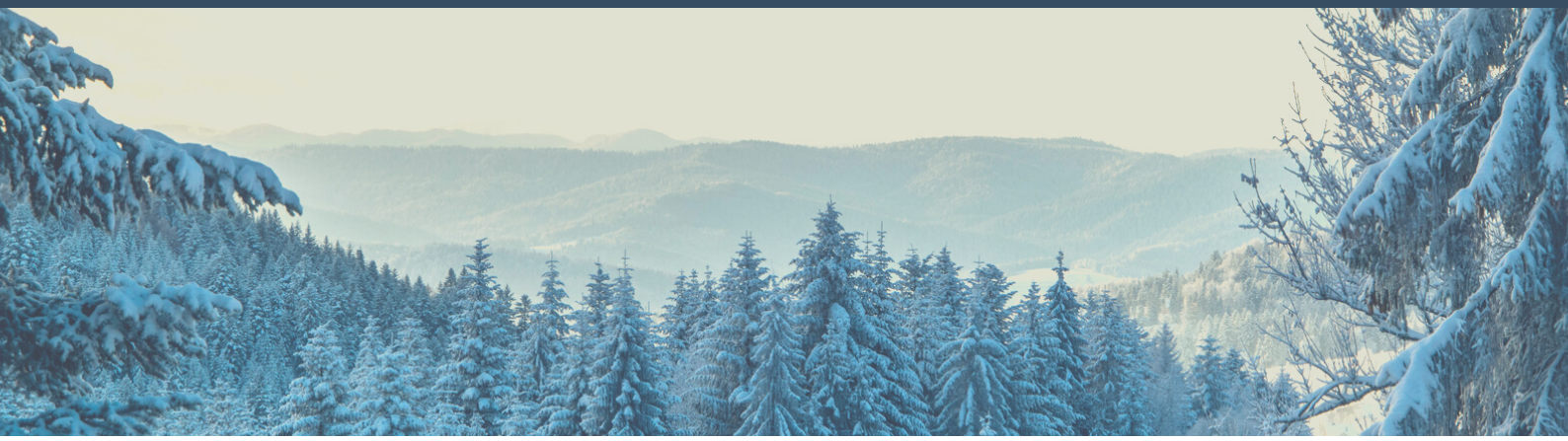
JANUARY 2022 | WINTER EDITION

BIOMEDICAL ENGINEERING IN REGENERATIVE MEDICINE



This issue's must-read articles:

Volume 5

A Letter from Dr. Li:

In 2021, we overcame many challenges, whether it was the continuation of the pandemic or our journeys to reach goals in diverse areas of our lives. But we were also able to continue to explore in the environment we all knew best: the laboratory.

In addition to several publications (one of which was selected to be featured in the frontiers in the cell and developmental biology collection the editor's pick 2021: highlights in stem cell research), we are perhaps most proud of our students for their successful presentations across several meetings, including the Orthopaedic Research Society (ORS) annual meeting. As a team, we also were awarded several pilot grants from our very own WMed School, an essential step in reaching our larger goals of NIH and DOD grants in the coming years. Congratulations!

As well, we increased strived to focus on cultivating a sense of collaboration and teamwork. As a team, we would like to extend our appreciation to all our members and collaborators.

Looking ahead, we firmly believe that 'Regenerative Medicine is the Future Medicine.' And through collaboration and innovation, we can build the foundation for future successes in the research and strive to push the boundaries of this statement.

We will strive to consistently uphold 'Teamwork and collaboration make the dream work!'

From my family to yours, we extend our warmest wishes for the new year. Stay healthy and safe!

Best regards,

Yong Li

Yong Li, MD, PhD

Lab News and Events:

WHERE WE ARE NOW

Orthopaedic Surgery Research Symposium for Medical Students in Michigan (OSRS):

Eight students from Dr. Li's Lab of WMed, as well as many other medical schools in Michigan, came to Detroit to present their abstracts and posters on the symposium at Oakland Beaumont Medical School. Our team gave five poster presentations and one podium talk (please see detail on pages 3 and 4), Dec 1, 2021.

Highlight: Editor's Pick:

Dr. Li's article entitled "Hypoxia in Cell Reprogramming and the Epigenetic Regulations" published in the journal of 'Frontiers in Cell and Developmental Biology' was selected as one (of 12 articles) in the Editor's Pick 2021: Highlights in Stem Cell Research. https://www.frontiersin.org/research-topics/30610/editors-pick-2021-highlights-in-stem-cell-research#overview

Organizations:

Dr. Li was invited to organize the '3rd Euro conference on sports medicine-fitness and physiotherapy', which will be held on Oct 17-18, 2022, Paris, France. https://www.longdom.com/sportsmedicine/ocm

Additionally, Dr. Li was invited to be a keynote speaker and section chair on the the '7th Annual World Congress of Orthopaedics 2022', which will be held in Lisbon, Portugal, on July 20-22, 2022.

Attended conferences:

Dr. Li Participated and involved discussion on the International Society for Stem Cell Research (ISSCR) digital meeting: Developing Standards for Stem Cell Research, Oct 7, 14, 20, 25, 2021.

Dr. Akkouch was invited to serve as Judge and moderator for scientific session on "Biofabrication of musculoskeletal tissues" at the 6th world congress of the Tissue Engineering and Regenerative Medicine International Society (TERMIS2021), Nov 15-19, 2021.

Dr. Akkouch participated in the 3D-cember: The Global Event to Celebrate 3D Biology. The event highlights new developments in 3D tissue biology in the limelight, Dec 3, 2021.

Pilot Research Grants:

Dr. Akkouch PI and Dr. Sawyer Co-PI, were awarded a Pilot Research Grant for their project entitled "3D printed meshes with antimicrobial activity for hernia repair" Oct 18, 2021.

Dr. Li awarded a Pilot Research Grant for their project entitled "Use of Blood Clots to Improve Skin Wounds" on Dec 9, 2021. The co-PI is Dr. Sawyer.

Asian Pacific American Medical Student (APAMS) Conference:

Students from the Li Lab, Son Tran and Andre Ksajikian had the oportunity to give a poster presentation at the national APAMS conference at Columbus, Ohio, Jan 8, 2022

Lab News and Events:

Orthopaedic Surgery Research Symposium for Medical Students in Michigan

Five Poster Presentations:

Risk Factors for Manipulation Under Anesthesia Following Total Knee Arthroplasty: A Systematic Review and Meta-analysis: Presented by Son Tran, Juliana Overbey, Andre Ksajikian, and Sumit Patel MD, this project investigated the healing effects of implanted isogenic blood clots loaded with Mesenchymal Stem Cells (MSCs) into bone defects. This study aims to compile and evaluate risk factors for manipulation under anesthesia (MUA) following primary total knee arthroplasty. Our goal is to provide surgeons with the information they need to better inform patients of the need for future procedures.

