**William L. Murphy, Ph.D.**
*Associate Professor*
*Department of Biomedical Engineering*
*Director of the Stem Cell and Regenerative Medicine Center*
*The University of Wisconsin*

## Biomaterials that Mimic and Harness Biological Signals

Control over the signals that cells encounter in their local environment is a common theme in natural tissue formation, and also an emerging theme in functional tissue engineering strategies. This concept is particularly important in stem cell-based applications, in which local signals can dictate cell fate decisions. Nature often achieves intricate control over local soluble signaling via non-covalent sequestering. Inspired by nature, we are interested in creating biomaterials that actively sequester soluble molecules, and thereby control cell behavior. In one approach, our recent studies show that modular growth factors can be designed to "decorate" the surface of natural and synthetic biomaterials, including orthopedic implants, allogenic bone grafts, and tissue engineering scaffolds. Results demonstrate enhanced osteogenic differentiation of mesenchymal stem cells and improved new bone formation *in vivo*. In addition, soluble growth factors present in standard biological environments can be harnessed via specific molecular sequestering strategies. We have recently used this type of specific molecular sequestering strategy as a broad mechanism to up-regulate or down-regulate growth factor signaling, and cell behaviors can be controlled by modulating the nature of the sequestering interaction (e.g. binding site, affinity, avidity). In summary, our recent studies show that tailored interactions between signaling molecules and biomaterials may provide an adaptable mechanism to control cell behavior in regenerative medicine applications.

**Brendan Harley, Ph.D.**
*Assistant Professor*
*Department of Chemical and Biomolecular Engineering*
*University of Illinois at Urbana-Champaign*

## Patterning Biomaterials for Tissue Engineering

The extracellular matrix (ECM) is a complex organization of structural proteins such as collagens and proteoglycans. Heterogeneous tissues with spatially and temporally modulated properties and their biomaterial mimics play an important role in organism physiology and regenerative medicine. With the understanding that the microstructure, mechanics, and composition of the ECM is dynamic and often spatially patterned or heterogeneous over the length-scale of traditional biomaterials, there has recently been significant effort aimed at moving away from static, monolithic biomaterials towards instructive biomaterials that provide specialized cell behavioral cues in spatially and temporally defined manners. We have been developing patterned, tunable biomaterial systems to explore the practical significance of how cell/matrix cues can be optimized to improve biomaterial regenerative potential and the mechanistic details of how individual (stem) cells sense, integrate, and respond to multiple microenvironmental signals. We are integrating anisotropic and multi-compartment collagen scaffolds with photolithography-based biomolecule patterning tools for the regenerative repair of orthopedic defects. We are also creating multi-gradient biomaterials to investigate fundamental questions regarding niche-mediated regulation of hematopoietic stem cell (HSC) behavior. Here microfluidic tools aid our investigation of the role played by matrix elasticity, ligand presentation, and paracrine-mediated signaling on HSC fate.

**J. Zach Hilt, Ph.D.**
*Associate Professor*
*Department of Chemical and Materials Engineering*
*University of Kentucky*

## Nanocomposite Polymer Networks: From Controlled Synthesis to Applications as Biomaterials

In my laboratory, we apply chemical engineering fundamentals to the rational design, synthesis, and application of novel nanoparticle systems and macromolecular materials. We are particularly interested in designing and applying advanced materials based on nanocomposite hydrogels. Although the majority of hydrogel applications have been in biology and medicine, there is great promise for these materials to impact other areas, especially as nanocomposites.

Nanocomposite hydrogels are a new class of advanced materials, which have recently attracted interest as biomaterials and intelligent materials. The incorporation of nanoparticles into a hydrogel matrix can provide enhancement to properties (e.g., mechanical, electrical, etc.) or introduce new and unique properties such as remote actuation. These resultant properties of the nanocomposites can be easily tailored by manipulating the composition of the hydrogel and the nanoparticulate material. Here, our broad activities in the development and application of hydrogel nanocomposites will be presented. In particular, hydrogel nanocomposites with magnetic particles will be highlighted, which have been demonstrated as potential candidates in combination cancer therapies, pulsatile drug delivery, and soft actuator applications. In addition, our recently developed methodology for synthesizing stable functionalized nanoparticles with improved properties, where nanoparticles are isolated, functionalized, and then released (ISOFURE platform), will be presented.

**Yoon Yeo, Ph.D.**
*Assistant Professor*
*Department of Industrial and Physical Pharmacy*
*Department of Biomedical Engineering*
*Purdue University*

## Zwitterionic Chitosan Derivative as a New Functional Biomaterial

Chitosan is a cationic polymer of natural origin and has been widely explored as a pharmaceutical excipient for a broad range of biomedical applications. While generally considered safe and biocompatible, chitosan has the ability to induce inflammatory reactions, which varies with the physical and chemical properties. We hypothesized that the previously reported zwitterionic chitosan (ZWC) derivative had relatively low pro-inflammatory potential because of the aqueous solubility and reduced amine content. To test this, we compared various chitosans with different aqueous solubilities or primary amine contents with respect to the intraperitoneal (IP) biocompatibility and the propensity to induce pro-inflammatory cytokine production from macrophages. ZWC was relatively well tolerated in ICR mice after IP administration and had no pro-inflammatory effect on naïve macrophages. Comparison with other chitosans indicates that these properties are mainly due to the aqueous solubility at neutral pH and relatively low molecular weight of ZWC. Interestingly, ZWC had unique ability to suppress cytokine/chemokine production in macrophages challenged with lipopolysaccharide (LPS). This effect is likely due to the strong affinity of ZWC to LPS, which inactivates the pro-inflammatory function of LPS, and appears to be related to the reduced amine content. Our finding warrants further investigation of ZWC as a functional biomaterial.

**Chien-Chi Lin, Ph.D.**
*Assistant Professor*
*Department of Biomedical Engineering*
*Indianapolis University-Purdue University at Indianapolis*

## PEG-peptide Hydrogels Formed by Thiol-Ene Photo-Click Reactions for Controlled Release and Tissue Engineering Applications

Poly(ethylene glycol) or PEG-based hydrogels have been widely used as platforms for 3D cell culture and for protein and cell delivery. PEG hydrogels formed by step-growth thiol-ene photopolymerizations have recently emerged as attractive and versatile matrices for tissue regeneration applications. Compared to chain-growth PEG diacrylate (PEGDA) hydrogels, thiol-ene photo-click hydrogels have homogeneous network structures, higher monomer conversion, and enhanced mechanical properties. Thiol-ene click reactions also preserve all of the preferential properties offered by photopolymerizations, specifically rapid and spatial-temporal controlled over gelation kinetics. In addition, thiol-ene PEG hydrogels can be rendered biodegradable by using enzyme sensitive peptide sequences as crosslinkers.

Here, we synthesized PEG-peptide hydrogels by thiol-ene photo-click reactions that were both hydrolytic and enzymatic degradable. The degradation profiles were highly tunable within therapeutically relevant time scales. More importantly, these diverse degradation profiles were achieved using simple hydrogel chemistry. We also studied biophysical properties and cytocompatibility of step-growth photopolymerizations using pancreatic beta cells (MIN6), which are prone to cellular damage by radical species. We found that step-growth thiol-ene click reactions are highly cytocompatible for pancreatic beta cells, even when cells were encapsulated at very low density. MIN6 beta cells not only had high viability in thiol-ene click hydrogels, they also proliferated to form cell spheroids. Furthermore, when a chymotrypsin Invited Session Speakers sensitive peptide sequence was used as gel crosslinker, insulin-secreting cell spheroids were rapidly and safely recovered (within 5min) from the thiol-ene hydrogels via a surface erosion mechanism. These studies demonstrated the advantages and versatility of thiol-ene hydrogels for tissue engineering and regenerative medicine applications.

**Anirban Sen Gupta, Ph.D.**
*Assistant Professor*
*Department of Biomedical Engineering*
*Case Western Reserve University*

## Biomimetic Approaches in Rational Design of a Synthetic Platelet

Platelet transfusion is clinically important for treating congenital or induced platelet disorders, and also in managing bleeding complications during acute surgery or in traumatic injury. Current natural platelet-based transfusion products (e.g. cold-stored platelet concentrates) are expensive, have short storage life (3-7 days), and pose significant risks of contamination and immunoreactions. Hence there is considerable clinical interest in platelet-mimetic synthetic hemostats that are less expensive, have long storage life, and can avoid the biological risks. The two main hemostatic functions of natural platelets are to 'promote adhesion' to the vascular injury site by binding to proteins like von Willebrand factor (vWf) and collagen, and to 'promote aggregation' via fibrinogen-mediated inter-platelet binding of the platelet surface integrin GPIIb-IIIa. Past research in synthetic platelet substitutes have mostly focused on amplifying only the 'aggregation' function by developing particle constructs surface-modified with pro-aggregatory proteins (e.g. fibrinogen) or peptides (e.g. RGD). These constructs do not have the property of adhesion-specificity to vascular injury site proteins and hence have minimal control over the location of 'aggregation'. This may pose the risk of thromboembolic complications from free-floating aggregates. We rationalize that particle constructs that mimic the dual hemostatic properties of platelets simultaneously, i.e., matrix adhesion and platelet aggregation promotion under hemodynamic shear, can act as highly effective synthetic platelets. Also, to exhibit the platelet-mimetic dual hemostatic properties, the particle constructs will need to marginate to the vessel wall in a hemodynamic flow environment. For natural platelets this



transport towards the wall is guided by morphological parameters like platelet's spheroidal shape and dimensions. Hence we rationalize that particle constructs with spheroidal shapes and specific aspect ratios will have a higher probability of marginating to the wall under hemodynamic flow and hence have a higher efficiency to exhibit the platelet-mimetic biological functions. Hence our research is focused on independently optimizing the size, shape and surface-modification parameters of particle constructs to identify the ideal properties for platelet mimicry and subsequently integrate them on one construct for an effective synthetic platelet design.

