased, whereas, trabecular thickness and separation were increased while femoral, subchondral sclerotic bone was observed. By immunohistochemistry, Safranin-O staining was decreased and MMP-13 labeling was increased in the cartilage of knee joints of HMWTg mice compared to the Vector. These changes suggest that the osteoarthritic phenotype is more severe in the HMWTg mice.



Conclusion: Overall, these results suggest that overexpression of HMWFGF2 leads to signs of severe OA and offers insight into a molecular mechanism that causes the disease, while being a potential therapeutic target to treat it.

Acknowledgements: This work was supported by ROI NIDDK 098566-01A1, HD22610, and UCHC Biomedical Science Graduate Program.

Biography:

A.A. Liberal Arts and Science: Naugatuck Valley Community College, Waterbury, CT

B.S. Biology: Post University, Waterbury, CT

M.A. Biomolecular Sciences: Central CT State University, New Britain, CT

PhD in Biomedical Science; Skeletal, Craniofacial and Oral Biology – 1st year

As a first year student, I am currently exploring the various career options after graduation. I chose UCHC because it offers an incredibly translational program which combines laboratory research with health and medicine.



Investigating the role of BMP signalling on hepcidin expression in cancer cells

Manz D¹, Torti S.V²

Affiliations

¹ University of Connecticut

²Department of Molecular Biology and Biophysics, The University of Connecticut Health Center, Farmington, CT

Abstract: Hepcidin is a key protein involved in the regulation of intracellular and systemic iron. At the cellular level hepcidin promotes an elevation of intracellular iron levels by degradation of the transmembrane iron export protein ferroportin. Increased intracellular iron has been described in various cancer cells including breast, liver and prostate. In liver cells it has been established that Bone morphogenetic proteins (BMP) play a significant role in inducing hepcidin expression. Therefore our hypothesis is that BMPs, specifically BMP7 and BMP6, regulate iron homeostasis in breast and prostate cancer cells by modulating hepcidin transcript levels.

Methods: Breast and prostate cancer cells grown in vitro were treated with recombinant BMPs and/or a BMP signaling inhibitor (compound LDN – 193189). RNA was isolated from harvested cells and RT-PCR was used to evaluate relative levels of hepcidin transcript.

Results: Hepcidin transcripts were not significantly induced in MDA-MB-231 breast cancer cells treated with BMP6 or BMP7, and endogenous levels of hepcidin were not lowered by treatment with the BMP inhibitor. DU145 prostate cancer cells showed an increase in hepcidin in response to BMP7, but were only modestly responsive to the BMP inhibitor.

Conclusion: These results suggest that novel mechanisms may regulate hepcidin synthesis in cancer cells. Indeed, subsequent research has revealed that MDA-MB-231 breast cancer cells are more responsive to Interleukin-6 mediated regulation of hepcidin than BMP regulation; other breast cancer cell lines however, such as MCF-7 cells, may be more responsive to BMPs and thus warrant further study.

Acknowledgements: This work was supported by grant R01-CA171101 from the National Cancer Institute, National Institutes of Health. David was supported by grant NIH T90-DE021989-02.

Biography: David graduated from the University of Connecticut with a major in biological Sciences and a minor in business. He is currently in his second year of the combined D.M.D./Ph.D. program at the University of Connecticut Health Center. Being a former intern at the University of Connecticut Health Center, David felt very comfortable with the culture and opportunities available here. David aspires to integrate clinical practice with advancing knowledge to innovate and ultimately to provide patients with better care.



Using hESCs as an Isogenic Model to Investigate the Epigenetic Regulation of FKBP5

Evan Yu, Kristen Martins-Taylor and Marc Lalande

University of Connecticut Health Center

Objective: FKBP5 is a protein associated with predisposition for PTSD after traumatic experiences through long-term epigenetic modifications involving its associated gene, *FKBP5*. *FKBP5* contains a SNP (rs1360780) with two alleles, A and G. Patients with the homozygous A allele are at a higher risk for PTSD than patients with the homozygous G allele. The goal of this study was to create isogenic hESC lines for the homozygous risk and protected variants. The lines could then be differentiated into neural progenitors to study the effects of simulated stress on the epigenetics and gene expression of FKBP5.

Methods: TALENs were used to create double-stranded breaks near *FKBP5* rs1360780 in H9 hESC lines. A plasmid was used for homologous recombination to insert a neomycin cassette flanked by LoxP and either A or G at the SNP to generate homozygous lines A1L and G3L. The lines were transfected with a plasmid with EGFPcre. After transfection, fluorescent colonies were isolated to acquire colonies that had undergone intracellular recombination at the LoxP sites to remove the neomycin cassette.

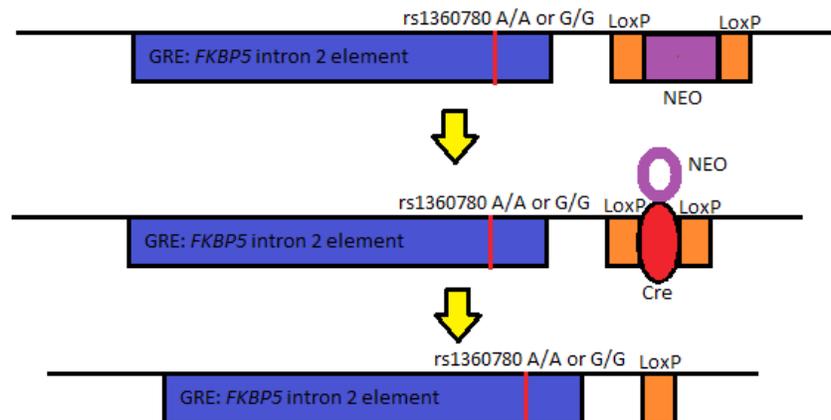


Figure 1: Model of Cre recombinase catalyzed intracellular recombination to excise the neomycin cassette in A1L and G3L lines.

Regions flanking the LoxP and neomycin cassette were PCR amplified to screen for neomycin cassette removal.

Results: The neomycin cassette was removed in one clone of G3L and A1L. Using Cre recombinase to remove the neomycin cassette left a 34 base pair LoxP site within the recombined cell lines so they are not completely isogenic.

Conclusions: With near isogenic lines obtained, we plan to differentiate them into neural progenitors for use as an in-vitro model of developing human brains. To simulate traumatic experiences, we plan to treat the neural progenitors at different stages of development with dexamethasone and assay for changes in *FKBP5* methylation and gene expression. The A1L and G3L lines were not completely isogenic because one LoxP site remains. An alternative method to make completely isogenic lines would be to use a CRISPR reaction and direct Cas9 nuclease to create double stranded breaks around the both LoxP sites to be removed by non-homologous end joining.

Acknowledgements: This work was supported by Dr. Lalande's endowment; NIDCR; T90DE021989-03

References:

Klengel, T, et al. "Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions." *Nature Neuroscience* 2013 Jan;16(1):33-41. doi: 10.1038/nn.3275. Epub 2012 Dec 2.

Biography:

B.S. Biochemistry: Boston College, Chestnut Hill MA
DMD/PhD in Skeletal, Craniofacial and Oral Biology – 1st year

After completing this program, I would like to do a residency in a dental specialty and gain an appointment within a department at a dental school. The division of my time as dental school faculty, between teaching, clinical work, and research will depend on my ability to develop as a scholar and researcher while at UCHC and in my future career. I chose UCHC because it provides its dental students with a comprehensive didactic background in medicine and strong mentorship in the realm of dental research in addition to foundational dental clinical skills training.



Expression of DMP1-mCherry/DSPP-Cerulean Transgenes in Primary Pulp Cultures

Anushree Banerjee, Karen Sagomyants, Barbara Rodgers, Peter Maye and Mina Mina

School of Dental Medicine, University of Connecticut Health Center

Transgenic mouse lines in which Green Fluorescence Protein (GFP) expression is under the control of tissue and stage specific promoters have provided powerful experimental tools for identification and isolation of cells at specific stages of differentiation in many lineages including odontoblast lineage. However the available animal models express reporters in both odontoblasts and osteoblasts making it a challenge to delineate the two cell types in dental pulp cultures. We have generated a DMP1-mCherry and DSPP-Cerulean double transgenic animal that allows identification and isolation of odontoblasts at later stages of differentiation and provides a way to distinguish between odontoblasts and osteoblasts.

Objectives: To characterize the expression of DMP1-mCherry and DSPP-Cerulean transgenes during the mineralization of primary pulp cultures.