Effects of Pirfenidone on Fibroblast Proliferation and Gene Expression: Presented by Andre Ksajikian, Juliana Overbey, Son Tran, and Sumit Patel MD, this project investigates the suppressive effects of pirfenidone, an anti-inflammatory drug approved for lung fibrosis, on inflammation and the Wnt/GSK-3β/β-catenin and transforming growth factor (TGF)-β1/Smad2/3 cell signaling pathways that regulate fibroblast activation and proliferation. With the prevalence of iatrogenic skin, soft-tissue, and joint fibrosis after various types of procedures, the aim of this project is to explore Pirfenidone as a potential therapeutic to decrease scarring, joint stiffness, inflammation and improve patient satisfaction after surgery.

Hypoxic conditioning makes muscle cells express anti-apoptosis protein XIAP and improves cell survival
Presented by Jefferson DeKloe, this project explores the effects of hypoxia pre-conditioning on increases expression the anti-apoptosis protein X-linked inhibitor of apoptosis (XIAP) in muscle cells which may be an important factor in promoting cell survival. Understanding the mechanisms that favor muscle cell survival will guide the development of interventions that rely on muscle cell transplantation.

Left to right: Son, Victor, Leah, Erica, Juliana, Jefferson, Andre, Peter

Damaged Neurons Secrete Factors that Influence Myogenic Differentiation
Presented by Leah Liu, this project aims to determine whether neurons damaged by stretched or chemical injury produce factors to (1) initiate muscle atrophy and (2) modulate myogenic differentiation in vitro. This project provides novel insight into a potential mechanism of neuronal muscle atrophy and may reveal new targets to reverse or halt muscle atrophy to improve functional recovery after motor neuron damage.

Low Oxygen Culture Induction of Increased PD-L1 Expression in C2C12 Myoblasts
Presented by Juliana Overbey, this project questions if increased expression of programmed death ligand-1 (PD-L1) can improve the intramuscularly transplanted myoblasts' survival, thus enhancing the efficiency of myoblast transplantation. Findings support the notion that hypoxia exposure of myoblasts could be translated into a hypoxia-mediated preconditioning treatment of cell-based therapies before their transplantation into the patient to promote better cell survival and efficacy of treatment.

Lab News and Events:

Orthopaedic Surgery Research Symposium for Medical Students in Michigan

Poster Presentations and Podium Talk:

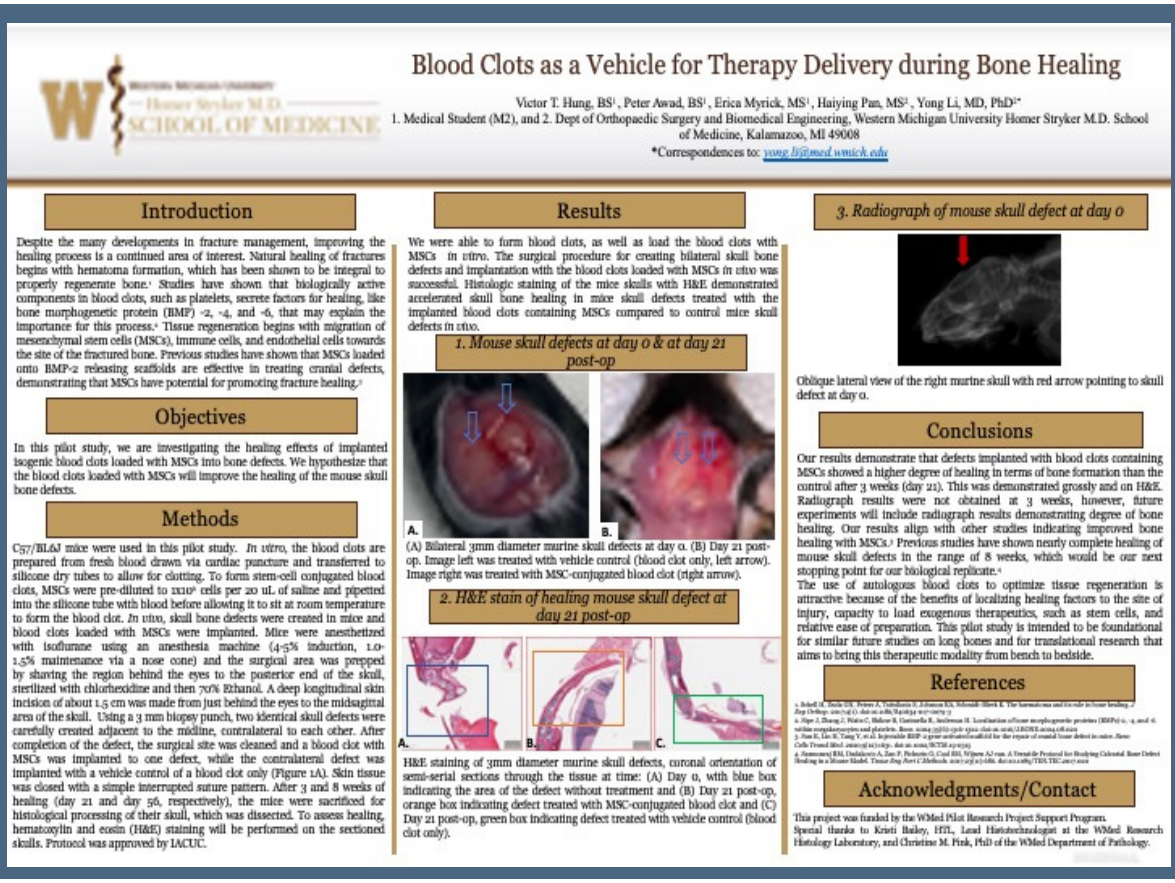
Blood Clots as a Vehicle to Deliver Therapeutics During Bone Healing*

Presented by Victor Hung, Peter Awad, and Erica Myrick, this project investigates the healing effects of implanted isogenic blood clots loaded with MSCs into bone defects.

The students also had the oportunity to give a podium talk and share their results demonstrating that defects implanted with blood clots containing MSCs showed a higher degree of healing in terms of bone formation than the negative control after 4 weeks, which aligns with other studies indicating improved bone healing with MSCs

The use of autologous blood clots to optimize tissue regeneration is attractive because of the benefits of localizing healing factors to the site of injury, capacity to load exogenous therapeutics, such as stem cells, and relative ease of preparation. This study is intended to be foundational for future translational research that aims to bring this therapeutic modality from bench to bedside.

https://oakland.edu/inmedicine/events/2021/OUWB-Orthopedic-Surgery-and-Sports-Medicine-Group-hosts-med-students-from-across-Michigan-



Blood Clots as a Vehicle for Therapy Delivery during Bone Healing

Victor T. Hung, BS[1], Peter Awad, BS[1], Erica Myrick, MS[1], Haiying Pan, MS[2], Yong Li, MD, PhD[2]*
1. Medical Student (M2), and 2. Dept of Orthopaedic Surgery and Biomedical Engineering, Western Michigan University Homer Stryker M.D. School of Medicine, Kalamazoo, MI 49008
*Correspondences to: yong.li@med.wmich.edu

Introduction

Despite the many developments in fracture management, improving the healing process is a continued area of interest. Natural healing of fractures begins with hematoma formation, which has been shown to be integral to properly regenerate bone.[1] Studies have shown that biologically active components in blood clots, such as platelets, secrete factors for healing, like bone morphogenetic protein (BMP) -2, -4, and -6, that may explain the importance for this process.[2] Tissue regeneration begins with migration of mesenchymal stem cells (MSCs), immune cells, and endothelial cells towards the site of the fractured bone. Previous studies have shown that MSCs loaded onto BMP-2 releasing scaffolds are effective in treating cranial defects, demonstrating that MSCs have potential for promoting fracture healing.[3]

Objectives

In this pilot study, we are investigating the healing effects of implanted isogenic blood clots loaded with MSCs into bone defects. We hypothesize that the blood clots loaded with MSCs will improve the healing of the mouse skull bone defects.