To test the biological parameters, we have developed liposomal constructs surface-modified by three hemostatically active peptides, namely a vWf-binding peptide (VBP) and a collagen-mimetic peptide (CMP) to enable matrix adhesion and stabilization under shear, and a GPIIb-IIIa-binding cyclic RGD peptide (cRGD) to enable platelet aggregation promotion specifically at the site of particle adhesion. We have studied the biological action of these constructs under ranges of shear on vWF/collagen-coated surfaces in a parallel plate flow chamber (PPFC). VBP and CMP modified constructs showed enhanced binding to the vWF/collagen mixed surface, compared to unmodified constructs. Liposomes bearing both VBP and CMP showed higher binding to the vWF/collagen-coated surface under shear, compared to liposomes bearing any one

peptide, suggesting cooperative action of the two adhesion mechanisms. cRGD-modified liposomes showed significant promotion of platelet aggregation, compared to unmodified liposomes. When integrated together, liposomes simultaneously bearing both ‘adhesive’ and ‘aggregatory’ functionalities (all three peptides) showed significant ability to adhere onto vWF/collagen surface and promote platelet aggregation on the surface at sites of particle adhesion, validating our design approach. In parallel studies, spheroidal polymeric particles about 2μ in major axis showed higher margination and retention to PPFC wall compared to spherical particles of the same volume. Our current research is focused on combining the morphological parameters with the biological parameters to identify the optimum shape, size and ligand surface-density for the constructs to exhibit maximum margination, adhesion and platelet aggregation promotion, towards establishing an effective design of synthetic platelets.

**Institution**: Illinois Institute of Technology
**Title**: The Role of Pore Size on Vascularization and Tissue Remodeling in PEG-based Hydrogels
**Authors**: Y.C. Chiu, M.H. Cheng, H. Engel, S.W. Kao, J.C. Larson, S. Gupta, and E.M. Brey
**Objective**: Porous scaffolds have been show to enhance scaffold vascualrization and tissue regeneration. In this study, we describe a method for generating porous PEG-based hydrogels, characterize the influence of synthesis conditions on polymer properties, and investigate the relationship between pore size and vascularization.
**Methods**: A particulate leaching technique was developed to generate porous PEG hydrogels with pore size ranging from 50-25, 100-50, and 150-100 mm. Porous PEG and PEG-poly(lactic acid) (PEG-PLLA) copolymer hydrogels were synthesized. Confocal microscopy, compression testing, and swelling analysis analyzed the hydrogels. Vascularization was exam by 3D culture and subfascia implantation.
**Results**: Both *in vitro* and *in vivo* studies agree with that the speed of neovascularization depended on pores size. *In vivo*, mature vasuclarized collagen was observed in the pores by week 2. While collagen rich tissue completely filled scaffolds in the 150-100 and 100-50 mm pore size groups, it was limited in the smallest size group. The largest pore size permitted the more rapid vessel invasion. The pore structure of the hydrogels monitored throughout degradation using confocal microscopy. Swelling ratios and compressive modulus were monitored to assess degradation. Degradation time could be controlled based on polymer percentage but not pore size.
**Conclusions**: Results indicated that neovascualrization of both *in vitro* and *in vivo* mediated by pore size. Degradation of PEG-PLLA porous hydrogel is based on polymer percentage. This study provides important insight into the role of physical structure on tissue response to porous PEG scaffolds.

**Institution**: The University of Wisconsin
**Title**: Hydrogels with well-defined peptide-hydrogel spacing and concentration
**Authors**: M. Wilson, S.J. Liliensiek, W.L. Murphy, and P.F. Nealey
**Objective**: Determine the extent peptide-hydrogel spacing in well-defined synthetic hydrogels controls the availability of bioactive cell adhesion ligands and the subsequent interactions with cells.
**Methods**: Different lengths of hydrophilic PEG spacers were used to separate an acryloyl functional group from the RGD cell adhesion ligand. Cell attachment and spreading assays probed the availability of the covalently bound ligands for cell interactions and competitive binding assays studied the specificity and relative affinity of the receptor-ligand interactions
**Results**: Using solid phase techniques, peptides can be conjugated to pure monodisperse heterobifunctional PEG reagents (Fmoc-NH-(PEG)x-COOH) where x is 5, 11 or 27 and functionalized with an acryloyl functional group. These monodisperse conjugates are easily purified. As the PEG spacer length increases, the RGD concentration required to support cell attachment and spreading decreases. The competitive detachment of hTCEpi cells in the presence of soluble linear RGD also shows non-linear dependence on the PEG spacer length, as more cells remained attached and spread on gels functionalized with longer PEG-RGD conjugates in comparison to the shorter PEG-RGD conjugates.
**Conclusions**: The described method for fabrication of pure, monodisperse acryloyl-PEGx-RGD conjugates provides an adaptable and controlled approach to investigate peptide-hydrogel spacing. This approach to present peptides is likely generalizable to most hydrogel chemistries and is highlighted by the ability to decrease the minimum peptide concentration needed for cell attachment and spreading. While the incorporation of one bioactive ligand, RGD, is shown, the approach can potentially be used for controllable incorporation of multiple cues that better mimic the native extracellular matrix. Hydrogel-peptide spacing critically impacts the presentation and availability of bioactive ligands and is a necessary consideration in the development of well-defined biomaterials.

**Institution**: Purdue University
**Title**: Thermoresponsive Collagen Peptide-Based Hydrogel for Three-Dimensional Cell Culture
**Authors**: C.M. Rubert-Pérez, A. Panitch and J.A. Chmielewski
**Objective**: We have developed collagen peptide-based hydrogels by attaching collagen mimetic peptides to a multi-arm PEG star polymer. These physical hydrogels possess thermoresponsive properties and promising viscoelastic properties for three-dimensional cell culture and the potential for differentiation of human mesenchymal stem cells (hMSCs).
**Methods**: Collagen peptides were synthesized by solid-phase peptide synthesis (SPPS). The resulting collagen peptide-based hydrogels were characterized by circular dichroism, rheology and scanning electron microscopy. For the cell culture experiments, hMSCs encapsulated within the hydrogels were submitted to MTS viability assays and to real-time PCR for gene expression experiments.
**Results**: 4% and 8% PSP-POG8 hydrogels were found to have the ability to melt into a liquid-like state near the melting temperature of the peptide and reform back into an elastic-solid at room temperature. By changing the length of the collagen peptide, the melting temperatures of these hydrogels were altered. In addition, the hydrogel possessed desirable stiffness to function as a three dimensional scaffold for the culture of hMSCs. Preliminary cell culture studies have shown the potential of the hydrogels to induce differentiation of hMSCs into osteocytes, as determined the overexpression of certain osteogenic genes after a week of cell growth.
**Conclusions**: We have developed a collagen peptide-based hydrogel with a highly porous morphology and appropriate viscoelastic properties in efforts to generate a matrix for the cell culture and the potential of stem cell differentiation. Also, the hydrogels were found to have a thermoresponsive feature due to the temperature dependent stability of the collagen triple helix. Since the collagen peptide plays crucial part in the properties of the hydrogel, altering the peptide sequence can be bring forward favorable changes to the material for a more ECM-like material with the addition of cell signaling or cell adhesion peptide sequences.

**Institution**: University of Kentucky
**Title**: Tailoring the Mineralized Surface with Parathyroid Hormone for Tissue Engineering Applications
**Authors**: J.N. Yewle, D.A. Puleo, and L.G. Bachas
**Objective**: To tailor the mineralized surfaces by site-specific immobilization of parathyroid hormone (PTH) through bifunctional hydrazine bisphosphonates (HBPs) and study their *in vitro* cell interactions.
**Methods**: Bone wafers were used as model mineralized surfaces. PTH was selectively oxidized with sodium periodate treatment, and the change in activity of PTH was determined. The oxidized PTH was site-selectively conjugated to HBPs of various length and hydrophilicity. The binding affinities of the conjugates to hydroxyapatite were determined. The conjugates were immobilized on the bone surface, and the cell interaction with the immobilized PTH was determined by measuring the cAMP produced by pre-osteoblasts.
**Results**: The activity of the oxidized PTH was found to be similar to that of the non-oxidized PTH. The hydroxyapatite binding studies demonstrated enhanced affinity of PTH to the mineral surface after its conjugation to HBPs. Quantification of the cAMP produced by pre-osteoblasts showed that immobilized PTH stimulated greater cell activity than did simply adsorbed PTH.
**Conclusions**: Site-specific conjugation of PTH to HBPs enhanced the affinity of the peptide to bone mineral. Furthermore, immobilization of PTH to mineralized surfaces through HBPs showed increased cell activity compared to adsorbed PTH. This approach of protein immobilization will be useful for tissue engineering and drug delivery applications.

**Institution**: Illinois Institute of Technology
**Title**: PEGDA Hydrogel Gradients Formed by PBFP Direct Cell Behavior
**Authors**: M.V. Turturro and G. Papavasiliou
**Objective**: Cell behavior is naturally guided by the spatial presentation of growth factors, immobilized extracellular matrix molecules and matrix stiffness. This study aims to recreate these gradients synthetically in poly(ethylene glycol) diacrylate (PEGDA) hydrogels with the goal of enhancing cell invasion and tissue formation.
**Methods**: PEGDA hydrogels containing gradients of elastic modulus and immobilized adhesion sequences were formed using perfusion based frontal polymerization (PBFP). The effect of these gradients on cell behavior was evaluated by seeding aggregates of fibroblast cells on the surface (2D) or within (3D) the hydrogels and monitoring cellular outgrowth over time.
**Results**: PBFP resulted in PEGDA hydrogels with up to a 47% decrease in crosslink density, an 81% decrease in elastic modulus and a 61% decrease in YRGDS concentration over 8 mm. The magnitude of PBFP gradients was manipulated through variations in polymerization conditions. *In vitro* cell studies indicate that by day 14 fibroblast aggregates seeded on the surface of these gradients spread twice as far in the direction parallel to the gradient than in the perpendicular direction. Aggregates seeded within PBFP hydrogels exhibit directed invasion both up and down the measured gradients.
**Conclusions**: PEGDA hydrogels formed by PBFP resulted in simultaneous and tunable gradients in physical and mechanical properties as well as immobilized YRGDS. These gradients are capable of stimulating directional cellular outgrowth in 2D as well as cell invasion in 3D. Future studies will focus on further quantifying the observed 3D response as a function of both gradient magnitude and position of the aggregate along the gradient. PBFP shows great promise for generating synthetic scaffolds capable of enhancing directed cell invasion in 3D systems and may benefit numerous tissue-engineering applications.