Methods: Primary cultures derived from the coronal portion of dental pulp from DMP1-mCherry/DSPP-Cerulean double transgenic animals were used to examine the stage-specific activation of these transgenes during in vitro mineralization and to examine the changes in the percentage of cells expressing these transgenes (GFP+) at various time points by FACS analysis.

Results: Primary pulp cultures were composed of a heterogeneous group of cells with respect to cell morphology and expression of DMP1-mCherry. A small population expressed low level of DMP1-mCherry prior to mineralization. The number of DMP1-mCherry+ cells increased with time in cultures and the appearance of late markers of odontoblast differentiation. There was also a close correlation between the areas of the cultures expressing DMP1-mCherry and areas of mineralization identified by Calcein staining. DSPP-Cerulean expression appeared around day 10-11 in DMP1-mCherry+ cells in the mineralized nodules with increases thereafter.

Conclusions: In our mouse model, the expression of DMP1-mCherry and DSPP-Cerulean transgenes mimicked the expression of endogenous *Dmp1* and *Dspp*

transcripts and thus, presents a potential tool to distinguish odontoblast and osteoblast cell lineages in dental pulp culture.

Acknowledgements: Supported by Grant R01-DE016689.

Biography:

BDS, Mumbai India

MS, University of Medicine & Dentistry New Jersey

PhD in Biomedical Sciences, Skeletal Craniofacial and Oral Biology (SCOB) - 2nd year

I aspire to become a proficient clinician-scientist and the PhD program at University of Connecticut provides an excellent environment to train and work with accomplished minds from diverse backgrounds that will help me achieve my professional goals.



Collagen Hydrogel as Synthetic Three Dimensional Environment for studying bone and brain cells under Mechanical Stimulation through Ultrasound

Fayekah Assanah, James Veronick, Alexandra Nicaise, Steve Crocker and Yusuf Khan

University of Connecticut Health Center

Objectives: Various tissue engineering approaches, including those for bone and neural regeneration [1, 2], utilize cells encapsulated within a collagen hydrogel to understand how a three dimensional (3-D) environment can influence cell behavior. Our work seeks to expand this approach by incorporating low-level mechanical forces as both a stimulator of repair and a mimic of pathology. Using ultrasound, a source of such mechanical energy that is delivered as acoustic pressure waves, we can deliver very low-level forces in a spatially and temporally controlled manner [3]. We have also designed collagen tissue mimetics of varying mechanical properties to simulate both injectable scaffolds (bone) and pathological tissue states (neural).

Methods: In order to conduct cell studies, mechanical properties (storage and loss modulus) of various collagen hydrogels (ranging from 1mg/mL - 5mg/mL: resembling both injectable bone scaffolds and brain tissue mechanics) were tested through rheology. A Type I collagen hydrogel (1mg/mL) was used to encapsulate osteoblast precursor (MC3T3) cells and subsequently exposed to low-intensity pulsed ultrasound (LIPUS) for 1, 3, and 7 days for 20 minutes/day. qRT-PCR was used to quantify the mRNA expression of osteogenic markers. Similarly, 2-D cultures of primary astrocytes were exposed to different intensities of low intensity sinusoidal ultrasound for 5 minutes on the 5th day of culture. ELISA, immunostaining and qRT-PCR was used to identify cell behavior and response to such mechanical stimuli.

Results: Rheology testing showed that with increasing concentration of collagen, the storage and loss modulus of the hydrogel increases. Osteoblasts encapsulated in 3-D collagen hydrogel when exposed to LIPUS showed varied biochemical responses. Ultrasound application to astrocytes in 2-D cultures appeared to influence overall cell morphology.

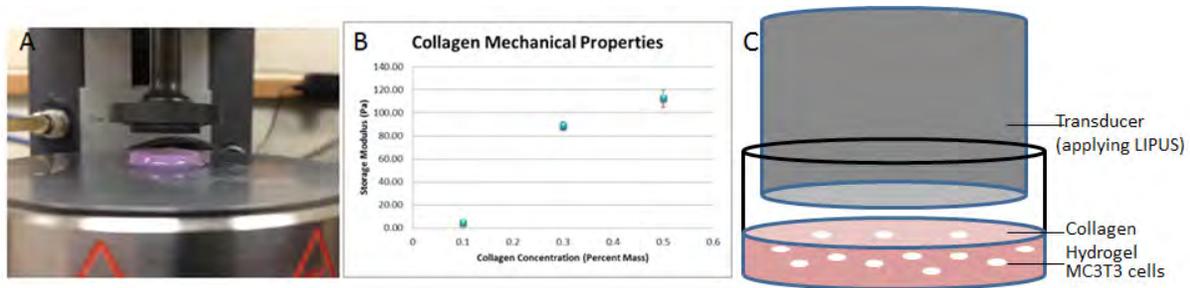


Figure 1: (A) Mechanical testing of collagen hydrogel through rheology. (B) Storage Modulus of hydrogel increases with collagen concentration. (C) Set-up of the hydrophone applying LIPUS to cells encapsulated in 3D collagen hydrogel.

Conclusions: Through this study we have established a method of delivering low-level mechanical stimulation (via ultrasound) to cells on 2-D surfaces and suspended in 3-D collagen hydrogel. It is seen that low levels of ultrasound is able to stimulate structural changes in astrocytes in 2-D cultures and influence biochemical signaling in osteoblasts in 3-D cultures.

References:

- [1] Ferreira A. M, Gentile P, Chiono V, Ciardelli G, Collagen for bone tissue regeneration, Acta Biomaterialia 2012.
- [2] Suri S, Schmidt C. E, Cell-Laden Hydrogel Constructs of Hyaluronic Acid, Collagen, and Laminin for Neural Tissue Engineering, Tissue Engineering 2010.
- [3] Mundi R, Petis S, Kaloty R, Shetty V, Bhandari M, Low-intensity pulsed ultrasound: Fracture healing, Indian J Orthop 2009.

Biography:

B.S. Electrical Engineering, University of Virginia, Charlottesville VA
 M.S. Biomedical Engineering, New Jersey Institute of Technology, Newark NJ
 PhD in Biomedical Science; Skeletal, Craniofacial and Oral Biology – 2nd year

I am a second year PhD candidate in Biomedical Sciences Program at UCHC. My research interest lies in translational research and I am inclined to accomplish my Doctorate Degree in the field of Biomaterials/Biomechanics and Tissue engineering. The fundamental knowledge obtained in this research would ultimately render me a higher platform to pursue my career as a scientist in research labs. I chose UCHC as my degree institute because it offers a diverse research background particularly in the field of translational research and medicine. I am sure that my exposure to such research and working with the reputed faculty at UCHC would be indispensable towards my career growth.



The fungus among us.....

Studies on the mycobiome of the human mouth.

Linda D. Strausbaugh, PhD
Professor of Genetics & Genomics
Department of Molecular and Cell Biology
University of Connecticut

Fungi are a large, complex group of organisms that occupy every conceivable environmental niche. They are opportunistic pathogens that are increasingly recognized as emerging threats. While a large amount of knowledge has been accumulated about bacteria in the mouth, fungal communities remain relatively poorly understood. As an entry point into the airways and gastrointestinal tract, fungi in the mouth are relevant to several biocompartments. We have surveyed the human oral mycobiome using massively parallel, high throughput sequencing of internal transcribed spacer 1 (ITS1) amplicons from saliva, following a new and robust extraction method. We also developed and employed improved methods for sequence-based taxonomy assignments and nomenclature to describe the fungal genera present in the healthy human mouth. In comparison with the other two similar metagenomic studies, and several earlier culture-based ones, our findings change the current conception of the oral mycobiome, especially with the discovery of the high prevalence and abundance of the genus *Malassezia*. Previously identified as an important pathogen of the skin, and recently reported as the predominant fungal genus at the nostril and backs of the head and ear, ours was the first finding of *Malassezia* in the human mouth. Findings from this study were in good agreement with others on the existence of many consensus members of the core mycobiome, and on unique patterns for individual subjects. Preliminary findings from studies on the mycobiome of chemotherapy patients suggest that differences in the compositions of fungal communities are associated with the development of oral lesions. The results indicate that a balance between different members in the fungal community might be an important tipping point between healthy and diseased states. Our project has provided a roadmap for enhancing the likely biological relevance of sequence-based fungal surveys, and built the foundation for understanding the role of fungi in health and disease of the oral cavity.