Methods

C57/BL6J mice were used in this pilot study. *In vitro*, the blood clots are prepared from fresh blood drawn via cardiac puncture and transferred to silicone dry tubes to allow for clotting. To form stem-cell conjugated blood clots, MSCs were pre-diluted to $1x10^6$ cells per 20 uL of saline and pipetted into the silicone tube with blood before allowing it to sit at room temperature to form the blood clot. *In vivo*, skull bone defects were created in mice and blood clots loaded with MSCs were implanted. Mice were anesthetized with isoflurane using an anesthesia machine (4-5% induction, 1.0-1.5% maintenance via a nose cone) and the surgical area was prepped by shaving the region behind the eyes to the posterior end of the skull, sterilized with chlorhexidine and then 70% Ethanol. A deep longitudinal skin incision of about 1.5 cm was made from just behind the eyes to the midsagittal area of the skull. Using a 3 mm biopsy punch, two identical skull defects were carefully created adjacent to the midline, contralateral to each other. After completion of the defect, the surgical site was cleaned and a blood clot with MSCs was implanted to one defect, while the contralateral defect was implanted with a vehicle control of a blood clot only (Figure 1A). Skin tissue was closed with a simple interrupted suture pattern. After 3 and 8 weeks of healing (day 21 and day 56, respectively), the mice were sacrificed for histological processing of their skull, which was dissected. To assess healing, hematoxylin and eosin (H&E) staining will be performed on the sectioned skulls. Protocol was approved by IACUC.

Results

We were able to form blood clots, as well as load the blood clots with MSCs *in vitro*. The surgical procedure for creating bilateral skull bone defects and implantation with the blood clots loaded with MSCs *in vivo* was successful. Histologic staining of the mice skulls with H&E demonstrated accelerated skull bone healing in mice skull defects treated with the implanted blood clots containing MSCs compared to control mice skull defects *in vivo*.

1. Mouse skull defects at day 0 & at day 21 post-op

(A) Bilateral 3mm diameter murine skull defects at day 0. (B) Day 21 post-op. Image left was treated with vehicle control (blood clot only, left arrow). Image right was treated with MSC-conjugated blood clot (right arrow).

2. H&E stain of healing mouse skull defect at day 21 post-op

H&E staining of 3mm diameter murine skull defects, coronal orientation of semi-serial sections through the tissue at time: (A) Day 0, with blue box indicating the area of the defect without treatment and (B) Day 21 post-op, orange box indicating defect treated with MSC-conjugated blood clot and (C) Day 21 post-op, green box indicating defect treated with vehicle control (blood clot only).

3. Radiograph of mouse skull defect at day 0

Oblique lateral view of the right murine skull with red arrow pointing to skull defect at day 0.

Conclusions

Our results demonstrate that defects implanted with blood clots containing MSCs showed a higher degree of healing in terms of bone formation than the control after 3 weeks (day 21). This was demonstrated grossly and on H&E. Radiograph results were not obtained at 3 weeks, however, future experiments will include radiograph results demonstrating degree of bone healing. Our results align with other studies indicating improved bone healing with MSCs.[3] Previous studies have shown nearly complete healing of mouse skull defects in the range of 8 weeks, which would be our next stopping point for our biological replicate.[4]
The use of autologous blood clots to optimize tissue regeneration is attractive because of the benefits of localizing healing factors to the site of injury, capacity to load exogenous therapeutics, such as stem cells, and relative ease of preparation. This pilot study is intended to be foundational for similar future studies on long bones and for translational research that aims to bring this therapeutic modality from bench to bedside.

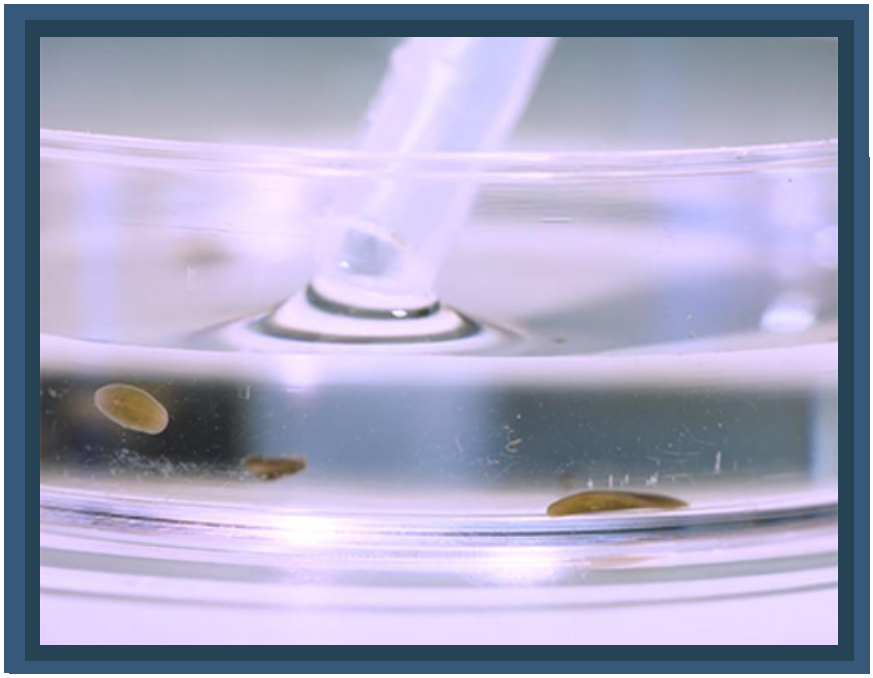
References

Acknowledgments/Contact

This project was funded by the WMed Pilot Research Project Support Program.
Special thanks to Kristi Bailey, HTL, Lead Histotechnologist at the WMed Research Histology Laboratory, and Christine M. Pink, PhD of the WMed Department of Pathology.