**Institution**: Purdue University
**Title**: Collagen Oligomers Enhance Matrix-Induced Vasculogenesis by Endothelial Colony Forming Cells
**Authors**: C.F. Whittington, P.J. Critser, K.Y. Teo, M.C. Yoder, B. Han, and S.L. Voytik-Harbin
**Objective**: Until recently, intermolecular cross-links have not been considered a major tunable parameter in 3D collagen matrix design because traditional collagen formulations lack mature cross-links. Here we show that intermolecular cross-links modulate matrix biophysical properties that are important for guiding vessel formation of endothelial colony forming cells (ECFC) *in vitro*.
**Methods**: Collagen monomers and oligomers were purified from pig skin and tendon to create formulations that differ in cross-link amount and chemistry. Matrix polymerization kinetics, microstructure, and viscoelastic and transport properties were quantified. 3D cultures of ECFC were also assessed for their vessel forming capacity (i.e. network and lumen formation).
**Results**: Intermolecular cross-linking (oligomers) increases interfibril branching that decreases projected pore size and diffusion coefficients and contributes to increased matrix stiffness. Preliminary comparisons of cross-link chemistry (skin and tendon oligomers) also show differences in microstructure and mechanics. Preliminary quantification of ECFC cultured within oligomeric and monomeric matrices (matched concentration and stiffness) suggests that oligomers support increased network formation, total vessel length, and average lumen area in long-term cultures. Furthermore, oligomer content modulates vessel formation through the matrix-integrin-cytoskeleton signaling pathway, as evidenced by increases in focal adhesion kinase (FAK) expression in oligomer matrices over that of monomer matrices at 48 hours.
**Conclusions**: By altering the collagen matrix on the molecular-level using collagen oligomers, we can modulate fibril- and matrix-level properties to guide and enhance ECFC vessel morphogenesis *in vitro*. Additionally, we can further define mechanisms by which collagen oligomers are able to regulate vascular network and lumen formation through the matrix-integrin-cytoskeleton signaling axis. For the first time, we have observed that naturally-occurring intermolecular cross-links can be used as a new, physiologically relevant design parameter for the development of polymerizable collagen formulations to be used in engineered tissue applications.

**Institution**: Case Western Reserve University
**Title**: Heteromultivalent Peptide Decoration on Nanoparticles for Enhancing Cell-specific Targeting and Shear-Stable Binding in Vascular Drug Delivery
**Authors**: C.L. Modery, M. Ravikumar, T.L. Wong, M.J. Dzuricky, N. Durongkaveroj, and A.S. Gupta
**Objective**: For particle-mediated drug targeting in vascular diseases, it is critical to ensure that the particulate vehicles can specifically bind vascular disease sites and can stay retained at these target sites under hemodynamic flow. We hypothesized and tested that targeting activated platelets via multiple ligand-receptor interactions can satisfy these design criteria.
**Methods**: We used liposomes as model delivery vehicles and decorated their surface with two different peptides that specifically target activated platelets via simultaneous binding with integrin áIIbâ3 (via RGD peptide) and P-selectin (via EWVDV peptide). These heteromultivalent constructs were studied for platelet-targeting specificity and platelet-binding stability under a dynamic flow environment.
**Results**: Receptor-specific blocking studies using fluorescently-tagged appropriate antibodies established that the RGD-modified liposomes specifically bind to integrin áIIbâ3 and the EWVDV-modified liposomes specifically bind to P-selectin on activated platelets. Flow cytometry analysis showed that liposomes surface-decorated with both peptides (dual-targeting) bound significantly more to activated platelets compared to liposomes with any one peptide (single-targeting), while unmodified liposomes showed only minimal non-specific binding. This established enhanced target-specificity for the heteromultivalent constructs. Binding studies using a parallel-plate flow chamber established that the dual-targeted liposomes stay retained on activated platelets significantly more than singly-targeted liposomes, under low-to-high (5-60 dynes/cm$^2$) shear flow.
**Conclusions**: We have successfully validated our hypothesis that simultaneous multi-receptor targeting using heteromultivalent peptide decoration on drug delivery vehicles leads to enhanced cell-specific targeting and shear-stable binding/retention in a hemodynamic flow environment. Our *in vitro* studies indicate that dual-targeting mechanisms directed at activated platelets can provide synergistic pathways of enhanced thrombus site-selectivity as well as stable attachment under flow. Further dimensional refinement and optimization of surface-functionalization of such heteromultivalent constructs can lead to effective vehicles for vascularly-targeted delivery of therapeutic and diagnostic agents.

**Institution**: Purdue University
**Title**: Drug Delivery Antimicrobial Peptide Hybrid for Targeting Intracellular Pathogens
**Authors**: M.A. Soofi and M.N. Seleem
**Objective**: The objective of this research was to design and assess the ability of a short synthetic cell penetrating peptide to deliver peptide nucleic acids (PNA) to target bacterial cells and to analyze the capacity in which the PNA-peptide conjugate could inhibit essential gene expression, and thus growth, of Salmonella.
**Methods**: Several antisense Peptide Nucleic Acid (PNA) constructs were designed to silence the expression of specific essential genes required for survival of Salmonella. Because naked PNA constructs cannot breach the cellular membrane, drug delivery was achieved by conjugating PNA to a synthetic cell penetrating peptide (CPP) that allowed for targeted delivery.
**Results**: Incubating 105 Salmonella colony forming units with 5 μM and 10 μM concentration of either anti- á-subunit or anti-sigma factor of RNA polymerase significantly reduced the viable cell count and growth rate of pure cultures whereas incubation with 15 μM eliminated Salmonella colony forming units within eight hours. A control PNA-peptide conjugate had no effect on bacterial growth or colony forming units. Clearance of the culture by the two PNA-peptide conjugates suggests that both were able to penetrate through the cellular membrane and bind to the target gene within Salmonella. The control conjugate demonstrates that the conjugates themselves do not render any antibacterial effects.
**Conclusions**: The growth of Salmonella can be inhibited *in vitro* (pure culture) and in a macrophage cell line with anti-sense gene constructs consisting of a peptide nucleic acid (PNA) fused to a CPP. The novelty of the biomolecular structure and the efficient antisense strategy of PNA suggest that conjugated CPP molecules represent an effective PNA delivery mechanism and this technology may play a role in addressing the urgent need for novel therapeutics to combat antibiotic resistant strains. Moreover, this approach presents the possibility of reducing the duration of treatment and the side effects of traditional antibiotics to treat chronic intracellular infections.

**Institution**: Vanderbilt University
**Title**: Smart Nanoparticle for MMP-7 Proximity-Activated siRNA Delivery
**Authors**: H. Li, T. Werfel, C.E. Nelson, S.S. Yu, T.D. Giorgio, and C.L. Duvall
**Objective**: An MMP-7-responsive element was incorporated onto a smart polymeric nanoparticle (SPN). The SPN has a cationic corona for siRNA condensation and a pH-responsive, endosomolytic core that mediates efficient siRNA cytosolic delivery. This design is selectively activated and can penetrate into cells only once it has been activated by MMP-7, and thus employs proximity-activated targeting (PAT).
**Methods**: An MMP-7 cleavable peptide was conjugated to a reversible addition-fragmentation chain transfer (RAFT) chain transfer agent (CTA) and a PEG to form a macroCTA. MacroCTA was used to polymerize a peptide-functionalized diblock copolymer PEG-peptide-P(DMAEMA)-b-P(DMAEMA-BMA-PAA). GPC, TEM, DLS and flow cytometry were used to verify SPN architecture and siRNA activity.
**Results**: Polymeric micelles with ~70 nm diameter were synthesized and confirmed by zeta potential measurement and GPC to be susceptible to MMP-7 at physiologically relevant concentrations. The micelles became less stable in acidic condition indicating an important feature for endosomal escape. Flow cytometry analysis of FAM-siRNA uptake in MDA-MB-231 breast cancer cells indicated that activation by MMP-7 increased cellular internalization of siRNA-loaded PAT-SPNs.
**Conclusions**: A multifunctional, smart siRNA carrier PAT-SPN, was constructed. This polymer-based system was demonstrated to form micelles that are MMP- and pH-responsive. This novel nanoscale vehicle is designed for targeted, effective siRNA delivery to breast cancer metastases.

**Institution**: University of Kentucky
**Title**: Synthesis and Characterization of Curcumin Based Poly(β-Amino Ester) Antioxidant Nanoparticles to Control Cellular Oxidative Stress
**Authors**: P. Gupta, J.Z. Hilt, and T.D. Dziubla
**Objective**: Oxidative stress is a pathophysiological condition defined by an increased production of reactive oxygen species, resulting in cellular damage. Antioxidants are capable of intercepting ROS, thereby preventing oxidative stress state, but poor pharmacokinetics limit their use. In this work, we synthesize curcumin based biodegradable polymer nanoparticles to control cellular oxidative stress.
**Methods**: Curcumin was conjugated into biodegradable Poly-Beta-Amino-Esters (PBAE), which was then formulated into nanoparticles. These nanoparticles were then infused into the endothelial cells. Degradation of nanoparticles with respect to time, cytotoxicity towards the endothelial cells, and their ability to prevent oxidative stress were studied in DCF florescence assays.
**Results**: Hydrolytic degradation of the hydrophobic biodegradable polymeric nanoparticles within the endothelial cells resulted in the release of curcumin, which in turn performs its anti-oxidant function to suppress oxidative stress. Degradation and hence rate of curcumin release varied with varied polymer composition. More hydrophobic polymer nanoparticles degraded slowly as compared to more hydrophilic ones.
**Conclusions**: Curcumin loaded PBAE showed effective anti-oxidant activity through hydrolytic degradation of polymer releasing free curcumin, and varying the composition of hydrophilic and hydrophobic entities in the polymer was used as a tool to optimize its degradation time. So, curcumin, an anti- anti-cancerous, anti-inflammatory agent and anti-oxidant effects but poorly soluble in aqueous media was now available through its conjugated form to prevent ROS production and stop cell damage.