Biography

Education

B.S. with Honors, Wright State University, 1972, *summa cum laude*

Ph.D. (Genetics), Wesleyan University, 1977

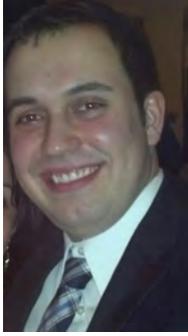
Post-doctoral, the Johns Hopkins University, the University of Pennsylvania, 1977-80

Linda Strausbaugh's research interests are in genome evolution and DNA identity typing for biomedical, forensic and ancestry applications. She designed, obtained funding to create, and directed for a decade, UConn's Center for Applied Genetics and Technology, a state-of-the-art facility supporting integrated genomics research and education. Her research has been funded by the NSF, NIH, NIJ, and Alfred P. Sloan Foundation. Strausbaugh's current research focuses on the use of Next Generation sequencing methods to investigate the roles of fungal communities in health and disease in the oral cavity. Strausbaugh has been active in several scientific societies and previously served terms in the elective office of Executive Secretary for the Society for Molecular Biology and Evolution and Secretary of the American Genetic Association.

At the State level, Strausbaugh was a member of the Governor's Crime Lab Working Group and participated in the recruitment of the Jackson Lab facility to the University of Connecticut. She has also been involved in the genetic perspectives on Venture Smith, a formerly enslaved African who became a land owner and respected resident of Connecticut. Strausbaugh's groups also typed a segment of skull purported to be that of Adolf Hitler. The latter two projects were featured in television and news reports worldwide.

Professor Strausbaugh is recognized locally and nationally as an education innovator; she was a finalist in the 2010 Connecticut Women of Innovation competition in the category of Academic Leadership and Innovation. She was named a 1997 Teaching Fellow of the University of Connecticut, was awarded the 2009 Alumni Association Award for Excellence in Teaching at the Undergraduate Level, and was a 2010 Top Nominee for the national Robert Foster Cherry Award for Great Teaching. She is active in local and national diversity initiatives and was named a 1998 NEBHE Faculty Mentor of the Year. Dr. Strausbaugh has served as mentor and research supervisor to dozens of undergraduate and graduate students.

She conceived of, and directed for 13 years, the Professional Science Masters in Applied Genomics and works routinely with dozens of biotechnology, genomics and pharmaceutical companies. The Applied Genomics PSM program won a Silver Prize from the 2006 Connecticut Quality Improvement Awards, was featured in several prominent journals (e.g. *Science*, *Nature Medicine*, *Science Careers*). Strausbaugh chaired and was a member of the Council of Graduate School's PSM National Advisory Board.



miR-433 Regulation of Osteoblast Circadian Rhythm and TGF β Signaling

Spenser S. Smith, Tiziana Franceschetti, Neba Dole, Catherine Kessler, Anne M. Delany

Center for Molecular Medicine, University of Connecticut Health Center

miRNAs are small non-coding RNAs that primarily function to down regulate the expression of target mRNAs; and several lines of evidence suggest that miR-433 has negative effects on osteoblast differentiation. For example, miR-433 is expressed at a higher level in bone marrow stromal cells from a low bone mass mouse, C57Bl/6, compared with a high bone mass mouse C3H/HeJ. In human fetal osteoblastic cells (hFOB1.19), expression of miR-433 decreased as osteoblastic differentiation progressed. Functionally, knockdown of miR-433 increased osteoblastic differentiation and collagen synthesis.

To investigate the molecular mechanism for miR-433 effects on osteoblastogenesis, we used a bioinformatic approach and determined that miR-433 potentially targets several positive regulators of TGF β signaling. Using a TGF β -responsive luciferase reporter, we demonstrated that miR-433 inhibits TGF β signaling. Using luciferase-3' UTR reporter assays and Western blot analysis, we are examining whether miR-433 targets SMAD2, SMAD4, TGFBR1, and TGFBR2. Since miRNAs and their targets often participate in regulatory loops, we tested the effect of TGF β on miR-433 levels and found that TGF β decreases miR-433 expression. Intriguingly, we also found that miR-433 is regulated in a circadian manner in vivo. Circadian regulators have a profound impact on bone mass, and we are determining the role of miR-433 in the circadian rhythm in vitro and in vivo. Overall, miR-433 appears to be an important regulator of osteoblast function and differentiation, and there is still much to learn about its impact on skeletal tissue.

Acknowledgements: This work is supported by NIH/NIAMS AR44877 and the Skeletal, Craniofacial, and Oral Biology Training Grant.

Biography: I graduated from The College of Idaho in 2008 with a B.A. in Biology and History. I am currently a third year Ph.D Biomedical Science student in Dr. Anne Delany's lab in the Skeletal, Craniofacial, and Oral Biology Area of Concentration. I was attracted to UCHC because of the bone research program. My career aspirations are to obtain a position in academia as a professor teaching as well as researching bone biology.

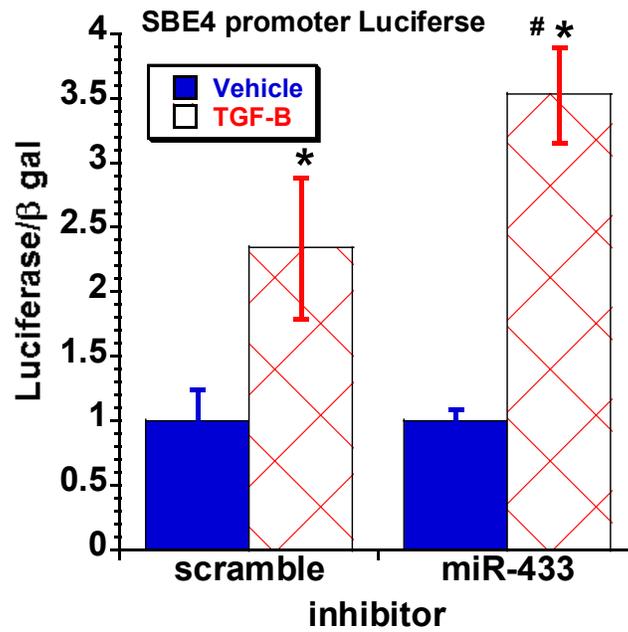


FIG. 1. Examination of miR-433 regulation of TGF β signaling. Human fetal osteoblast cells (hFOB) were transfected with the SBE4 luciferase reporter construct, a synthetic promoter containing SMAD 3/4 binding elements. hFOB cells were transfected with either a scramble or miR-433 inhibitor and treated with vehicle or 5ng/ml TGF β for 24 hours. Cells were measured for luciferase activity and normalized to β -galactosidase. *, Significantly different from vehicle, $p < 0.05$. #, Significantly different from scramble TGF β , $p < 0.05$.



A single nucleotide polymorphism in osteonectin 3' untranslated region regulates bone volume and is targeted by miR-433

Neha S. Dole, M.S.¹, Kristina Kapinas, Ph.D.¹, Catherine B. Kessler, B.S.¹, Siu-Pok Yee, Ph.D.², Douglas J. Adams, Ph.D.³, Renata C. Pereira, Ph.D.⁴, and Anne M. Delany, Ph.D.¹

¹Center for Molecular Medicine, UConn Health Center

²Gene Targeting and Transgenic Facility, UConn Health Center

³Department of Orthopaedic Surgery, UConn Health Center

⁴Mattel Children's Hospital, University of California, Los Angeles

Osteonectin is an extracellular matrix protein that regulates collagen fibril organization and promotes osteoblast differentiation. Osteonectin-null and haploinsufficient mice are osteopenic. Previously, haplotypes consisting of 3 SNPs in the 3' untranslated region (UTR) of osteonectin were associated with bone mass in a cohort of men with idiopathic osteoporosis (1). Specifically, haplotype A (1046C_1599G_1970T) was found at a higher frequency in severely affected osteoporosis patients, whereas haplotype B (1046C_1599C_1970T) was found at a higher frequency in less affected patients and healthy controls. We postulated that SNP 1599G/C (rs1054204) in the osteonectin 3' UTR may affect bone mass and modulate osteonectin expression.

To elucidate the role of SNP 1599 in regulating gene expression, luciferase reporter constructs containing haplotype A or B osteonectin-3'UTR were transfected into hFOB1.19 cells (human fetal osteoblast cell line). Our results demonstrated that SNP 1599G/C affects gene regulation, and that haplotype A has a repressive effect on gene expression compared to B. Bioinformatics revealed a novel potential microRNA-433 (miR-433) binding site at SNP1599G in haplotype A-3'UTR. Using haplotype A and B luciferase reporter constructs, we found that miR-433 inhibitor relieved repression of the haplotype A, but not B, 3' UTR reporter construct. This suggests that miR-433 differentially targets the haplotype A and B 3' UTR.