Lab News and Events:

Presentation by Dr. Wendy Scott Beane



Lessons from Planaria: Separating Wound Healing from Regeneration, and Quantum Control of Stem Cells

On November 18th, 2021, **Dr. Beane** presented her lab's latest research investigating the roles of reactive oxygen species (ROS) signaling during tissue repair using the planarian regeneration model system. Her lab is currently focused on two main areas: 1) parsing how ROS are used during wound healing versus regeneration, and 2) how weak magnetic field exposure might be used to manipulate stem cell activities during new tissue growth by altering ROS levels in vivo. In summary, their findings suggest that regeneration and wound healing are controlled by separate ROS-mediated signaling programs. These pathways are distinguishable by differential threshold and gene expression changes 1 hour post injury. Furthermore, their data reveal that ROS accumulation is required for stem cell-mediated regeneration, and weak magnetic fields can be used to both increase ROS (and new tissue growth) or inhibit ROS (and growth) depending on field strength.

Thank you, Dr. Wendy Scott Beane

Lab News and Events:

Another Noteworthy Event: Collaboration vs. Laboratory tour

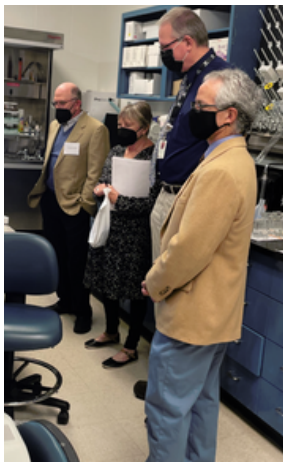
On November 11th, 2021, Our BioMedical Engineering research team, led by Dr. Li, held a tour of the research facilities at WMed's Upjohn campus. We were pleased to host more than twenty esteemed faculties from WMed and Western Michigan University, including five Chairmen (Drs. Kenter, Liu, Rothstein, Sawyer and Spitsbergen) and two associate deans (Drs. Kenter and Vanden Heuvel). Researchers had the opportunity to discuss current investigations and collaborations. Additionally, newly renovated research facilities and updated equipment were showcased in the following areas of the Upjohn campus:

- 4th floor Biomedical Engineering lab
- 4th floor Pathology/Histology lab, and Genetic study lab
- 4th floor Toxicology lab
- 5th floor Immunology and Core facilities
- 7th floor Pathology and Surgical Skill lab

Dr. Keith Kenter and **Dr. Gregory Vanden Heuvel** concluded the event with an excellent summary at the end of the tour. In consideration of continued successful collaboration, faculties from both WMed and WMU look forward to increasing our partnerships in science and education. All welcome the organization of mini-symposia/retreats in the coming year. Furthermore, we are excited to welcome the new student researchers from WMU, with the intent to host additional researchers at the master's and Ph.D. levels in collaboration with WMU.

It is exciting to see the further partnership with the faculties from WMed and WMU who participated in the tour of our Upjohn campus!



Publications:

Boivin J, Tolsma R, Awad P, **Kenter K**, **Li Y.** The Biological Use of Platelet-Rich Plasma in Skeletal Muscle Injury and Repair. *Am J Sports Med.* 2021; doi:10.1177/03635465211061606. *The America Journal of Sports Medicine is widely regarded as the top choice in journals for orthopedic sports medicine*! *Impact Factor: 6.2*

Lu A, Guo P, **Pan HY,** Tseng C, Sinha KM, Yang F, Scibetta A, Cui Y, Huard M, Zhong L, Ravuri S, Huard J. Enhancement of myogenic potential of muscle progenitor cells and muscle healing during pregnancy. *FASEB J.* 2021;35(3):e21378. doi: 10.1096/fj.202001914R.

Li Y, Darabi R. Role of epigenetics in cellular reprogramming; from iPSCs to disease modeling and cell therapy [published online ahead of print, 2021 Oct 19]. *J Cell Biochem.* 2021;10.1002/jcb.30164. doi:10.1002/jcb.30164

Heffernan M.J., Song B., **Bovid K.M.**, Leonardi C., Fornari E.D. Assessing the impact of the Pediatric Orthopaedic Society of North America (POSNA) visiting scholar program. *Journal of Pediatric Orthopaedics.* 2021;41:197-201

Bonatti HJR, Pruett TL, **Sawyer RG**. Portal Perfusion Through the Splenic Vein During Liver Procurement. *Exp Clin Transplant.* 2021 Dec;19(12):1286-1290. doi: 10.6002/ect.2021.0212. PMID: 34951347

Zhang AY, Marsh KM, Rastogi R, Wu D, Charles EJ, Kron IL, **Sawyer RG**, Yang Z. Topical Neck Cooling Prolongs Survival of Rats with Intra-Abdominal Feculent Sepsis by Activation of the Vagus Nerve. *Int J Mol Sci.* 2021 Sep 11;22(18):9828. doi: 10.3390/ijms22189828. PMID: 34575994

Huajian R, Qiongyuan H, Gefei W, Guosheng G, Jun C, Zhiwu H, Yanhan R, Mahmood G, **Sawyer RG,** Jianan R. Analysis of Later Stage Morbidity and Mortality after Pancreatic Surgery Because of Abdominal Trauma. *Surg Infect (Larchmt).* 2021 Dec;22(10):1031-1038. doi: 10.1089/sur.2020.246. Epub 2021 Jun 21. PMID: 34152863

Hranjec T, Estreicher M, Rogers B, Kohler L, Solomon R, Hennessy S, Cibulas M, Hurst D, Hegazy M, Lee J, Perez D, Doctor N, Kiffin C, Pigneri D, LaGuardia H, Shaw K, Arenas J, Rosenthal A, Katz RS, **Sawyer RG**, Pepe PE. Integral Use of Thromboelastography With Platelet Mapping to Guide Appropriate Treatment, Avoid Complications, and Improve Survival of Patients With Coronavirus Disease 2019-Related Coagulopathy. *Crit Care Explor.* 2020 Dec 21;2(12):e0287. doi: 10.1097/CCE.0000000000000287. eCollection 2020 Dec. PMID: 33381763

Publications (cont'd):

Srivastava T., Joshi T., Heruth D.P., Rezaiekhaligh M.H., Garola R.E., Zhou J., Boinpelly V.C., Ali M.F., Alon U.S., Sharma M., **Vanden Heuvel G.B.**, Mahajan P., Priya L., Jiang Y., McCarthy E.T., Savin V.J., Sharma R. A mouse model of prenatal exposure to Interleukin-6 to study the developmental origin of health and disease. *Scientific Reports.* 2021;11

Griesmann H., Mühl S., Riedel J., Theuerkorn K., Sipos B., Esposito I., **Heuvel G.B.V.**, Michl P. Cux1 enhances pancreatic cancer formation by synergizing with kras and inducing mek/erk-dependent proliferation. *Cancers.* 2021;13(10)

Ton E, Stevens C, Moorman P, Markey M, Hunter B, Bush R, **Jones P.** Thirteen Cases of Valeryl Fentanyl in Michigan: A Call for Expanding Opioid Testing. *Am J Forensic Med Pathol.* 2021 Dec 1;42(4):367-372. doi: 10.1097/PAF.0000000000000722. PMID: 34793410