**Institution**: Purdue University
**Title**: Functional Cellular Encapsulation By Means of Biomimetic Silica Membrane Deposition
**Authors**: D.B. Jaroch, N.D. Stull, M.C. Stensberg, J. Shi, R. Madangopal, M. Zeitchek, E.S. McLamore, W. Zhang, R. Mirmira, D.M. Porterfield, and J.L. Rickus
**Objective**: Living cells perform complex chemical processes on size and time scales artificial systems cannot match, acting as biological sensors, factories, and drug delivery devices. To utilize living cells in engineered constructs, we created stable protective cellular microenvironments using a bioinspired method for forming silica-based membranes at the surface of cells.
**Methods**: Endogenous proteins and polysaccharides surrounding a cell's surface are used as a site for silica nucleation in a saturated environment. Silica deposits preferentially on the outer surface of the cell. Subsequent dilution of the surrounding media halts bulk silica gelation resulting in a thin cell-specific membrane.
**Results**: We demonstrate silica layer formation at the surface of mammalian cells, bacterial biofilms, and pancreatic islets. Materials are characterized by SEM, and EDS. Cell death assays confirm cell survival and metabolite flux measurements confirm normal function and no major diffusion limitations. Exogenous proteins produced by diatoms to generate complex silica shells were also used as a template to form relatively ordered silica structures at the cell surface. We introduced silaffin proteins harvested from a diatom into the cell media. Subsequent addition of silicic acid results in the deposition of a condensed silica network with an ordered porous structure.
**Conclusions**: These hybrid silica-cellular constructs can be utilized in a range of industrial, environmental, and medical technologies. Bacterial encapsulation can be used in both domestic (municipal waste water treatment) and military (rapidly deployable, stable, and mature bioreactors) applications. We are also currently testing the silica shell as a stable biocompatible encapsulant to prevent immune system recognition of foreign cellular transplants in *in vivo* murine studies. Silica sol-gels are bioactive materials that integrate with the surrounding tissue forming a biocompatible interface. We hypothesize silicate tissue encapsulants will display long term biocompatibility and functionality, enabling islet transplant as a treatment for diabetes.

**Institution**: The University of Michigan
**Title**: Effect of Biomineral-Coated PLLA and PCL Porous Scaffolds on Bone Formation *In Vivo*
**Authors**: E. Saito, D. Suarez-Gonzalez, and W.L. Murphy,
**Objective**: To improve bone ingrowth into porous scaffolds, we combined solid freeform fabrication (SFF) techniques and biomineral coating to fabricate osteoconductive biodegradable scaffolds. The goal was to determine the effects of mineral coated porous scaffold made of two different biodegradable polymers, PLLA and PCL, on bone formation *in vivo*.
**Methods**: Four groups of the scaffolds (coated or uncoated PLLA and PCL) were fabricated using indirect SFF and modified SBF techniques. The caffolds were seeded with BMP-7 transduced fibroblasts and, subcutaneously implanted into mice for 3 and 10 weeks. The scaffolds were evaluated using micro-CT, SEM, mechanical testing, and histological techniques.
**Results**: Biomineral coatings on the PLLA and PCL scaffolds were confirmed using micro-CT and SEM. Micro-CT data and H&E staining showed that there was no difference of bone ingrowth between the scaffold groups at 3 weeks. However, the coated scaffolds had significantly more bone ingrowth with trabecular-like structures than the uncoated scaffolds at 10 weeks, and the bone ingrowth followed the designed scaffold architectures. The elastic moduli of the coated PLLA scaffolds significantly increased from 3 to 10 weeks; however, increase of the uncoated PLLA scaffolds was not significant. No such trend was seen in the coated and uncoated PCL scaffolds.
**Conclusions**: SFF scaffolds made of PLLA and PCL were successfully coated with biomineral layers using mSBF procedures. The biomineral layers improved *in vivo* bone ingrowth into porous scaffolds, and the designed SFF scaffolds with fully interconnected pore architectures also helped the bone tissues formed in the scaffolds. The improved bone ingrowth supports mechanical properties of PLLA scaffolds compensating for the loss of mechanical properties. PCL scaffolds, which have slower degradation than PLLA scaffolds, did not show effect of bone ingrowth on mechanical property indicating that material choice is also an important factor to design engineered bone scaffolds.

**Institution**: Case Western Reserve University
**Title**: *In Vivo* Persistence of Silicone Elastomer Used in Artificial Periosteum
**Authors**: S.R. Moore, U.R. Knothe, S. Milz, and M.L. Knothe Tate
**Objective**: This study assesses the degree of persistence of a non-biodegradable silicone elastomer membrane used as an artificial periosteum in an ovine model. Across three experimental groups and one control group, the persistent membrane area after 16 weeks is related to the total area of regenerated early and mature bone tissue.
**Methods**: Microscopically generated collages of giemsa and eosin-stained serial histological sections are produced to quantitatively measure areas of regenerated tissue and persisting membrane. Image thresholding is used to isolate membrane from surrounding tissue, followed by a pixel count to obtain the actual area in square millimeters.
**Results**: After 16 weeks of *in vivo* implantation, no significant difference in membrane area is seen between groups as revealed by a Kruskal-Wallis test ($p=0.273$), though significantly different areas of tissue regeneration are observed (control = $37\pm11$ mm$^2$, Group 1 = $33\pm10$ mm$^2$, Group 2 = $124\pm43$ mm$^2$, Group 3 = $228\pm54$ mm$^2$). Membrane areas are represented by the range of $92 \pm 119$ mm$^2$.
**Conclusions**: This study reveals that, in accordance with the non-degradable nature of the membrane, the material bulk persists regardless of healing success after 16 weeks in the ovine model. While the total area of the membrane is conserved, histological observations reveal breaks in the continuity of the solid membrane layer that may promote cell ingression. After a certain stage, the absence of a rigid barrier may prove responsible for more advanced tissue healing, as observed in Groups 2 and 3. Future studies quantifying the degree of membrane continuity will direct engineering of the second generation of artificial periosteal implants.

**Institution**: Purdue University
**Title**: Protein-Based Matrix for Cartilage Tissue Engineering and Stem Cell Differentiation
**Authors**: J.N. Renner, Y. Kim, and J.C. Liu
**Objective**: We are developing an artificial protein matrix with embedded biochemical cues to direct human mesenchymal stem cell (hMSC) differentiation and maintain the desired chondrogenic phenotype. A short peptide sequence derived from bone morphogenetic protein-2 (BMP-2) was characterized for its effect on chondrogenesis of hMSCs.
**Methods**: The BMP peptide was tested in a high-throughput pellet culture system, which was used to rapidly collect biochemical data such as glycosaminoglycan (GAG), total collagen, and DNA content, as well as alkaline phosphatase (AP) activity. Histology and gene expression measurements were also conducted and confirmed the high-throughput results.
**Results**: Comparable levels of GAG production were promoted by the peptide (100 μg/mL) and BMP-2 (200 ng/mL) over four weeks of culture. Histology revealed that the peptide promoted a more homogenous distribution of GAG than BMP-2 did. The BMP peptide directed human MSCs to increase collagen production after three weeks but at significantly lower levels compared to BMP-2. Treatment with BMP-2, but not the peptide, resulted in an increase in hypertrophic markers such as AP activity and gene expression of type X collagen.
**Conclusions**: The BMP peptide has previously been tested for its effect on bone formation, but has not been tested for its effect on hMSC chondrogenesis. Our results showed that the BMP peptide promoted GAG production similar to BMP-2 without increasing bone or fibrocartilage markers. This finding suggests that the BMP peptide could be an effective and valuable new tool for cartilage tissue engineering. Our lab is including the BMP peptide sequence in a protein-based matrix as a material-based cue for hMSC chondrogenesis.

**Institution**: Northwestern University
**Title**: Load Partitioning Between Phases in Bone During Fatigue
**Authors**: A.Singhal, S.R. Stock, and J.D. Almer
**Objective**: Irradiation is supplied to bones during sterilization and cancer therapy, and is known to have deleterious effects on the properties. The objective was to understand clearly the fatigue behavior of highly irradiated bone samples using high-energy x-ray diffraction, and correlate the observed mechanical properties with the microstructure using micro-computed tomography.
**Methods**: Bovine femur samples were tested at the Advanced Photon Source (APS), where simultaneous mechanical loading and diffraction measurements were conducted giving *in situ* strains. Samples were cyclically loaded in compression varying the stress range, frequency and mean stress. The fatigued samples were imaged using x-ray micro-tomography technique at APS.
**Results**: Strains in the hydroxyapatite (HAP) phase were found to decrease whereas strains in the collagen fibrils increased with increasing cycles. The rate at which the strains change depends on the frequency of loading, mean stress and the stress range studied. The apparent elastic modulus of the HAP and fibrils increased with cycles in some samples. In all the cases studied, samples tested at the highest stress range and lowest frequencies were found to have the greatest damage. Longitudinally oriented cracks were observed in the microstructure and existed in the interstitial bone, at lamellar interfaces and near Haversian canals.
**Conclusions**: It was concluded that strains in HAP decrease because of interfacial damage due to cyclic loading and irradiation. Irradiation results in decarboxylation at the HAP-collagen interface. Lower frequencies allow more time for collagen deformation (viscoelastic), thus greater damage. Higher stresses result in greater plastic deformation of collagen as well as interfacial damage. At the microstructural level, low frequencies allow microcracks to propagate along interfaces, whereas high frequencies result in cracks bypassing the microstructural features. In this first study of the *in situ* fatigue behavior of bone, irradiation was thus shown to have deleterious effects on the fatigue behavior of bone.