To determine the impact of SNP 1599 on osteonectin expression and bone mass in vivo, a knock-in approach was used to replace the mouse osteonectin 3' UTR with human haplotype A or B 3' UTR. Bones from mice bearing haplotype A contained less osteonectin compared to B. The bone phenotype of mice homozygous for haplotype A and haplotype B was analyzed by μ CT and histomorphometry. Haplotype B mice had higher bone formation rate and gained more trabecular bone from 10 to 20 weeks of age compared to haplotype A. When parathyroid hormone was administered intermittently, haplotype B mice gained more cortical bone area than A. Altogether, this indicated that in comparison to haplotype B, haplotype A mice have lower bone osteonectin, decreased bone formation and are less responsive to the anabolic effect of intermittent PTH.

Overall, we assigned a physiological function to a common osteonectin allele, providing support for its contribution to the complex trait of skeletal phenotype.

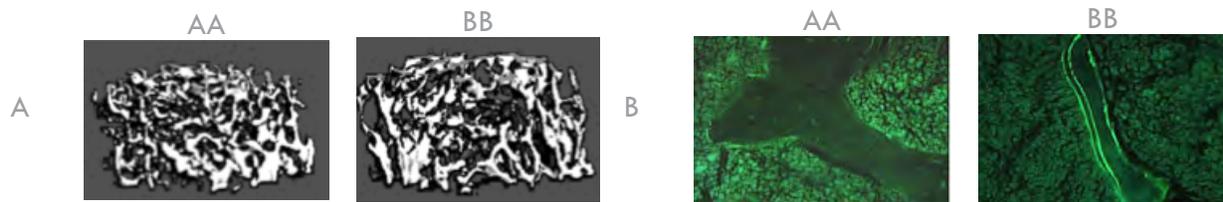


Figure 1: (A) Representative microCT images from femora of 20-week old haplotype A and B knock-in mice. (B) Representative histomorphometry images of Calcein double-labeled bone forming surfaces in femoral trabecular bone from 14-week old haplotype A and B knock-in mice.

Acknowledgements: This work was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health under Award Number AR044877 (to Dr. Anne Delany).

References: 1. Delany AM, McMahon DJ, Powell JS, Greenberg DA, Kurland ES (2008) Osteonectin/SPARC polymorphisms in Caucasian men with idiopathic osteoporosis. *Osteoporos Int.* 19(7): 969-78.

Biography:

B.Tech. Biotechnology: Dr. D.Y Patil Institute of Biotechnology and Bioinformatics, Mumbai, India.

M.S. Biotechnology: Texas Tech University, Lubbock, TX.

PhD in Biomedical Science; Skeletal, Craniofacial and Oral Biology - 4th year

I am interested in pursuing a career in an academic setting, but I am open to working in a science-based industry. I intend to continue my future research in the bone biology field to gain a better insight into the molecular mechanism underlying the bone diseases. I chose UConn Health Center as it has one of the best skeletal biology programs in the US.



miRNA-29 Promotes Osteoclastogenesis Through Regulation of Osteoclast Commitment and Migration

*Tiziana Franceschetti, Ph.D.¹, Catherine B. Kessler, B.S.¹,
Sun-Kyeong Lee, Ph.D.², & Anne M. Delany, Ph.D.¹*

Affiliations

¹ Center for Molecular Medicine,

² Center on Aging, University of Connecticut Health Center

Osteoclast differentiation is complex and tightly regulated at multiple levels. microRNAs (miRNAs) are key post-transcriptional regulators of gene expression. Although disrupted miRNA processing results in osteopetrosis, the function of specific miRNAs in osteoclasts is elusive.

We analyzed the role of the miR-29 (a/b/c) family in osteoclast differentiation, using primary cultures of mouse bone marrow-derived macrophages. Retroviral-mediated miR-29a knock-down decreased osteoclast formation and size. qRT-PCR showed that expression of the miR-29 family increased during osteoclast differentiation, in concert with mRNAs for TRAP and Cathepsin K. Similar regulation was observed in the monocytic cell line RAW264.7.

To determine the function of miR-29 in osteoclastogenesis, we used as a model stably transduced RAW264.7 cells expressing a doxycycline (DOX)-inducible miR-29 competitive inhibitor, or miR-29 “sponge” construct. miR-29 knock-down impaired osteoclastic commitment and migration of pre-osteoclasts. However, miR-29 knock-down did not affect cell viability, actin ring formation, or apoptosis in mature osteoclasts.

To better understand how miR-29 regulates osteoclast function, we validated miR-29 target genes using Luciferase-3' UTR (untranslated region) reporter assays and specific miR-29 inhibitors. miR-29 negatively regulated RNAs critical for cytoskeletal organization, including Cell Division Control protein 42 (Cdc42) and SLIT-ROBO Rho GTPase activating protein 2 (srGAP2). Moreover, miR-29 targeted RNAs associated with the macrophage lineage: G protein-coupled receptor 85 (GPR85), Nuclear Factor I/A (NFIA), and CD93. In addition, Calcitonin receptor (CTR), which regulates osteoclast survival and resorption, is a novel miR-29 target.

Thus, miR-29 is a positive regulator of osteoclast formation, and targets RNAs important for cytoskeletal organization, commitment and osteoclast function. We hypothesize that miR-29 controls the tempo and amplitude of osteoclast differentiation. An expanded understanding of how miRNAs regulate osteoclast formation and activity may lead to new therapeutics for skeletal disease.

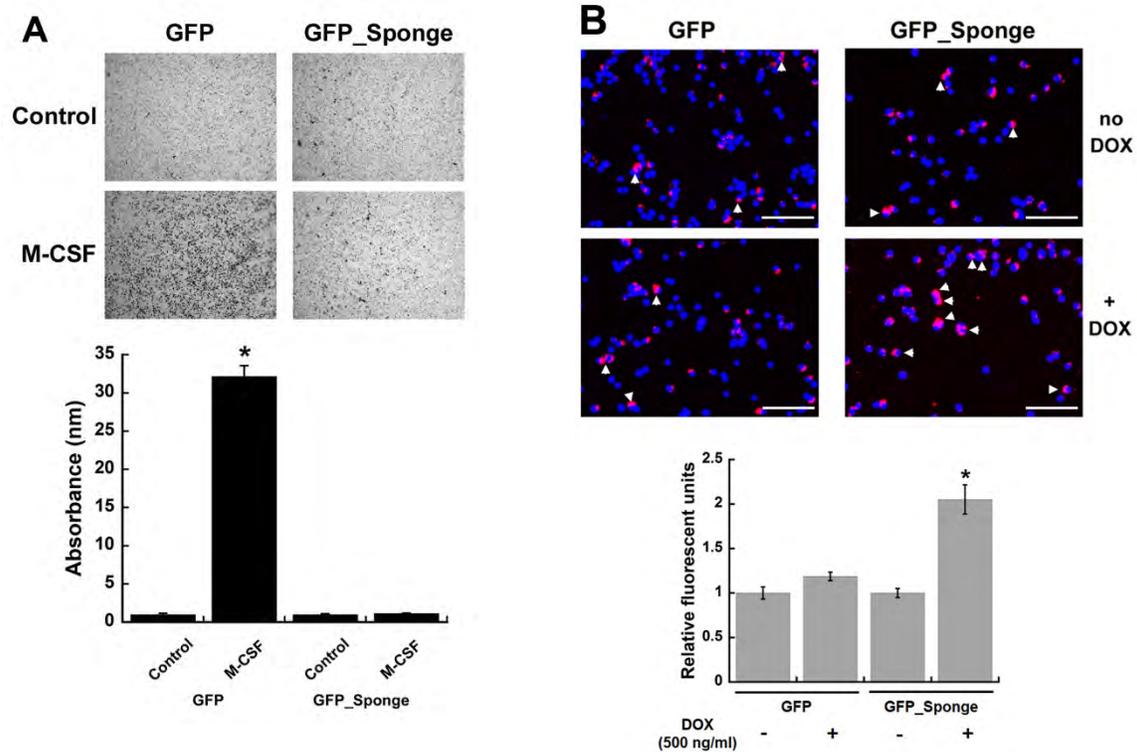


Figure 1. Effects of miR-29 knock-down in the RAW264.7 cell line. *A*, Inhibition of miR-29 activity impairs migration of RAW264.7 cells toward M-CSF. *B*, Knock-down of miR-29 increases RAW264.7 phagocytosis and commitment to the macrophage lineage. * $p < 0.05$

Acknowledgements: This work was supported by Grant Number AR44877 (AMD) from NIAMS/NIH.