Morton E, Prahlow JA, Ianni J, **Jones P Jr**. Novel Evaluation of Submandibular Salivary Gland Tissue for Use as an Alternative Postmortem Toxicology Specimen. *Am J Forensic Med Pathol.* 2021 Dec 1;42(4):328-334. doi: 10.1097/PAF.0000000000000708. PMID: 34475318

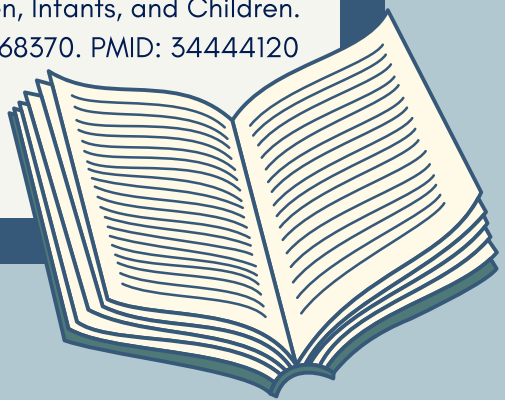
Cintron-Colon AF, Almeida-Alves G, VanGyseghem JM, **Spitsbergen JM.** GDNF to the rescue: GDNF delivery effects on motor neurons and nerves, and muscle re-innervation after peripheral nerve injuries. *Neural Regen Res.* 2022 ;17(4):748-753. doi: 10.4103/1673-5374.322446. PMID: 34472460.

Hack SJ, Kinsey LJ, **Beane WS**.An Open Question: Is Non-Ionizing Radiation a Tool for Controlling Apoptosis-Induced Proliferation? *Int J Mol Sci.* 2021 Oct 16;22(20):11159. doi: 10.3390/ijms222011159. PMID: 34681819

Kim S, Miller M, Tallarico A, Helder S, **Liu Y, Lee S.** Relationships between physical characteristics and biomechanics of lower extremity during the squat.
J Exerc Sci Fit. 2021 Oct;19(4):269-277. doi: 10.1016/j.jesf.2021.09.002. Epub 2021 Oct 4.PMID: 34712337

Cone SM, **Lee S**. Lower Limb Force Asymmetries During Landing and Jumping Exercises: A Pilot Study. *Int J Exerc Sci.* 2021(1);14(1):544-551. eCollection 2021.PMID: 34055145

Rojhani A , Ouyang P, Gullon-Rivera A , Dale TM. Dietary Quality of Pregnant Women Participating in the Special Supplemental Nutrition Program for Women, Infants, and Children. *Int J Environ Res Public Health.* 2021;18(16):8370. doi: 10.3390/ijerph18168370. PMID: 34444120



FEATURED STUDENT SCIENTIST:

Jefferson DeKloein, from Dr. Li's lab

Hypoxic conditioning makes muscle cells express anti-apoptosis protein XIAP and improves cell survival

INTRODUCTION: Cell death is a significant challenge in myoblast transplantation. Transplantation of myoblasts has been shown to be beneficial in replacing diseased or injured skeletal muscles (1, 2). A large share of the transplanted cells die within the first 24 hours (3). Here we show that hypoxic pre-conditioning can increase cell survival in severe hypoxia and under oxidative stress. This hypoxia pre-conditioning increases expression the anti-apoptosis protein X-linked inhibitor of apoptosis (XIAP) in muscle cells which may be an important factor in promoting cell survival.

METHODS: C2C12 cells were cultured in simulated hypoxia (HYP) at 5% O2 for 24, 48, and 72 hours and controls had room oxygen (21% O2). HYP cells and control cells were then exposed to severe hypoxia (1% O2) or oxidative stress (100µM H2O2) for 12, 24, and 48 hours. Cell viability was tested after severe hypoxia and oxidative stress. Cells were then mixed with trypan blue (1:1) and counted with Corning's CytoSmart Cell Counter (Corning, Life Sci). Cells pre-conditioned for 48hours and control cells were stained with DAPI after severe hypoxia to assess membrane integrity. Results were quantified using flow cytometry. Apoptotic gene expression after hypoxia preconditioning for 24 and 48 hours was measured using qPCR. XIAP expression was confirmed through Western Blot.

RESULTS: Hypoxia preconditioning reduced C2C12 cell death from oxidative stress (100µM H2O2) as well as severe hypoxia (1% O2) (Fig1.C&D). Compared to the 24 hour timepoint, the difference in survival was more pronounced after 48 hours of exposure to either oxidative stress or severe hypoxia. Flow cytometry analysis of DAPI stained cells showed a reduction in the percentage of dead cells from 1.496% (control) to 1.008% (Fig 1E). qPCR results showed increased expression of XIAP after 24 and 48 hours of hypoxia preconditioning (Fig 2A&B). Western blot analysis revealed XIAP protein levels were elevated after hypoxia preconditioning (Fig. 2C).

REFERENCES: *1. Skuk D, et al. First test of a "high-density injection" protocol for myogenic cell transplantation throughout large volumes of muscles in a Duchenne muscular dystrophy patient: eighteen months follow-up. Neuromuscular Disord. 2007;17(1):38-46. 2. Beauchamp, JR., et al. Dynamics of myoblast transplantation reveal a discrete minority of precursors with stem cell–like properties as the myogenic source. J cell biology,1999:144(6);1113-22. 3. Duckett, CS et al. Human IAP-like protein regulates programmed cell death downstream of Bcl-xL and cytochrome c. Mol cell bio 1998:18(1), 608–615.*

FIGURES:

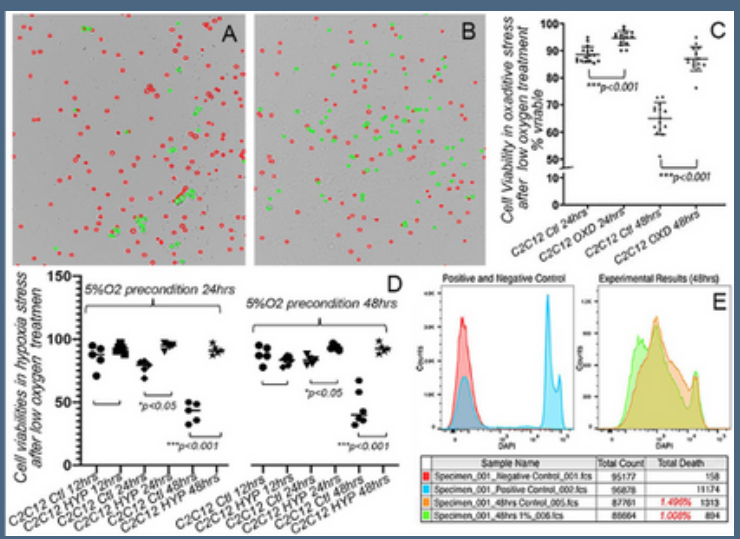


Figure 1. Hypoxia pre-conditioning increases cell survival in severe hypoxia. CytoSmart viability counts of Myoblasts (C2C12) exposed to severe hypoxia with preconditioning (B) compared to control (A) where green cells were determined viable and red non-viable. HYP preconditioning increases viability under oxidative stress (C) and severe hypoxia (D). Flow cytometry of DAPI staining (E) with red negative control, blue positive control. Orange control showing higher cell death (1.496%) than HYP preconditioned cells (1.008% cell death)