**Institution**: Purdue University
**Title**: A Canonical Biomechanical Vocal Fold Model
**Authors**: P. Bhattacharya and T.H. Siegmund
**Objective**: We aim to create rules of vocal fold (VF) model construction that ensure mechanical response correspondence between VF models and subject-specific vocal folds. Models are used in numerical and experimental studies to determine stresses in vocal fold tissue; they are geometrically abstracted to extract fundamental insight and simplify modeling and analysis.
**Methods**: Image-slice (CT/MRI) based data of a subject-specific VF is used to create a set of successively abstracted geometries. Body-cover partitioning is defined to account for VF tissue histology. The effect on VF model eigen frequencies due to variation in tissue biomechanical properties and geometric abstractions is analyzed.
**Results**: Compared to subject-specific geometry model (baseline), a higher degree of abstraction always corresponds to a larger deviation in model frequency (up to 50% in the range of tissue biomechanical properties). The canonical model is optimally abstracted, in that it significantly simplifies the VF geometry, but can be recalibrated consistently to match the baseline response. Models providing a marginally higher degree abstraction have significant deviation in frequency response. Quasi two-dimensional models cannot be recalibrated for their frequency response to match the subject-specific model; possibly due to complex support conditions accentuated by tissue biomechanical properties.
**Conclusions**: This analysis underlines the need to critically assess geometric abstraction in VF models with respect to mechanical response. Minor geometric modifications are shown to cause significant deviation in mechanical response when taken in conjunction with realistic tissue biomechanical properties. The automated design procedure presented here makes VF modeling based on subject-specific geometry more realizable by leveraging advances in clinical imaging techniques in creating canonical models.

**Institution**: Illinois Institute of Technology
**Title**: Investigation of Lysine-Acrylate Containing Poly(N-isopropylacrylamide) Hydrogels as Wound Dressings
**Authors**: B. Jiang, J.C. Larson, P.W. Drapala, V.H. Pérez-Luna, J.K. Kang-Mieler, and E.M. Brey
**Objective**: The objective of the study is to develop poly(N-isopropylacrylamide) (PNIPAAm) based hydrogels as potential wound dressing materials, with lysine-acrylate (A-lys) incorporated to promote tissue interaction and polyhexamethylene biguanide (PHMB) loaded to prevent infection. The effect of the wound dressing materials was evaluated *in vitro* and *in vivo*.
**Methods**: Hydrogels were synthesized via free radical polymerization of NIPAAm, PEG-DA-575 and A-lys. PHMB was loaded afterwards as antimicrobial agent. Cell adhesion was evaluated *in vitro* with varying concentration of A-lys, and antimicrobial activity was evaluated with varying concentration of PHMB. The hydrogel dressings were then evaluated *in vivo* with normal and infected wound healing animal model.
**Results**: A-Lys could be incorporated into the hydrogels to improve cellular interaction *in vitro* while maintaining hydrogel swelling properties and thermoresponsive behavior. PHMB could be encapsulated and released from the hydrogels and resulted in decreased bacteria counts within two hours. Application of the hydrogels to a rodent cutaneous wound healing model resulted in significantly increased in healing rate, compared to controls. Moreover, the hydrogels were also able to decrease bacteria levels in an infected wound model. These results suggest that PNIPAAm hydrogels containing A-lys are promising wound dressings due to their ability to promote healing and deliver active antimicrobial drugs to inhibit infection.
**Conclusions**: This study has described the synthesis and characterization of thermo-responsive hydrogels with cell adhesive and antimicrobial properties by incorporating with A-Lys and loading with PHMB. The hydrogels were evaluated as wound dressing materials in normal and infected animal wound models. Application of the dressings showed significant improvement in wound healing rate as well as wound surface infection treatment.

**Institution**: Case Western Reserve University
**Title**: Synthetic Platelets to Halt Bleeding After Trauma
**Authors**: A.J. Shoffstall, D.R. Campbell, K. Atkins, R. Groynom, L. Wu, S. Chang, B. Martyn-Dow, J. Ustin, and E.B. Lavik
**Objective**: Uncontrolled hemorrhage is a prevalent cause of death in both battlefield and civilian trauma. While many treatments exist to address external bleeding, there are few viable options for internal hemorrhage. Here, we investigate a synthetic platelet formulation that can be delivered intravenously to address internal hemorrhage.
**Methods**: The synthetic platelets are synthesized from a co-block polymer of poly(lactic-co-glycolic acid), poly-L-lysine, and poly(ethylene glycol). A targeting peptide is then conjugated to the particles to allow them to bind to activated, endogenous platelets. These particles are tested in rodent models of uncontrolled hemorrhage.
**Results**: Mean particle diameter is measured by DLS and SEM to be 200 - 300 nm. Biodistribution studies indicate the particles are taken up primarily in the liver, and have a short half-life in the plasma. The particles have been shown to significantly reduce bleeding compared to controls. This reduction in bleeding appears to improve survival up to 1 hour after injury.
**Conclusions**: This work shows that our synthetic platelet formulation has the potential to reduce bleeding in uncontrolled hemorrhage, and that administration of the particles after injury leads to improved survival outcomes in rats. These results suggest the need for further investigation of these particles to ascertain their safety and efficacy for administration during hemorrhagic events.

**Institution**: University of Kentucky
**Title**: *In Situ* Forming Drug Delivery Scaffold to Treat Avascular Necrosis
**Authors**: P.D. Fisher, J.Z. Hilt, and D.A. Puleo
**Objective**: The proposed treatment for avascular necrosis of the femoral head involves a direct local injection of an *in situ* forming drug delivery scaffold. N methyl pyrrolidone (NMP) and simvastatin act as osteogenic agents, and the system is designed to accomodate additional drugs.
**Methods**: A poly(lactic-co-glycolic acid) solution containing simvastatin-loaded poly(beta amino ester) hydrogel microsparticles rapidly precipitates in an aqueous environment to form a solid mechanical support. NMP is released in burst, while simvastatin releases via diffusion and hydrolysis of the microparticles. The scaffold itself is hydrolytically degraded.
**Results**: Free NMP was 50% released within 1 day and 80% released within 3 days. Simvastatin contained in microspheres showed little burst release, and prolonged release continued for at least 31 days. Daily simvastatin release began around 150ug and decreased to 20ug over the 31 day period. The scaffolds became noticeably softer approximately 2 weeks after injection, indicating degradation. Cell culture studies to test simvastatin and NMP dosage effects on osteogenic markers are ongoing.
**Conclusions**: The burst release of NMP due to its high aqueous miscibility has two-fold significance - first, it allowed for rapid precipitation of dissolved PLGA, providing acute mechanical support, and second, it provides an immediate osteogenic stimulus for surrounding cells in the damaged bone tissue. This effect is complemented by a prolonged release of simvastatin primarily due to degradation, with limited diffusion due to its hydrophobicity and lower aqueous solubility. This provides a steady local dosing of simvastatin that can promote bone growth over the course of several weeks. The PLGA scaffold itself degrades as well over the course of months, and as it degrades new bone can grow in its place.

**Institution**: Case Western University
**Title**: Quantifying Factors Behind *In Vitro-In Vivo* Correlation of Drug Release
**Authors**: A.C. Beiswenger, L. Solorio, A.A. Exner
**Objective**: The underlying variables leading to the disparity between the mass of drug released from phase sensitive *in situ* forming implants formed *in vivo* and those formed *in vitro* is complex. The surface-to-volume ratio and limitations on implant expansion were evaluated and compared to previous data from *in vivo* implant release.
**Methods**: Polymer solution loaded with sodium fluresecein was injected into agarose phantoms to form sheets (1.5 and 3.0mm), then placed in 37°C phosphate buffered saline (PBS) solution. Unconstrained spherical implants were formed by dropping polymer into PBS. The drug release was measured over one week and compared to *in vivo* data.
**Results**: Burst release of drug from implants formed *in vivo* after 24h was 77.2% reaching a plateau of 82.5% drug released after 48h *in vivo*, which was significantly higher than the 1.5mm, 3.0mm and spherical implants (48.9%, 27.8%m and 24.2% respectively after 24h). The release of drug from 3.0mm thick implants and unconstrained spherical implants were equivalent during the course of the first 24h. After 48h, burst release of drug occurred from the constrained implants, releasing 45% and 67% of drug (1.5mm and 3.0mm respectively). The release from unconstrained implants remained near zero order after 24h, releasing 54.2% after one week.
**Conclusions**: While release from the 1.5mm implants was greater than that of the 3.0mm implants due to the increase in surface-to-volume ratio (2.1 and 1.5, respectively), this difference is not significant enough to account for the elevated release occurring *in vivo*. We hypothesize that reaction forces generated at the injection site lead to elevated release from the implants, which we speculate resulted in the secondary burst of drug observed from the constrained implants. Therefore, we postulate that while surface-to-volume ratio is a factor contributing to elevated release, the poor correlation observed cannot be accounted for by changes in surface-to-volume ratio alone.

**Institution**: Cleveland State University
**Title**: Nitric Oxide Synthase Immobilized in Electrospun fibers: Towards Novel Nitric Oxide Release Membranes
**Authors**: B. Gunasekera, T. Bose, T. Kantz, M. Russo, H. Kalil, T. Lubysheva, G.E. Wnek, and M. Bayachou
**Objective**: Nitric oxide (NO) is a molecule known to counteract platelet aggregation, and thus can stop the thrombosis cascade on the surface of blood-contacting medical implants. Nitric oxide synthases are enzymes (NOSs) responsible for catalytic conversion of the substrates L-arginine to NO and L-citrulline. By using NO releasing biomaterial in their closest native characteristics to mammalian tissue, one may be able to solve the issue of thrombosis and restenosis on the surface of foreign devices implanted or used as part of cardiovascular procedures. Our objective in the current work is to use NOS enzymes trapped in electrospun fiber matrices as biocompatible platform for NO release.
**Methods**: In this project, we investigate embedding of nitric oxide synthase (NOS) as a functional component contained in aqueous pockets of electrospun biopolymer matrices; namely, polycaprolactone (PCL) and Polyurethane (PU). A guided stream of polymer solution containing suspended aqueous pockets of enzyme solution is directed towards a collector drum in strong electric field. Surface characterization is carried-out by Atomic Force Microscopic (AFM) imaging on the newly formed NOS-containing electrospun fibers. Further, the NOS-modified membranes are tested electrochemically using a characteristic electrocatalytic reaction mediated by entrapped NOS enzymes. Finally, the NOS-containing electrospun membranes are subject to assays under various conditions to determine the structural integrity of NOS enzymes and their enzymatic activity
**Results**: Morphology of the NOS containing nodes in individual microfibers imaged at different stages of electrospinning shows evidence of success of spin-trap process. Griess assay and hemoglobin deoxy assay shows quantitative release of NO from the NOS-modified fibers under physiologic conditions. This confirms electrochemical characterization using the NOS-mediated catalytic reduction reaction of exogenous NO of the same fibers.
**Conclusions**: Together, these results show that the native structure of the entrapped NOS in the aqueous pockets is conserved and is functional under physiologic conditions.