Biography:

Previous education:

2005 B.S. University of Ferrara (Italy)

2007 M.S. University of Ferrara (Italy)

2014 Ph.D. University of Connecticut Health Center

I recently graduated from the Ph.D. program in Biomedical Science. I would like to pursue a career as an independent investigator in academia or in industry. During my doctoral studies at UCHC, I had the opportunity to develop my project independently, but I was also exposed to topics of skeletal biology that were not directly involved in my thesis project, thanks to the continuous interaction with other laboratories of the strong bone research group at this University.



Polysaccharide-based Micro-Nano-Structures for Bone Repair and Regenerative Applications

Aja Aravamudan^{1,2,5}, Daisy Ramos^{1,2,3,4}, Matthew Harmon^{1,2,3,4}, and Dr. Sangamesh G. Kumbar^{1,2,3,4}

1. Institute for Regenerative Engineering, University of Connecticut Health Center

2. Raymond and Beverly Sackler Center for Biological, Physical and Engineering Sciences

3. Department of Orthopaedic Surgery, University of Connecticut Health Center

4. Department of Chemical, Materials and Biomolecular Engineering, University of Connecticut

Objectives: Approximately half a million individuals suffer from fractures every year and the demand for bone graft procedures are on a continuous increase. A variety of synthetic biodegradable polymers have been used to fabricate scaffolds for bone repair. However, their utility in transient biomedical applications such as implants is hampered due to acidic degradation products that can adversely affect the biocompatibility. Cellulose is a natural polymer forming the extracellular matrix of plants possessing both high mechanical strength and biocompatibility. We have fabricated three-dimensional porous sintered microspheres and collagen nanofiber functionalized composite scaffolds using a cellulose derivative for load bearing applications. The goal of this study was to determine whether these scaffolds have osteoinductive properties using human bone marrow derived mesenchymal stem cells (hMSCs).

Methods: In order to determine the osteoinductive potential of the Cellulose acetate (CA) and Cellulose acetate-collagen (CA-col) scaffolds, we cultured hMSCs on these scaffolds. We contrasted the differentiation of seeded hMSCs on these CA, CA-col scaffolds against poly (lactic-co-glycolic acid) (PLGA) and poly (lactic-co-glycolic acid)-collagen (PLGA-collagen) scaffolds. DNA content, alkaline phosphatase (ALP) content, and osteogenic gene expression [Runx2, Collagen 1A1 (Col1A1), Osteopontin (OPN) and Bone Sialoprotein (BSP)] were measured on these scaffolds and compared with each other.

Results: DNA content, reflecting cellular proliferation was similar between most of the scaffold groups. However, the collagen coated scaffolds showed increased DNA content, due to increased surface area and biomimetic property of the collagen nanofibers. Enzyme alkaline phosphatase (ALP) activity was maintained higher on CA and CA-col groups (Figure 1) even during the later time points. Runx2 expression, under osteoinduction was maintained at significantly high levels on the CA-col scaffolds than the other groups, indicating that the cells on CA-col scaffolds have a greater osteogenic potential. Late osteogenic gene BSP showed higher expression on CA-col scaffolds, indicating greater long-term osteoinduction on these scaffolds (Figure 1). This data suggests that the CA and CA-col scaffolds facilitate greater osteogenic progression of seeded hMSCs. While our previous results had established the mechanical competence and the osteoconductive ability of the CA and CA-col scaffolds, these results suggest that their ability for osteoinduction of hMSCs (*in vitro*) is comparable or better than PLGA scaffolds, which are approved by FDA and used widely.

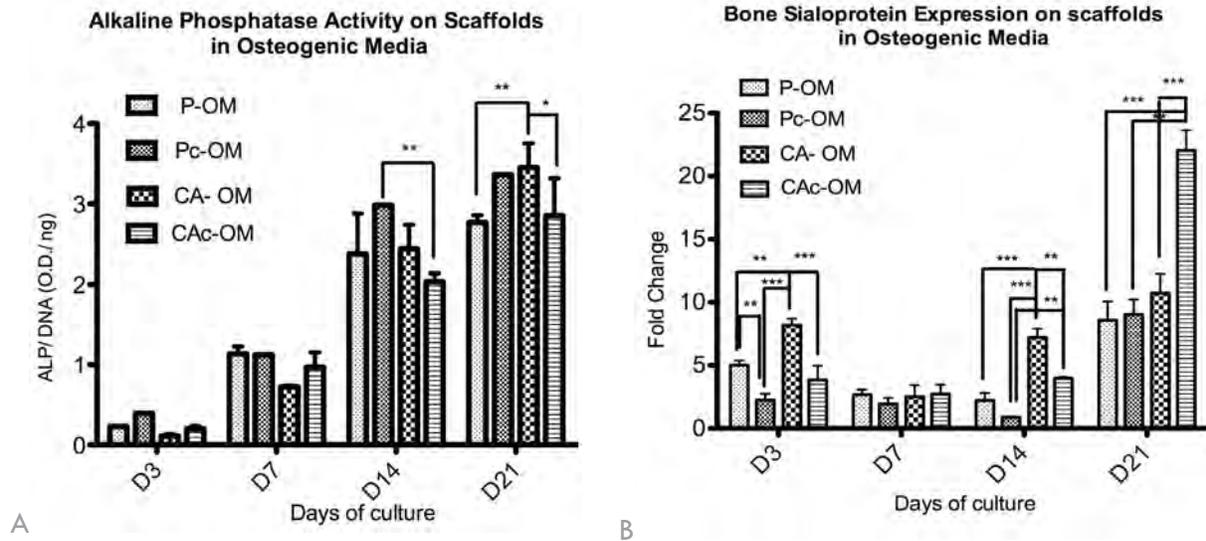


Figure 1: Osteogenic progression of hMSCs seeded on scaffolds *in vitro*. Here P indicates PLAGA, Pc indicates PLAGA-col, CA indicates CA, CAc indicates CA-col scaffold groups. OM indicates osteogenic media: (A) ALP activity of cells on scaffolds in osteogenic media- Cells seeded on CA scaffolds had highest ALP activity at day 21 of culture. (B) mRNA levels of late osteogenic marker BSP was greatest in CA-col scaffolds indicating better osteogenic maturation of seeded hMSCs (Normalized to cells cultured on tissue culture plastic)

Conclusions: The CA and CA-col scaffolds induced early osteogenic differentiation of the seeded hMSCs. The osteogenic progression of the seeded hMSCs was also greater on the CA and CA-col scaffolds. These results indicate that CA and CA-col scaffolds are osteoinductive and have great potential as scaffolds for bone regeneration. Experiments with animal models are in progress to evaluate the performance of these scaffolds *in vivo*.

Acknowledgements: Authors acknowledge the funding from Coulter Foundation, National Science Foundation (IIP-1355327, IIP- 1311907 and EFRI-1332329) and Raymond and Beverly Sackler Center.

Biography:

B.Tech., Biotechnology; Anna University, Chennai- 600025.

PhD in Biomedical Science; Skeletal, Craniofacial and Oral Biology - 5th year

I aspire to be in academia. I hope to teach and conduct research in the future. Being one of the leading institutes in biomedical research, UCHC was my first choice among the schools I had applied to. The research atmosphere and the guidance provided by professors in my department have helped me become a better student of science.



Localized Delivery of microRNAs from Nanofibers Enhances Extracellular Matrix Deposition: Post-transcriptional Regulation in Osteoblasts

Eric N. James¹, Anne M. Delany² and Lakshmi S. Nair¹

Affiliations

¹ Department of Othropaedic Surgery

² Center for Molecular Medicine

Background: Nanofiber scaffolds are attractive for bone tissue engineering, as they closely mimic the morphology of collagen fibrils in the natural extracellular matrix (ECM)¹. MicroRNAs (miR RNAs, miRs) are important regulators of bone maintenance and have emerged as powerful new therapeutic molecules². However, efficient tools to deliver miRNA mimics or antisense oligonucleotide inhibitors (antagomirs) to specific target tissues are limited².

The miR-29 family is well studied in bone. miR-29 inhibits the synthesis of ECM molecules, such as fibrillar collagens, as well as the non-collagen matrix protein, osteonectin. Osteonectin regulates collagen fibril assembly and is critical for normal bone remodeling². Inhibiting miR-29 activity increases ECM synthesis. The objective of this study is to develop a localized therapy for bone regeneration by combining nanostructured scaffolds with miR-29a inhibitors to enhance the production of ECM. We evaluated the ability of these scaffolds to increase ECM production by quantifying osteonectin *in vitro*.