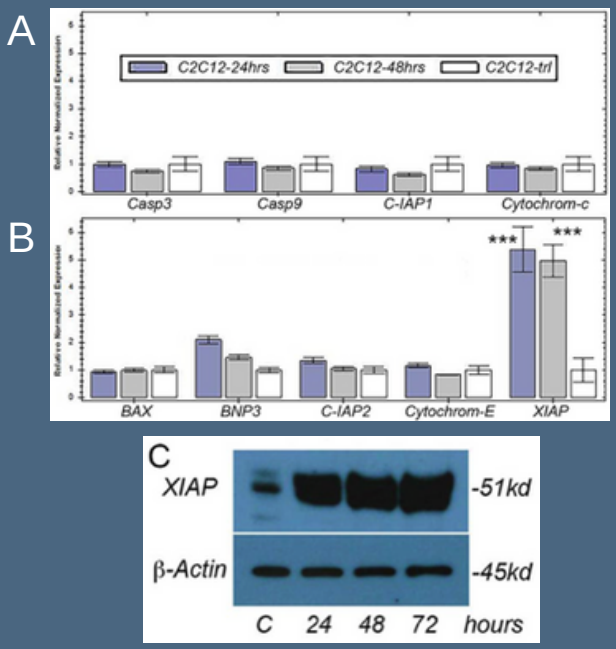


Figure 2. XIAP promotion. The screened apoptotic genes in HYP-treated C2C12 by qPCR, only XIAP is significantly increased in the low oxygen stimulation (A&B) and isconfirmed the expression of the proteins by Western blot (C). ***P<0.001.

FEATURED STUDENT SCIENTIST:

Jefferson DeKloein, Dr. Li's lab

Q: Classically, hypoxia stimulates the Hypoxia Induce Factors (HIFs) to activate the cascades in hypoxic conditions. Dose XIAP activation through HIF signaling pathways?

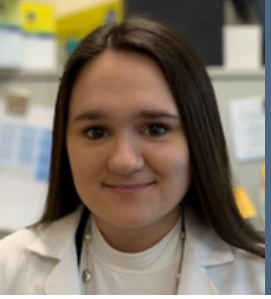
These experiments show both an increase in HIF and XIAP after hypoxia but the mechanism of how the two proteins interact has not fully been elucidated. In other sets of experiments, we have used Cobalt Chloride to stabilize HIF in normoxia. This stabilization of HIF also increased XIAP expression, suggesting that HIF is involved in the hypoxic stimulation of XIAP.

Q: This study was selected to investigate muscle cells. Are there any other lineages that have also been promoted the expression of XIAP during hypoxic conditions?

We have shown similar results in the NIH 3T3 line, a mouse fibroblast derived cell line, suggesting that this phenomenon is not limited to muscle cells. Tissues vary in their tolerance for hypoxia, and we might expect different results in cell lines derived from more sensitive tissues.

Q: What is your strategy to use the innovative discovery of XIAP expression in muscle cells with hypoxia stimulation? What is your next step in the research plan?

Currently, one limitation of muscle stem cell transplantation for the treatment of muscular dystrophies is the high rate of transplanted cell death after transplantation. We've demonstrated that hypoxic preconditioning of muscle cells can increase survival of those muscle cells to a variety of insults. The process of hypoxic conditioning is time consuming, so if we can target XIAP expression with pharmaceutical agents and achieve similar improvements to survival, this would allow for more efficient and successful muscle transplantation



FEATURED STUDENT SCIENTIST:

Lilly Ruell, from Dr. Akkouch's lab

The Effect of Non-oxidized LDL Particles and Oxidized LDL particles on Osteoblast Differentiation

INTRODUCTION: Oxidized-LDL particles are known to cause inflammation within the body and contribute to an increased risk of cardiovascular disease. Atherosclerotic calcification and bone mineralization share a number of common features. It is a highly organized process that is regulated by mechanisms similar to those involved in bone mineralization. There are many reports that cardiovascular disease and osteoporosis may go hand in hand. The mechanism to which this happens is not known. Lipoproteins and lipids may accumulate in the subendothelial matrix of human bone vessels as they do in the vascular endothelium. Since osteoblast cells are primarily adjacent to the subendothelial spaces, they may be exposed to inflammatory lipoproteins and lipids. We hypothesize that these lipoproteins, specifically LDL particles, play a role in this connection between cardiovascular disease and osteoporosis specifically contributing to the differentiation of osteoblast cells. Our study aims to demonstrate the cytotoxicity of oxidized LDL and non-oxidized LDL on bone cells and to understand their effect on inflammation and osteogenic/adipogenic differentiation of human osteoblasts.

RESEARCH PLAN: The goal of this study is to test the effect of non-oxidized LDL particles and oxidized LDL particles on osteoblast differentiation.

- Objective 1: To test the cytotoxicity of oxidized and non-oxidized LDL particles on osteoblasts using SAOS2, a human osteosarcoma cell line.
- Objective 2: To determine whether oxidized and non-oxidized LDL induce adipogenic differentiation of SAOS2 cells.

APPROACH: 1. Cytotoxicity: LDL will be oxidized using the Fenton Reaction. SAOS2 human osteosarcoma cells will be cultured using 10 concentrations of both oxidized LDL and non-oxidized LDL: 0, 1, 5, 10, 25, 50, 75, 100, 150, 200 (µg/mL). Supernatants will be taken at 6hr, 12hr, 24hr, 3 day, 5 day, 7day and tested using a lactate dehydrogenase cytotoxicity test. An alamar blue stain will also be performed at these time points in order to characterize the cell proliferation.
2. Differentiation: After determining optimal concentrations of both oxidized LDL and non-oxidized LDL as per objective one, these conditions will be used to study the role of lipoproteins in osteoblast differentiation. Cells will be examined for changes in morphology, stained using Alizarin red staining, their expression in gene markers will be analyzed via qPCR.

SIGNIFICANCE: We will be able to demonstrate the cytotoxicity of oxidized LDL and non-oxidized LDL on bone cells. We will also be able understand the effect of oxidized LDL and non-oxidized LDL on inflammation and osteogenic/adipogenic differentiation of human osteoblasts.

FEATURED STUDENT SCIENTIST:

Lilly Ruell, Dr. Akkouch's lab

Q: What are LDL Particles?