**Institution**: University of Kentucky
**Title**: Targeting of Antioxidant Polymer Nanoparticles for Inhibition of Vascular Oxidative Stress
**Authors**: D. Cochran, P. Wattamwar, R. Eitel, K.W. Anderson, and T.D. Dziubla
**Objective**: Our objective is to formulate antioxidant nanoparticles with a targeted antibody coating that can suppress oxidative stress; along with the ability of these nanoparticles to suppress oxidative stress. These particles should also be degradable, providing a therapeutic antioxidant concentration to protect against cellular death in response to injury.
**Methods**: Degradable antioxidant polymer nanoparticles were coated with an antibody directed against platelet endothelial cell adhesion molecule (anti-PECAM-1). These active targeting antibodies were then prophylactically administered to HUVECs prior to introduction of injury. Viability and generation of reactive oxygen species was then monitored for at minimum 24 hours after injury.
**Results**: Oxidative stress was induced in HUVECs through the use of iron oxide nanoparticles. ROS levels and viability measured through the use of DCF fluorescence and MTT assays, respectively. Targeted antioxidant polymer nanoparticles ranging from 0.8 mg/ml to 0.2 mg/ml were prophylactically administered before injury. For PTx 1000, we observe a dose dependent decrease in ROS levels and increase in viability as compared to no treatment. For PTx 2500, we observe a less pronounced effect, possibly due to the slower degradation kinetics provided by the higher molecular weight chains.
**Conclusions**: Anti-PECAM-1 targeted antioxidant nanoparticles were shown to adhere to vascular cells, and reduce oxidative stress in both static and ROS injury type models over a period of 24 hours. We observed a dose dependant increase of viability with the targeted antioxidants as compared to non specific antibody coated particles. This result reinforces the ability to selectively target cells for drug delivery through the use of site specific antibodies. Further research into novel targets can allow for precise and effective treatment of multitudes of injuries.

**Company**: Cook Medical

**Title**: Role of Biomaterials and Biocompatibility in Medical Devices
**Authors**: J.E. Barbick
**Abstract**: The importance of biomaterials and biocompatibility in the realm of medical devices has emerged in the past decades. Appropriate biomaterial choice affects device resistance to and interaction with physiological phenomena, including thrombosis, restenosis, tissue ingrowth, infection, wound healing, and biofilm formation. Several Cook products address these issues, and advances in biomaterials and coatings allow medical devices to be constantly improved, leading to care centered around what is best for the patient.

**Company**: Biomet, Inc.

**Title**: Antibiotic release from HA-coated porous metal structures under static and dynamic conditions LaCroix

**Authors**: A. LaCroix, S. Sundaramurthy, and G. Gupta

**Objective**: Orthopedic devices made with porous Ti6Al4V metal structures have historically demonstrated excellent biocompatibility. Their high surface area also provides potential for their use in drug delivery applications. Previous work has demonstrated that a hydroxyapatite (HA) coating can facilitate controlled release of antibiotics from porous metal discs. In this study the antibiotic release from HA-coated porous metal discs has been analyzed in static and dynamic conditions *in-vitro*. A combination of rifampin and minocycline was chosen because they are highly effective in preventing bacterial colonization of staphylococci strains that are a leading cause of infection.

**Methods**: Four porous Ti6Al4V metal discs were coated with HA using an electrodeposition process (37°C, pH 6.4). Both HA-coated and uncoated (control) discs were soaked in 10mL of methanol solution of rifampin and minocycline of concentration 40mg/mL for 30 minutes, then oven-dried at 40°C. Under static conditions separate sets of identical discs were soaked in 100mL PBS for 1, 4, 8, 24, 48, 120, 192, and 336h. All the samples were incubated in parallel under identical conditions. In the dynamic study, one set of discs were soaked in 25mL PBS solution, which was replenished at each of the abovementioned time points. All PBS solutions from both static and dynamic systems were analyzed using Ultra-High Performance Liquid Chromatography to determine the elution percentage of each antibiotic over time. After 7 days in static conditions, a zone of inhibition study was conducted to determine the antibacterial efficacy of the antibiotics present in the discs against *S. aureus*.

**Results**: After 14 days, degradation of minocycline was considerably higher in the static process compared to the dynamic process. On a 48h timescale, in either of the elution conditions, the HA-coating decreased the release of minocycline by 58 ± 12% and rifampin by 25 ± 7%. For either antibiotic, dynamic conditions increased antibiotic elution by 2.01 ± 0.32 times for HA-coated discs and by 1.44 ± 0.24 times for the control. Finally, microbiology testing showed that, even after 7 days, the antibiotics remaining on the discs were still bioactive.

**Conclusions**: HA-coated porous metal structures provided a more controlled antibiotic release over a 48h timescale, and also showed potential to reduce minocycline degradation and improve antibiotic efficacy over 14 days.

**Company**: Biomet, Inc.
**Title**: A Comparison of Anti-Inflammatory Properties of Whole Blood, Platelet-Rich Plasma, and an Autologous Protein Solution in IL-1β- and TNFα-Stimulated Chondrocytes
**Authors**: K. O'Shaughnessey, A. Matuska, J. Woodell-May, and J. Hoeppner
**Objective**: Autologous intra-articular injections of platelet-rich plasma (PRP) have been investigated as a potential treatment for cartilage degeneration. Degradation of the cartilage matrix, seen in early stage osteoarthritis (OA), is believed to be driven by inflammatory cytokines such as IL-1 and TNFα. These cytokines induce cells in the joint to produce matrix metalloproteinases (MMPs) that in turn are responsible for degradation of the cartilage matrix. An autologous protein solution (APS), which is derived from PRP and contains anti-inflammatory cytokines, has been developed. The purpose of this study was to determine if APS can inhibit the production of MMP-13 from IL-1β- and TNFα-stimulated chondrocytes more effectively than PRP.
**Methods**: *Preparation of samples* - Nine mL of PRP was prepared from 90 mL fresh whole blood (8.3% v/v ACD-A) using two disposable separation devices containing a tuned density buoy (Biomet Biologics). Six mL of the combined PRP output was transferred to a disposable device containing polyacrylamide beads. The device was centrifuged and the APS was collected. Cells in the whole blood (WB), remaining PRP, and APS were lysed by processing 500 µl of each sample at 5°C with protease inhibitor and cell disruption glass beads in a cell disrupter. The samples were centrifuged at 5°C for 20 minutes at 13,000 rpm (11,500 g), and the lysate was removed and stored (-50°C) until use in the assay. *Cell Assay* - Human knee articular chondrocytes (NHAC, P5, Lonza Inc.) were seeded in 12-well plates at 20,000 cells/$cm^2$ in 2 mL growth media. Two hours prior to the assay, media was exchanged with serum-free. The treatment wells were pre-incubated with 25 µl WB, PRP, or APS in a trans-well insert (Corning Inc.) for 2 hours before addition of recombinant IL-1β (5 ng/mL) and TNFα (100 ng/mL). Negative control was untreated media, and positive control contained only IL-1β (5ng/mL) and TNFα (100 ng/mL). Following incubation at 37°C and 5% $CO_2$ for 24 hours, the supernatant was removed and frozen at -50°C. Cells were trypsinized and counted. The supernatant was assayed for MMP-13 by ELISA, and MMP-13 production was normalized to cell number. *Statistical Analysis* - A single-factor ANOVA with a post-hoc Fisher LSD test ($\alpha$=0.05) was performed to determine statistically significant differences.
**Results**: APS was more capable of inhibiting MMP-13 production by IL-1β- and TNFα-stimulated chondrocytes than an equal dose of WB ($p<0.0001$) or PRP ($p=0.0003$). WB had no effect on MMP-13 production compared to the positive control ($p = 0.4$). PRP and APS inhibited approximately 45% and 70% of MMP-13 production, respectively. The ability of APS to block MMP-13 production better than PRP is likely due to its increased concentrations of anti-inflammatory cytokines. Previous studies have shown APS has a greater fold increase of anti-inflammatory cytokine concentrations from WB.
**Conclusions**: APS has been shown to inhibit production of MMP-13, a protein known to be responsible for cartilage degradation, more effectively than WB or PRP. This is likely because APS contains higher concentrations of anti-inflammatory cytokines. These

results suggest that APS may be more effective as a potential treatment for early OA, and further studies are warranted.

**Institution**: Purdue University
**Title**: Using Peptides to Engineer Bioactive Implants that Facilitate Vocal Fold Regeneration
**Authors**: A.M. Kosinski, M. Sivasankar, and A. Panitch
**Objective**: The development of a peptide based bioactive component for use in a hydrogel system that upon implantation will facilitate the regeneration and functional restoration of damaged vocal folds over the long term.
**Methods**: Assessment of this peptide based bioactive component will initially be achieved by elucidating how adherent immortalized human vocal fold fibroblasts (I-HVFFs) respond to varying strengths of the surface bound peptide-based biological adherence signal GRGDS. Variance of signal strength will be accomplished using a novel approach that involves peptidyl-branching.
**Results**: Varying the strength of the surface bound peptide-based biological adherence signal GRGDS via peptidyl-branching, caused I-HVFFs to adhere so strongly to substrates covalently modified with branched peptides that contained a stronger GRGDS adherence signal. That spreading and growth of I-HVFFs on these substrates was impaired compared to substrates modified with a weaker GRGDS adherence signal.
**Conclusions**: Many current materials (e.g. hyaluronic acid or calcium hydroxylapatite) used in treating various vocal fold disorders (e.g. glottal incompetence) have poor cell adhering properties thereby impairing their ability to facilitate regeneration and functional restoration of damaged vocal folds over the long term. We hope that by modifying these materials with our branched GRGDS peptides we will be able to facilitate infiltration and adherence of native vocal fold fibroblasts into these materials, thereby, increasing the likelihood that these fibroblasts will secrete extracellular matrix as the material they infiltrated and adhered to breaks down over the long term.