Methods: Gelatin was dissolved in trifluoroethanol to obtain a 7.5% solution. Scramble (control), miR 29a inhibitor with TKO transfection reagent was added to the gelatin solution, to yield concentrations of approximately 50nM/scaffold. Electrospinning was performed to fabricate miRNA-loaded nanofibers. Release kinetics of miRNAs were quantified by NanoDrop spectrometry. Delivery and bioactivity of miR-29a inhibitor was determined by Western Blot analysis of target gene osteonectin. Pico green assay was performed to quantify DNA content.

Results & Discussion: Dy547 labeled miRNA (scramble) encapsulated in electrospun gelatin nanofibers were uniform and bead-free (**Figure 1**). Sustained miR-29a inhibitor release from the gelatin nanofibers was observed over a period of 72hrs (**Figure 2**). miR-29a negatively regulates osteonectin by binding to its mRNA, causing instability and disrupting translation. Thus, introducing a miR-29a inhibitor will enhance osteonectin expression. Western blot analysis revealed that MC3T3-E1 cells seeded on miR-29a inhibitor loaded gelatin fibers and scramble loaded nanofibers (3D) showed comparable transfectability to cells on miR-29a inhibitor or scramble on glass coverslips (2D) after 24h. Further, both 2D and 3D groups with miR-29a inhibitor had significantly increased expression of osteonectin compared to scramble controls (**Figure 3 A, B**). DNA quantification revealed no differences cell number.

Conclusions: The study demonstrated the feasibility of producing miR-29a inhibitor loaded nanofibers as an ECM stimulating scaffold. Gelatin nanofibers locally delivered bioactive miR-29a inhibitor in a sustained manner, inducing the expression of the critical ECM component, osteonectin. Applications for this novel technology include the ability to deliver transient RNA-based therapy, without potential for cell transformation. Further, this approach is flexible, with the potential to deliver any miRNA inhibitor or mimic. The unique bioactivity of miRNA-based therapeutics, combined with ECM mimicking nanostructured scaffolds serves as a novel platform for localized therapy for bone regeneration.

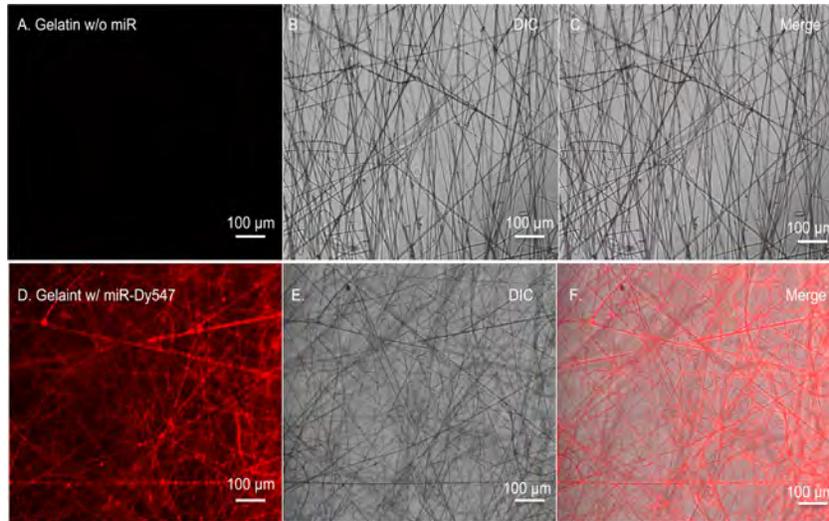


Figure 1. Fluorescence micrographs of Dy547 conjugated miRNA incorporated into gelatin nanofibers. A-C) Unloaded gelatin nanofibers and D-F) gelatin nanofibers loaded with fluorescently labeled miRNAs. Differential interference contrast (DIC) image, fluorescent miRNAs (red) (scale 100 μ m).

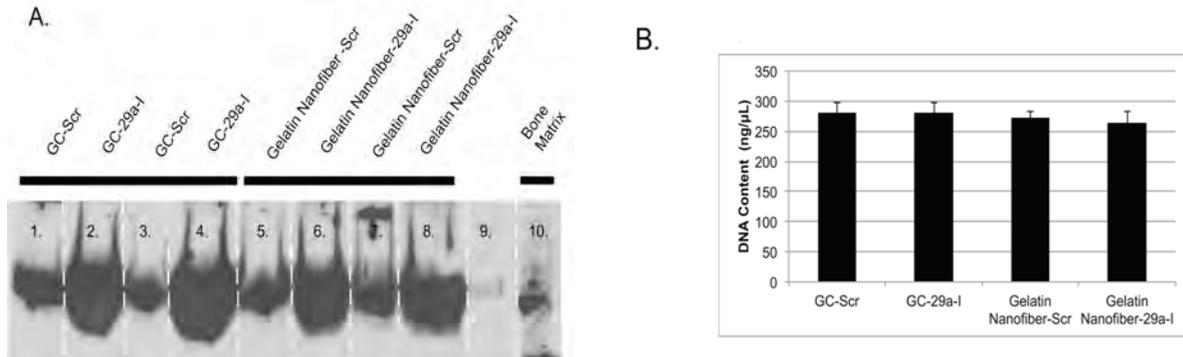


Figure 3. Osteonectin protein secreted from transfected MC3T3-E1 cells seeded on 2-D cover slips or miR-29a inhibitor loaded gelatin nanofibers A) Western blot analysis of osteonectin was performed 24h after cells were seeded on scaffolds, B) DNA content of cells cultured on glass coverslips and miRNA loaded nanofibers for 24h No statistical differences were found among groups DNA content.

References:

1. James EN and Nair LS. J Biomed Nanotechnol 2014;10(8)500-507
2. Kapinas K, Kessler CB, Delany AM. J Cell Biochem 2009;108;216-24

Acknowledgements: This work was funded by Financial support from the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health under Award Numbers R044877 (to AMD), AR061575 (to LSN)

Biography: I earned my B.S. in Biology at Stillman College, Tuscaloosa AL in 2006. I continued my education and earned a Professional Science Master's degree in the applied genetics, genomics and bioinformatics program in 2009 at the University of Connecticut, Storrs. This marks my 4th year as a Biomedical PhD candidate. I choose UCHC based on multidisciplinary projects that were being performed in the Skeletal Craniofacial and Oral Biology program. My future milestone is to obtain a postdoctoral position at an institution in the U.S. that allows me to conduct quality tissue engineering research while also serving as a part-time instructor to college students. My ultimate goal is to obtain a permanent position at a research institute and successfully secure grant funding for my own research goals.



Evaluation of nanofiber-permeated scaffolds for bone repair in a transgenic mouse model

Clarke Nelson, Yusuf Khan, David Rowe, Cato T. Laurencin

Statement of Purpose: Sintered composite microsphere matrices have shown potential as autograft replacements, but mineralization is often limited to the periphery in static culture. Fibrous networks of the physical scale of collagen ECM in bone may increase cellular retention and migration leading to improved bone repair. While a fibrous structure alone would have limited clinical utility due to no load-bearing potential, we propose to increase mineralization throughout a mechanically stable microsphere matrix using a secondary, nanofibrous phase within its pore structure. We hypothesize that a nanofiber mesh within the pore structure may increase cell residence and bone tissue formation in a critical sized defect.

Methods: Sintered, composite microsphere matrices were fabricated according to reported procedure, submerged in a 1% solution PLLA in DMF, and cooled to allow thermally induced phase separation to occur. In two experiments, scaffolds were either implanted without cells for 6 weeks in calvarial defects of transgenic mice carrying a collagen type I GFP reporter, or seeded with bone marrow stromal cells from a separate GFP-reporter and implanted for 6 weeks in the same animal model. Cryohistology of the non-decalcified implants were evaluated for TRAP, ALP, GFP, and active mineralizing surfaces. For the cell-seeded experiment, mineralized tissue was additionally quantified using the indirect measure of electron density through MicroCT.