LDL Particles, known as low-density lipoprotein particles, are bi-products from fat transport. In circulation over time these particles can penetrate the artery wall and form plaque. This plaque can form blockages and rupture leading to heart attack and strokes. LDL Particles contain approximately 50% cholesterol (free and esterified), 25% proteins, 20% phospholipids and 5% triglycerides. The LDL particles house LDL cholesterol.

Q: How do osteoblasts come in contact with LDL Particles?

Lipoproteins and lipids may accumulate in the subendothelial matrix of human bone vessels as they do in the vascular endothelium. Since osteoblast cells are primarily adjacent to the subendothelial spaces, they can be exposed to these inflammatory lipoproteins and lipids.

Q: What is the difference between LDL and HDL? Which one is considered bad for our health?

LDL stands for low-density lipoprotein and HDL stands for high-density lipoprotein. LDL is known as bad cholesterol because it transports cholesterol to our arteries where it then can accumulate in artery walls. LDL is approximately 50% cholesterol and 25% protein. LDL contain proteins called B-100 proteins. Higher amounts of LDL increases risk of atherosclerosis. HDL is known as good cholesterol because it transports cholesterol to our liver for expulsion from the body. HDL consist of 20% cholesterol and 50% protein. HDL particles contain A-I and A-II proteins.

New Collaborating Faculty Members:

Gregory Vanden Heuvel, PhD

Dr. Vanden Heuvel, is Associate Dean for Research and Professor of Biomedical Sciences of WMed. He has worked in the kidney development field for over thirty years, and in the polycystic kidney disease field for over twenty-five years. His research goals have been to understand the molecular mechanisms that regulate cell growth and differentiation during kidney formation and to determine whether these mechanisms are altered in the progression of polycystic kidney disease (PKD). He has supervised over 50 students in his laboratory at all levels, many of whom have gone on to careers in scientific research and medicine.

John M Spitsbergen, PhD

Dr. Spitsbergen is the Chairman and Professor of Dept. of Biological Science, at WMU. The focus of Dr. Spitsbergen's research is on understanding the regulation of neurotrophic factor expression in target tissues of the peripheral nervous system (blood vessels, cardiac muscle and skeletal muscle) and to determine the consequences of altered neurotrophic factor expression with sedentary aging and with exercise.

Wendy Scott Beane, PhD

Dr. Beane, is a Presidential Professor of Innovation and Associate Professor of Biological Sciences at WMU. She obtained her Ph.D. in Cellular, Molecular, and Developmental Biology from Duke University and did her postdoctoral fellowship at Harvard and Tufts Universities in ion channel regulation of stem-cell mediated regeneration. In particular, her lab is interested in non-invasive ways, such as weak magnetic field exposure, to control stem cell activity in regenerative medicine and carcinogenesis. By utilizing an interdisciplinary approach, this research aims to determine whether quantum effects can be used therapeutically to control tissue growth in vivo.

New Collaborating Faculty Members:

Jered Cornelison, PhD

Dr. Cornelison, is currently an Assistant Professor (promoting to Associate Professor in July 2022) in the Department of Pathology and WMed Research Integrity Officer. Dr. Cornelison has multiple presentations and publications on topics including human identification, pediatric trauma, cranial fracture healing, decalcification of human bone, skeletal processing, research integrity, and bioarcheology.

Robert Baker, MD, PhD

Dr. Baker is the Director of the Primary Care Sports Medicine Fellowship at Western Michigan University School of Medicine Clinics. In addition, he is the Team Physician for Western Michigan University and Professor of Clinical Medicine at Western Michigan University School of Medicine and Michigan State University. His particular areas of interest include: Exercise-Induced Asthma, Athletes with diabetes and other chronic diseases, back pain in adolescent and adult athletes, and non-surgical orthopedic injuries in athletes.

Kristi Bailey B.S., HTL

Kristi Bailey is the Lead Histotechnologist of the Research Histology Lab located at the Western Michigan University Homer Stryker M.D. School of Medicine since the summer of 2016. She graduated with a B.S. in Biochemistry and Biology from Western Michigan University. The lab is part of the Pathology department and provides histology services to the Office of the Medical Examiner, as well as internal and external research collaborators. Areas of expertise include immunohistochemistry, bone processing, neuropathology, and veterinary research.

New Resident Members:

Ayooluwa Ayoola, MD

Ayooluwa Ayoola, MD, is a resident physician in the Orthopaedic Surgery Residency Program at Western Michigan University Homer Stryker M.D. School of Medicine. She received her undergraduate degree in Mechanical Engineering from Calvin University. She received her medical degree from Wayne State University in Detroit, Michigan. At Wayne State University she worked on research projects focused on patient outcomes following joint replacements. Her research and clinical interests include improving access to healthcare to underserved populations, as well as improving patient outcomes following orthopedic interventions.

Shaan Manawar, MD

Shaan Manawar, MD earned his B.S. in Biomolecular Science from the University of Michigan and medical degree from WMed. During medical school, his research interests included long-term outcomes on robotic total hip arthroplasty. Currently, his research interests are in the socio-economic factors related to SCFE patients and management of critical long bone defects. Shaan is currently working on a potential translational bone matrix project in Dr. Li's lab.

New Student Members:

Avery Waldron

Avery is a second-year medical engineering master's student who graduated with a B.S. in Mechanical Engineering from the University of Michigan. Currently he is studying the accuracy of the "push/thumb test" that occurs during orthopedic procedures, specifically joint replacements.

OUR RESEARCH TEAM

FACULTY

Adil Akkouch, PhD	• Assistant Professor, Program in Medical Engineering Assistant Professor, Department of Orthopaedic Surgery
Keith Kenter, MD	• Associate Dean, Clinical Affairs; Chair, Department of Orthopaedic Surgery Professor, Program in Medical Engineering Professor, Department of Orthopaedic Surgery
Yong Li, MD PhD	• Chief, Medical Engineering, Department of Orthopaedic Surgery Professor, Program in Medical Engineering Professor, Department of Orthopaedic Surgery
Haiying Pan, MS MBA	• Lab manager, Li Lab

LAB MEMBERS
Akkouch Lab

CURRENT PROJECT

Guston Zervoudakis, MD	Adipokines on the pathogenesis of osteoarthritis
Letty Thotahill (M4)	3D printable organ-on-a-chip microfluidic devices
Emily Beck (M3)	Role of CUX1 on scoliosis pathogenesis
Zeena Qiryaqoz (M3)	Barrier Constructs for Bone Engineering
Lucas Bezerra (M2)	Effect of blood flow restriction on bone density
Shelby Chaney (M2)	Cartilage spheroids fabrication using 3D printing
Monty Randhawa (M2)	Biomimetic scaffolds with enhanced mechanical properties
Suhaib Ellythy (M1)	Cytotoxicity levels of Nb and Sr on osteoblasts
Hong Phan (M1)	MicroRNAs in osteogenesis and adipogenesis
Kunal Ranat (M1)	Meshes and scaffolds via 3D bioprinting to research
Ravi Trivedi (M1)	Effect of acetylcholine in bone healing
Michelle Zhang (M1)	Synthesis of sensitive, antibacterial biomaterial
Lilly Ruell (MS in Medical Engineering)	Bioactive inflammatory lipoproteins (LDL) in regulation of osteogenesis and adipogenesis
Avery Waldron (MS in Medical Engineering)	3D printed bone substitute characterization and mechanical properties