**Institution**: Case Western Reserve University
**Title**: Using Ultrasound Imaging to Predict Drug Release From *In Situ* Forming Implants
**Authors**: A.M. Olear, L. Solorio, A.C. Beiswenger, and A.A. Exner
**Objective**: Recent studies have shown diagnostic ultrasound to be an effective method for characterizing phase inversion and predicting initial drug release from *in situ* forming implants. To further validate this strategy, this study investigated the relationship of phase inversion and drug release with three drugs of various properties.
**Methods**: Sodium fluorescein, 1,1'-dioctadecyl-3,3,3'3'-tetramethylindocarbocyanine perchlorate (DiI), bovine serum albumin (BSA) and Doxorubicin were investigated as model drugs from Poly (DL-lactide-co-gylcolide)/N-methyl pyrrolidinone polymer systems. They were individually evaluated in studies measuring *in vitro* dissolution, *in vitro* ultrasound imaging, pH, scanning electron microscope imaging and *in vivo* analysis.
**Results**: Doxorubicin and fluorescein released a similar drug mass for a 10 d period. DiI released less than 3% over 14 days, while BSA appeared to delay release caused by polymer degradation. Nonlinear mathematical fitting was used to correlate drug release and phase inversion ($R^2 > 0.93$ all formulations).
**Conclusions**: Fluorescein was shown to be a good predictor for doxorubicin drug release; but due to differences in the matrix/drug interactions, phase inversion was markedly different. The hydrophobic DiI was not readily released and increased hydrophobicity of the implant. The observed delay in polymer degradation by BSA was attributed to its buffering capacity at pH 3.5, thus reducing the mass of drug released from the implant. Since the mass release from the implants can be correlated to the rate of phase inversion, the image data provides a means to evaluate implants noninvasively, providing insight into *in vivo* implant behavior.

**Institution**: Case Western Reserve University
**Title**: Block-copolymer Micelles for Delivery of Photosensitizer Pc 4 for PDT
**Authors**: A. Master, M. Rodriguez, M. Kenney, N. Oleinick, and A.S. Gupta
**Objective**: Photodynamic Therapy (PDT) is a promising modality for cancer treatment where photoactivation of a photosensitizer (PS) drug causes cell death. Formulation of PS for delivery to cancer cells without non-specific uptake can increase the therapeutic efficacy of PDT. Hence, we are investigating the formulation of the PS Pc 4 using block-copolymer-based micelles.
**Methods**: Micelles were developed from poly(ethylene glycol)-co-poly(ε-caprolactone) (PEG-PCL). The critical micelle concentration (CMC) was determined by a standard pyrene assay. Pc 4 was encapsulated in the micellar core and cell experiments to determine subcellular localization and PDT efficacy were completed on MCF-7c3 human breast cancer cells.
**Results**: A high encapsulation efficiency of 70% was found along with an association constant of 1.7 x 103 M-1 which is comparable to association constant values reported for several hydrophobic photosensitizers in lipidic vehicles. Subcellular localization experiments revealed that the Pc 4 loaded micelles become localized in lysosomes whereas free PS becomes localized in the mitochondria. This difference in subcellular localization may have an effect on the cell killing efficiency of the micellar formulation. PDT studies using standard MTT and Live/Dead assays show that Pc 4 loaded micelles cause significant cell death following photoirradiation.
**Conclusions**: Micelle-formulated Pc 4 was used to do preliminary PDT studies on MCF-7c3 breast cancer cells. The results show effective cell killing upon photoactivation. Hence micelle-encapsulated Pc 4 has considerable promise towards developing targeted Pc 4-PDT for cancer.

**Institution**: Purdue University
**Title**: Collagen-Binding Peptidoglycan's Influence on Fibrillogenesis and Mechanics
**Authors**: A.K. Ramaswamy, J.E. Paderi, K.A. Stuart, and A. Panitch
**Objective**: Collagen, the most prevalent protein in the human body, and its organization is of utmost importance within the field of tissue engineering. We have previously shown that a biomimetic collagen-binding peptidoglycan, designed to function similar to the native proteoglycan decorin, can be used to manipulate collagen assembly. Applications of this peptidoglycan include collagen-based engineering initiatives, specifically future investigations regarding breast cancer tissue development.
**Methods**: The synthesized peptidoglycan contains a peptide sequence modeled to compliment active binding sites on collagen monomers. By attaching a sugar chain (dermatan sulfate) to the tail of the peptide, we formed a peptidoglycan similar in morphology to Decorin within physiological conditions. The effect of the peptidoglycan on collagen fibril formation was examined by monitoring collagen gel turbidity. Furthermore, the mechanical properties of collagen gels with peptidoglycan were examined via rheology.
**Results**: Turbidity data revealed that the peptidoglycan significantly delays fibrillogenesis while controlling fibril diameter, mimicking decorin. Rheological data shows that the integration of this peptidoglycan significantly increases collagen gel strength. Both the fibrillogenesis of the collagen fibers and mechanical properties of the scaffolds were further modulated and controlled by adjusting the number of peptides attached per dermatan sulfate backbone. Human coronary artery endothelial cells remained viable when incubated within the presence of collagen gels bonded with the peptidoglycan.
**Conclusions**: This work indicates that the peptidoglycan can be used to modulate the collagenous stroma tissue surrounding breast cancer cells, providing an external environmental influence on cancerous tissue formation.

**Institution**: Purdue University
**Title**: Development of Fast-Dissolving Polymer Films for Drug Release from Balloon Catheters
**Authors**: R.A. Scott and A. Panitch
**Objective**: The delivery of therapeutics from angioplasty balloons is an important strategy for treating restenosis associated with complications from coronary revascularization procedures. Release of drugs from fast-dissolving polymer films coated on the balloons is one technique for localized delivery. This work aimed to characterize the development of fast-dissolving polymer films for drug delivery during angioplasty
**Methods**: Polymer films, consisting of poly(vinyl alcohol), poly(ethylene glycol), and decorin-mimic, were fabricated using a spin coating technique. The time required for film dissolution was examined. Dynamic light scattering was utilized to determine the size of polymer fragments after film dissolution. Release of the decorin-mimic from the film was determined via an alcian blue assay.
**Results**: The amount of polymer and decorin-mimic incorporated within the film was dependent on the spin coating speed, where decreased speed resulted in an increase of film weight. All films dissolved in less than one minute; however, altering the polymer composition within the films resulted in varied dissolution speeds. While the size of polymer fragments from the dissolved films was found to be larger than plain polymer, the size of the fragments was similar for all film compositions. Furthermore, the alcian blue assay indicated that decorin-mimic was released from the polymer film.
**Conclusions**: The use of drug-eluting balloons during the revascularization procedures provides less damage and more homogeneous drug delivery to the vessel wall, compared to drug-eluting stents. This work indicates that fast-dissolving films can be fabricated from hydrophilic polymers such as poly(vinyl alcohol) and poly(ethylene glycol), providing suitable matrices for drug delivery from angioplasty balloons. Future work will investigate the functionality of the decorin-mimic after film dissolution, examining its effect on platelet activation and endothelial cell migration.

**Institution**: Purdue University
**Title**: Collagen Peptide-Based Biomaterials as Magnetic Resonance Imaging Agents
**Authors**: D.M. Ernenwein and J.A. Chmielewski
**Objective**: Using our metal-triggered self-assembly collagen strategy, we wanted to design micron sized particles containing multiple DOTA ligands which in turn could coordinate Gd(III), resulting in the development of MRI active biomaterial species. Two different strategies were examined: DOTA-containing His-tag peptides and DOTA-containing collagen-based peptides.
**Methods**: Structural morphology was determined by scanning electron microscopy, whereas the incorporation and quantification of Zn(II) and Gd(III) metal ions were verified by EDX and ICP-MS. MRI T1-weighted images and relaxivity values were obtained on a 3 T and 9.1 T MRI. Cellular cytotoxicity was also performed by MTS assay.
**Results**: Micron-sized collagen-based particles can be generated with the addition of Zn(II) with minimal structural change upon Gd(III) post-synthesis addition. EDX and ICP-MS detected Gd(III) and Zn(II) within the microflorette structures. Microflorettes containing 5% DOTA-His6 with the self-assembling collagen peptide, NCoH, were determined to be more efficient at Gd(III) uptake than the DOTA-containing collagen peptide, NHdota, by ICP-MS and MRI imaging. No appreciable cellular toxicity was induced by metal loaded microflorettes with HeLa cells after 24 h.
**Conclusions**: Gd(III)-loaded microflorettes were developed through the incorporation of DOTA ligands within His-tagged peptides and collagen self-assembling peptides, DOTA-His6 and NHdota. Up to 15% of the DOTA containing peptides could be incorporated within the core of the microflorettes without disrupting microflorette formation or paramagnetic properties as determined by SEM and T1-weighted MRI images, respectively. We have generated a ~2-fold more active MRI agent than commercial agents, Magnevist and Dotarem, by reducing the relaxation rates of water with our designed DOTA-containing microflorettes. These experiments have laid the groundwork for microflorettes to be utilized as a new class of MRI active agents.