Results: For the non-cell seeded *in vivo* study, hybrid scaffolds demonstrated a trend towards higher levels of bone tissue formation (1.2% vs. 0.5%, $p > 0.05$) and higher levels of the osteoclast marker TRAP (2.7% vs. 0.01%, $p < 0.05$) in the presence of nanofiber-infused scaffolds compared to control scaffolds. In the cell seeded *in vivo* experiment, active bone labeling was higher in hybrid scaffolds compared to control scaffolds (11% vs. 6.8%, $p < 0.01$). TRAP activity was also higher (13% vs. 5.5%, $p < 0.001$). Electron density as measured by MicroCT was also higher in hybrid scaffolds compared to controls in cell-seeded scaffolds (21% vs. 11%, $p < 0.002$). There was no statistical differences in either the amount of host or donor cells positive for collagen type I promoter activity in either scaffold.

Conclusions: Improvement of current bone graft substitute technology with advanced, ECM-mimetic features has the potential to improve bone regeneration in critical sized defects. In support of our hypothesis, the amount of alizarin complexone deposited on active bone forming surfaces was greater in nanofiber-permeated, non-cell seeded scaffolds. With the addition of cultured bone marrow stromal cells, both the levels of absolute bone label deposition and the relative levels of bone level deposition was increased in hybrid scaffolds compared to control scaffolds. Furthermore, the presence of areas of active osteoclasts further suggests this newly deposited tissue may

potentially have increased turnover and integration into host tissue, an excellent attribute for a bone graft substitute. While promising, the exact mechanism by which these fibers may be engendering an enhanced regenerative capacity needs to be analyzed. In the future, more osteoclast specific studies need to be accomplished to determine the nature of the increased TRAP activity on these scaffolds.

Acknowledgements: Department of Defense DAMD W81XWH11-10262, M.D./Ph.D program

Biography: B.S., Physics, Vanderbilt University 2007. Currently 6th year M.D./Ph.D student. Aspirations toward a career in the surgical application of advanced, translational biomaterials and astronautics.

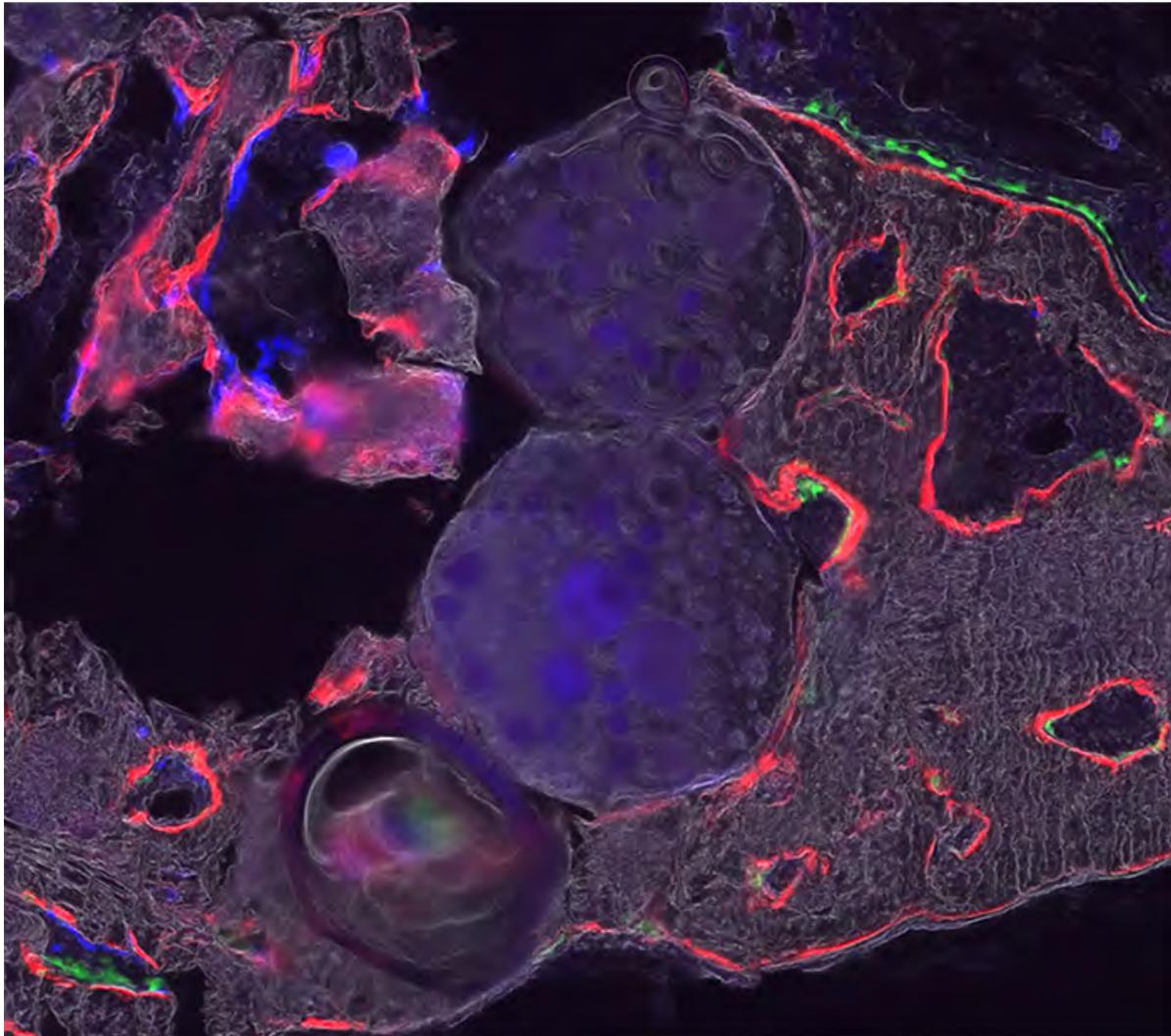


Figure 1, detail of hybrid, nanofiber-permeated scaffold after 6 weeks in a cranial defect. Microspheres visible embedded in newly formed mineralized tissue labeled red with alizarin complexone. Host cells positive for collagen type I promoter activity report green color, and donor cells positive for collagen type I promoter activity report blue color in this image. Association of collagen type I promoter activity with areas of active bone deposition is evidence for osteoblast activity.



The Cellular and Matrix Dynamics of the Enthesis

*Nat Dyment¹, Andrew Breidenbach², Andrea Schwartz³,
Lindsey Aschbacher-Smith⁴, Rulang Jiang⁴, David
Butler², Steve Thomopoulos³, David Rowe¹*

Affiliations

¹ University of Connecticut Health Center

² University of Cincinnati

³ Washington University in St. Louis

⁴ Cincinnati Children's Hospital Research Foundation

Abstract:

The enthesis is the attachment site where soft tendons or ligaments attach to rigid bone. Several chronic pathologies and injuries affect the entheses for a variety of tendons or ligaments. The injuries that often occur in chronic tendinopathies are particularly problematic to repair. An improved understanding of the dynamics of the cells and matrix within the enthesis during the normal processes of growth and natural healing may provide insight into how to improve repair or prevent the progression of these chronic injuries. Commonly injured entheses are in direct or zonal insertions that transition from the tendon/ligament body through zones of unmineralized and mineralized fibrocartilage to anchor into bone. The focus of this study is on the transition from the unmineralized to mineralized fibrocartilage, as this transition is important in maintaining normal mechanical function of the enthesis and is often altered in chronic pathologies.

Hedgehog signaling regulates chondrocyte hypertrophy in the growth plate and Indian hedgehog is highly expressed in the unmineralized fibrocartilage of the enthesis. However, the function of hedgehog signaling in the enthesis is not fully understood. Therefore, the objective of this study is to determine 1) the direction and rate of mineralization in the enthesis during growth, 2) the positioning of hedgehog cells in the enthesis during growth, and 3) the influence of hedgehog signaling on enthesis mineralization.

A combination of transgenic mice and multiple mineralization labeling was used to monitor the dynamics of the enthesis during growth. We found that new mineralized fibrocartilage was made in the direction of the tendon body, away from the underlying bone. We also found that the mineral apposition rate in the enthesis varied amongst the patellar, supraspinatus, and Achilles tendons. In order to determine whether the cells in the enthesis followed a similar pattern, we used the Gli1-CreERT2 mice crossed with Ai14-tdTomato Cre reporter mice to label hedgehog positive cells in the enthesis. We found that after initial labeling, the Gli1-Ai14+ cells were isolated to the unmineralized fibrocartilage in the enthesis (Fig. 1A). Following a 4-week chase, the distal region of the labeled Gli1 cells were now within mineralized fibrocartilage, indicating that these cells differentiated and mineralized the surrounding matrix (Fig. 1B). Finally, since hedgehog signaling was initially isolated to the unmineralized fibrocartilage but became mineralized after 4 weeks post-labeling, we next determined whether hedgehog signaling had an effect on this mineralization. To do so, we crossed Scleraxis (ScxCre) mice, which labels cells throughout the tendon length, with smoothed floxed (Smo^{fllox}) mice to target deletion of hedgehog signaling in the tendon enthesis. We found that the mineralized fibrocartilage of the entheses in these mice were severely impaired. The area of mineralized fibrocartilage was reduced and the organization of the tidemark was disrupted (Fig. 1C). These data in combination indicate that hedgehog signaling is required for cells within the enthesis to differentiate from unmineralized

to mineralized fibrocartilage. This may provide insight into future repair strategies as we must first anchor collagen down to bone during the repair process and then have cells within the repair differentiate properly through coordinated hedgehog signaling to mineralize the fibrocartilage at the insertion in order to create a sufficient anchor that can function mechanically.