OUR RESEARCH TEAM

LAB MEMBERS *Li Lab*	CURRENT PROJECT
Jordan Boivin, MD (PGY2)	PRP preparation and application
Michael Rahl, MD (PGY2)	Osteoarthritis pain & sacroiliac joint dysfunction
Kelsey Sheets, MD (PGY2)	Adolescent idiopathic scoliosis, and fibrosis
Sumit Patel, M.D, M.S (PGY1)	Fibrosis in orthopaedic tissue healing
Ayooluwa Ayoola, MD (PGY1)	Improving patient outcomes following orthopedic interventions
Shaan Manawar, MD (PGY1)	Use decellularization tissue for bone healing
Loyall Harris (M3)	Exosomes in hypoxia stimulated myogenic differentiation
Rachael Tolsma (M3)	Neuronal differentiation of hypoxia induced stem cells Use of PRP and blood clots in regenerative medicine
Peter Awad (M2)	Blood clots in orthopaedic tissue injury and repair
Jefferson DeKloe (M2)	Cobalt chloride inducing hypoxia-like markers
Victor Hung (M2)	Blood clots in orthopaedic tissue injury repair
Andre Ksajikian (M2)	Fibrosis in musculoskeletal tissue healing
Leah Liu (M2)	Mechanisms of neurogenic muscle atrophy
Max Olson (M2)	Hypoxia treatment for transplanted organs
Juliana Overbey (M2)	Fibrosis in musculoskeletal tissue healing Hypoxia preconditioning treatment on PD-L1 expression
Son Tran (M2)	Fibrosis in musculoskeletal tissue healing
Eliza VanZweden (M2)	Use of blood clots for tissue regeneration
Erica Myrick (M2)	Blood clots in tissue injury repair
Sultan Elhaj (M1)	Blood clots in skin wound healing
Delour Haj (M1)	Blood clots in diabetic tissue injury repair
Mallory Wright (M1)	Hypoxia stimulate muscle regeneration
Joseph Preziosi (M1)	Hypoxia promote XIAP to prevent cell death
Kumail Naqvi (M1)	Blood clots use for tissue regeneration and repair
Leilani Nguyen (M1)	Blood clots use for tissue regeneration and repair
Justin Lee (M1)	Hypoxia stimulates MMP and vEGF expression in muscle cells
Kevin Chen (M1)	Hypoxic condition stimulates angiogenesis in muscle

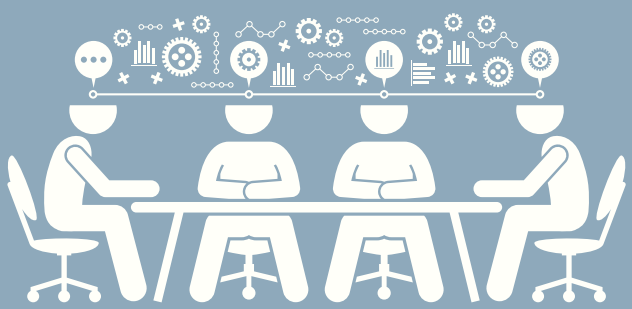
OUR COLLABORATING FACULTY

WMed

Robert Baker, MD, PhD	Professor and Director of the Primary Care Sports Medicine Fellowship, Dept. of Orthopaedic Surgery and Clinic Medicine
Karen Bovid, MD	Assistant Professor, Department of Orthopaedic Surgery, and Department of Pediatric and Adolescent Medicine.
Jered Cornelison, PhD	Assistant Professor, Department of Pathology,
Prentiss Jones, PhD	Associate Professor, Department of Pathology
Christine Pink, PhD	Assistant Professor, Department of Pathology
Robert Sawyer, MD	Chairman and Professor, Department of Surgery, Program in Medical Engineering Professor
Tyler Snoap, MD	Clinical Assistant Professor, Department of Orthopaedic Surgery
Gregory Vanden Heuvel , PhD	Associate Dean for Research, and Professor in the Department of Biomedical Sciences
Ramona Wallace, DO	Assistant Professor, Department of Family and Community Medicine
Joseph K Weistroffer, MD	Residency Program Director, Department of Orthopaedic Surgery Assistant Professor, Department of Orthopaedic Surgery

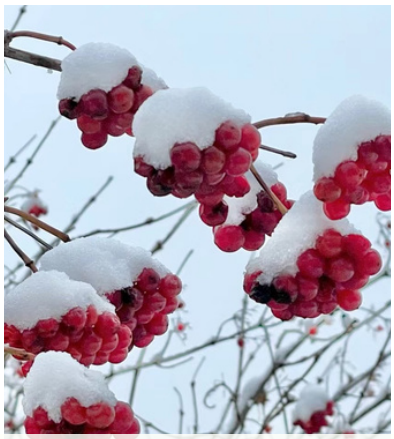
WMU

Wendy Scott Beane, PhD	Presidential Professor of Innovation and Associate Professor, Dept. of Biological Sciences
Jeremy S Duncan, PhD	Assistant Professor, Dept. of Biological Sciences
Stephen E Kaczmarek, PhD	Associate Professor, Carbonate petrology
Cindy Linn, PhD	Professor, Dept. of Biological Sciences
Yuanlong Liu, PhD	Chairman and Professor, Measurement and Evaluation, Dept. of Human Performance and Health Education
Ping Ouyang, PhD	Assistant Professor, Nutrition and Dietetics
Arezoo Rojhani, PhD	Associate Professor, Nutrition and Dietetics, Undergraduate Program Director
Sangwoo Lee, PhD	Associate Professor of Exercise Science
John M Spitsbergen, PhD	Chairman and Professor of Dept. of Biological Science



Winter Pictures

Key West, FL; from Dr. Li



From Dr. Liu

From Dr. Liu

Delour and Mallory with M1 friends in Tennessee's Great Smoky Mountains

Snowy igloos in Suttons Bay, MI
- From Mallory.

From Dr. Li

Victor Hung, Erica Myrick, and Peter Awad (left to right) presenting their blood clot project at OSRS

Happy Chase, Dr. Li's dog

Sunset at Key West, FL; from Dr. Li

Son Tran and Andre Ksajikian at the Asian Pacific American Medical Student Association Conference

Students from the Li lab in Detroit post OSRS

Students from the Li lab in Detroit post OSRS

Editorial Team

Andre Ksajikian
Editor in Chief

Peter Awad
Associate Editor

Mallory Wright
Associate Editor

Past Editors in Chief:

Rachael Tolsma, Winter 2020;
Juliana Overbey, Spring 2021;
Leah Liu, Summer 2021;
Son Tran, Fall 2021.