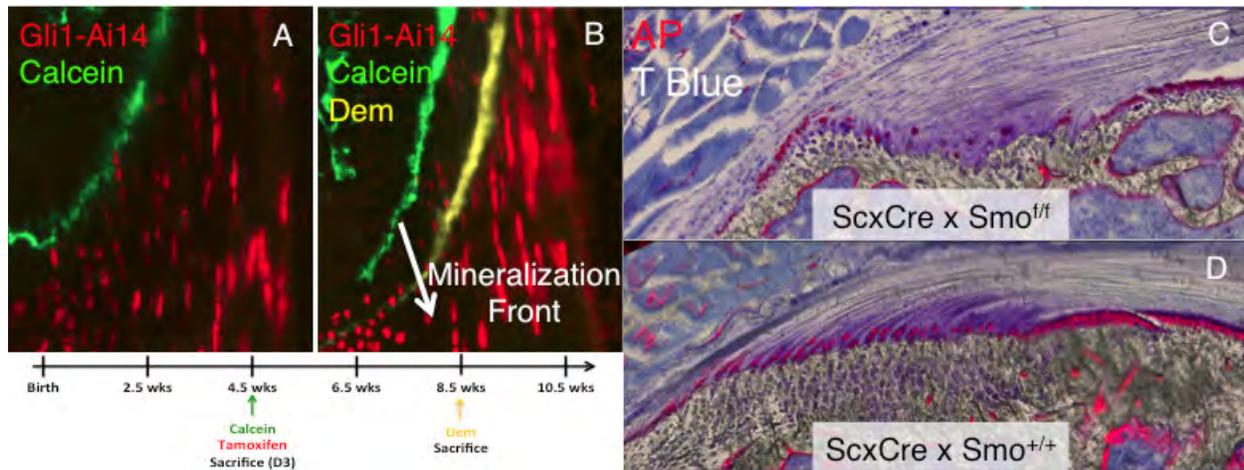


Fig. 1. A-B) Lineage trace of Gli1-Ai14+ cells from unmineralized fibrocartilage in the patellar tendon enthesis to mineralized fibrocartilage (B). Tamoxifen and calcein were delivered at 4 weeks of age followed by demeclocycline (Dem) at 8 weeks of age. Mice were analyzed at 3 days (A) and 28 days (B) following tamoxifen injection. C-D) Knockdown of hedgehog signaling in the enthesis leads to severely impaired mineralized fibrocartilage formation with reductions of alkaline phosphatase (AP) activity near the tidemark of the supraspinatus tendon.

Acknowledgements: This work was supported by National Institutes of Health grants AR56943, AR46574, and AR054713, AR063702 from NIAMS and 5T90DE021989 from NIDCR.

Biography:

Education

University of Illinois at Urbana Champaign, B.S., Materials Science and Engineering, 2001-2005
University of Cincinnati, PhD, Biomedical Engineering, 2005-2011

Position: T90 Postdoctoral Fellow

Year in Program: 3rd

Career Aspirations: My goal is to remain in academia as a principal investigator with research focused on improving repair of musculoskeletal injuries.

Why UCHC: My PhD work focused on using tendon development as a guide for improving tissue engineering strategies to improve repair. This work was a part of an NIH Bioengineering Research Partnership grant for which Dr. David Rowe was a consultant. I came to Dr. Rowe's lab to improve our understanding of tendon differentiation by identifying genetic markers that define stages within the lineage and the signaling pathways that regulate differentiation. We are now using Cre lineage tracing models to characterize progenitors cells as they differentiate into mature tendon cells.

Career Panel

Alumna Panelists



I-Ping Chen, DDS, PhD

Assistant Professor

Oral Health and Diagnostic Sciences

University of Connecticut School of Dental Medicine

Dr. Chen is Assistant Professor in the Division of Endodontology, Department of Oral Health and Diagnostic Sciences, in the School of Dental Medicine at the University of Connecticut Health Center. She earned her D.D.S at the China Medical University in Taiwan in 1998 and a certificate in Endodontics and Masters in Oral Biology at the University of Pennsylvania in 2005. Subsequently, she entered the Skeletal, Craniofacial and Oral Biology Program at the University of Connecticut Health Center and earned a Ph.D. in Biomedical Sciences in 2009.

Dr. Chen's research interest is on the pathogenesis of a rare genetic bone disorders, specifically craniometaphyseal dysplasia (CMD). She uses the mouse model as well as the patient-specific induced pluripotent stem cells (iPS cells). In 2010, Dr. Chen has been awarded a NIDCR Dentist/Scientist K99/R00 Career Award entitled "The use of patient-specific iPS cells to identify osteoclast defects in CMD".

Jessica Costa, DMD, PhD

Postdoctoral Fellow

Center for Molecular Medicine

UConn Health

Career Panel

Alumna Panelists



Irene K. Reed, Ph.D.
Assistant Professor of Biology
University of Saint Joseph

Dr. Irene Reed earned her B.S. degrees in Forensic Science and Biology from the University of New Haven, and in 2010 earned her Ph.D. in Biomedical Science with a concentration in Molecular Biology and Biochemistry from the University of Connecticut Health Center in Farmington, CT. While she was a graduate student, she was an adjunct professor at the University of Saint Joseph where she taught general biology and microbiology laboratories. In the Fall of 2010, she joined the University of Saint Joseph Biology Department as a Visiting Assistant Professor, and obtained a tenure track position the following year.

USJ is a small private institution with a strong focus on teaching and scholarship. Irene is currently engaged in bench research with undergraduate students focused on the role of regulatory microRNAs in human breast cancer, and she is actively pursuing grant funding through several institutions and Biotechnology companies for research support. She is dedicated to student centered research at the University, and is also interested in collaborating with larger institutions. Mentored students also present at regional undergraduate research conferences and are encouraged to publish their work. Currently, one of Irene's students has a first author paper under review.

Menu

Catering provided by the Kitchen at Billings Forge

The Kitchen at Billings Forge, home of a cozy Kitchen café and farm-to-table catering business, offers job skills training and high-quality employment opportunities to residents of the Billings Forge and the Frog Hollow areas of Hartford. The Kitchen brings together employment, education, access to healthy eating options, and a connection to the environment using hands-on training, community interactions, and a shared love for food.

Breakfast and Morning Break

- **Mini Scones**
 - *Flavors may include Seasonal Fruit, Chocolate, Cinnamon & Sugar, Pumpkin & Cranberry*
- **The Kitchen Muffins**
 - *Flavors may include Seasonal Fruit Muffin, Carrot Raisin, Cranberry, Cinnamon Raisin Spice & Buttermilk Spice*
- **House Made Breakfast Breads**
 - *Flavors may include: Lemon Tea, Pecan Raisin, Blueberry Crumble, Plain Croissant & Coffee Cake*
- **Assorted Vegetarian Mini Quiches**
- **Fresh Fruit Platter**
- **Bean & Leaf Coffee**
- **Harney & Sons Teas**
- **Assorted Fruit Juice**
- **Bottled Water**

Lunch

- **Boxed Lunches**
 - *Roasted Turkey Sandwich with creamy parmesan spread, shaved parmesan, romaine lettuce served with Greek orzo salad, chips and house dessert*
 - *Curry Chicken Salad Sandwich with mango chutney & arugula served with Greek orzo salad, chips and house dessert*
 - *Grilled Eggplant Sandwich with dried fruit ketchup, spinach served with Greek orzo salad, chips and house dessert*
- **Hosmer Mountain sodas & seltzers**
- **Bottled Water**

Thank You

Thank you for participating in today's event. We look forward to seeing you at the SCOB Symposium next year.