**Institution**: Case Western Reserve University
**Title**: Effect of BSA and DiI on Drug Release from *In Situ* Forming Polymer Implants
**Authors**: D. Sundarapandiyan, A.M. Olear, A.C. Beiswenger, L. Solorio, and A.A. Exner
**Objective**: *In situ* forming implant systems have a characteristic release profile consisting of a burst phase, a diffusion driven release phase, and a polymer degradation facilitated phase. This study will explore methods to create a more clinically relevant, zero-order, release profile through the addition of excipients BSA (bovine serum albumin) and DiI (1,1'-dioctadecyl-3,3,3'3'-tetramethylindocarbocyanine perchlorate).
**Methods**: Poly(lactic-co-glycolic acid) (PLGA) was dissolved in 1-methyl-2-pyrrolidinone(NMP) solvent mixed with sodium fluorescein (mock drug), and excipient. The burst and diffusion phases of sodium-fluorescein released from DiI-infused implants was evaluated for 14 days, along with a 28-day release study monitoring degradation facilitated release from BSA infused implants.
**Results**: The addition of BSA does not alter the burst and diffusion driven periods of release, but does alter the onset of the degradation facilitated release phase. For the control, 0.05% BSA, 1% BSA, and 4% BSA, the increase in release during the degradation phase became noticeable after 75, 125, 125, and 300 h respectively. Release profiles of DiI infused implants showed a 10% reduction in the amount of fluorescein released from the high concentration implants during the initial burst phase when compared to the control, within the first 50 hours, while the onset of the degradation phase remained unchanged.
**Conclusions**: Observing that increased concentrations of BSA delay the onset of the degradation facilitated release phase, we speculate that BSA acts as a buffer, eliminating degradation until a level of the polymer's acid-byproduct saturation is reached. At this point, BSA's buffering ability is lost, and degradation proceeds. DiI studies indicated that the implant hydrophobicity increased with the increase in excipient concentration, reducing the interconnectivity between pores, and thereby decreasing release during both the burst and diffusion driven phases. Further understanding of these methods to alter release kinetics is a significant step towards a clinically beneficial model of controlled drug release from *in situ* polymer implants.

**Institution**: Purdue University
**Title**: Development of a Core-Shell Nanoparticle System for Targeted Drug Delivery
**Authors**: F.C. Knight, J.L. Brugnano, A.M. Kosinski, and A. Panitch
**Objective**: A major challenge in drug delivery is overcoming physiological barriers to allow a drug to reach its therapeutic target. Pharmaceuticals can be loaded into nanoparticles used as targeted delivery vehicles that protect the drug and direct it to the proper area of the body. For this project, the aim was to develop a targeted, thermoresponsive core-shell nanoparticle system.
**Methods**: Chemical methods were used to form the poly(lactic-co-glycolic acid) core and poly(N-isopropylacrylamide-co-acrylic acid) shell of the nanoparticles. Particles were functionalized with a collagen II-binding peptide (15% biotinylated). A biotin-streptavidin assay was used to detect peptide attachment and particle binding to collagen II. Nanoparticles were incubated with human cells to assess biocompatibility.
**Results**: Measurements of nanoparticle diameter by dynamic light scattering over a range of temperatures confirmed the thermoresponsive behavior of the particles due to PNIPAm; on average, particles were roughly 475 nm in diameter at 25°C, and they shrank to approximately 350 nm at 37°C. Analysis of biotin-streptavidin assays reveal peptide attachment and binding to collagen II, although not always to the desired degree. Initial biocompatibility results show negligible cell toxicity and inflammation at nanoparticle concentrations up to 4 mg/mL.
**Conclusions**: Initial results regarding functionalization and biocompatibility show that this system holds promise as a vehicle for targeted delivery of therapeutics. However, work remains to be done in optimizing the chemistry used to attach peptide to the surface of the nanoparticles; this is integral to the targeting aspect of the delivery system. This work is also significant in that the system described can be easily adapted for other targeting needs, e.g., by using a different peptide.

**Institution**: University of Notre Dame
**Title**: Pseudomonas Aeruginosa Swarms Efficiently by Fast Propagation of a Cell Wave into Developing Tendrils
**Authors**: H. Du, Z. Xu, O. Kim, W.M. Leevy, J.D. Shrout, and M. Alber
**Objective**: Pseudomonas aeruginosa colonizes new territory on exposed surfaces by the process of swarming. Within the range of agar that supports swarming, branched tendril patterns are often, but not always, observed for P. aeruginosa swarms. Formation of these tendrils requires production of the surfactant rhamnolipid (RL), which is regulated by quorum sensing.
**Methods**: We use the multiscale computational model to study P. aeruginosa swarming. Off-lattice model is utilized to model individual P. aeruginosa cells in which a cell is represented by three connected nodes. Macroscopic nutrient level, production and propagation of QS signals and RL are modeled using convection-diffusion-reaction equations. We employ a thin viscous fluid flow equation obtained by a lubrication approximation of the Navier-Stokes equations to describe the liquid layer.
**Results**: Our experiments show that cells and RL propagate as high density waves that spread symmetrically as rings. These rings promote accumulation of high density cell groups toward the tendril tips, which leads to the development of secondary swarm tendrils. Model simulations suggest that distribution of RL on the surface of the liquid film assists bacterial colonization by creating a surface tension gradient that drives liquid spreading at the edge of the colony resulting in tendril formation and swarm expansion.
**Conclusions**: Our simulations suggest that the dominant influence of RL upon formation of tendrils is the extraction of water to increase the height of the thin liquid film. Analysis of these experiments and simulations suggests that constant formation of new tendrils and directed motion of cells into these tendrils provide a highly efficient strategy to colonize surfaces.

**Institution**: Purdue University
**Title**: Intracellular Drug Delivery of an MK2 Inhibitor Using Cell-Penetrating Peptides
**Authors**: J.L. Brugnano and A. Panitch
**Objective**: Cell-penetrating peptides (CPPs) are short amino acid sequences that facilitate intracellular uptake of cargo. Intracellular access is thought to occur via one or more modes of endocytosis. In this submission, we characterize the efficacy and intracellular uptake of a drug (MK2-inhibitor peptide), consisting of two domains: a CPP and a MK2-inhibitor.
**Methods**: The MK2-inhibitor peptides were synthesized and determination of intracellular uptake occured with a FITC label. Efficacy was determined by evaluating the ability of the MK2-inhibitor peptides to reduce proinflammatory cytokine production (evaluated by ELISA) in stimulated human monocytes. Intracellular uptake was characterized using confocal microscopy and markers of endocytosis.
**Results**: Four different MK2-inhibitor peptides were synthesized, each containing the same MK2-inhibitor domain with a unique CPP domain. Controls consisted of the CPPs alone. Changing the CPP domain had an effect on the concentration required to demonstrate efficacy. Stimulated human monocytes showed reduced proinflammatory cytokine production without apparent toxicity when treated with the MK2-inhibitor peptides compared to controls. However, concentrations required to decrease proinflammatory cytokine production ranged from 3 mM to 1000 mM, depending on the CPP sequence. In some cases, the CPP alone decreased proinflammatory cytokine production. Visual confirmation of intracellular uptake suggests that uptake occurs via endocytosis.
**Conclusions**: Our results demonstrate that CPPs themselves can be biologically active. This finding is significant because CPPs have traditionally been viewed as inert molecules that simply deliver cargo. Appropriate controls are needed to ensure that the activity typically attributed to the cargo is a result of the cargo and not the CPP itself. Additionally, the differences in concentration required for *in vitro* efficacy suggest that CPP uptake could be cell-type specific.

**Institution**: Purdue University
**Title**: Smoking Induced Biomechanical Changes to Voice
**Authors**: J.E. Kelleher, T. Siegmund, and R.W. Chan
**Objective**: This study examines biomechanical properties in the vocal folds of non-smokers and smokers and aims to explore consequences following from these properties within the context of voice physiology.
**Methods**: The vocal folds from human excised larynges of six male non-smokers and three male smokers were tested in uniaxial tension. An optical method was employed to determine the stretch of three segments in the anterior-posterior direction. Subsequently, the spatial distribution of the elastic moduli was assessed.
**Results**: For the non-smokers, we discover a significant gradient in modulus with the middle segment on average 8 and 9 times stiffer than the anterior and posterior segments, respectively. In smokers, however, the gradient is largely absent. For non-smokers - exhibiting a strong modulus gradient - the vocal fold deflection of the first eigenmode was predicted to be broadly distributed along the anterior-posterior length of the vocal fold while for smokers – with a rather homogeneously distributed modulus – the deflection of the vocal fold is predicted to be more focused at the mid coronal location.
**Conclusions**: The results suggest that insufficient glottal closure may exist in the vocal folds of smokers as a result of changes to the biomechanical properties of the vocal fold tissue. This would lead to a breathy voice which has been clinically documented. The present results could have implications for clinicians and physicians. Currently, one standard procedure for poor glottal closure is to inject a biomaterial into the vocal folds to add volume to the tissue to enable better closure. However, more precise injection into the mid-coronal section with a biomaterial that would stiffen the vocal fold may yield better results.

**Institution**: Purdue University
**Title**: Optimization of the Mechanical and Cellular Properties of Electrochemically Aligned Collagen Threads
**Authors**: J.A. Uquillas, V. Kishore, and O. Akkus
**Objective**: The current study aimed to optimize the crosslinking conditions of ELAC threads by investigating the effects of solvent composition (1X PBS, 70%, 80%, 90%, and 100% ethanol) and genipin concentration (0%, 0.1%, 0.625%, 2%, and 6%) on the mechanical properties, crosslinking degree and cellular compatibility of ELAC threads.
**Methods**: The mechanical properties and crosslinking degree of ELAC threads were determined using monotonic mechanical tests and 2,4,6-trinitrobenzenesulfonic acid (TNBS) assay respectively. Alamar blue assay was used to evaluate the adhesion and proliferation of human mesenchymal stem cells (hMSCs) on ELAC threads.
**Results**: The results indicated that ELAC threads crosslinked with 0.625% genipin in 1X PBS exhibited an ultimate tensile stress of 30-40 MPa with only 26% of the available amine groups crosslinked. However, elevating the concentration of genipin to 2% and changing the solvent to 90% ethanol significantly enhanced the ultimate tensile stress of ELAC threads up to 110 MPa with a crosslinking degree of 65%. Furthermore, significantly higher adhesion and proliferation of hMSCs was observed on ELAC threads crosslinked with 2% genipin in 90% ethanol compared to 0.625% genipin in 1X PBS.
**Conclusions**: We conclude that mechanically competent ELAC threads may be synthesized by efficiently crosslinking them using optimal concentrations of genipin and ethanol/water solvents. These ELAC threads have significant potential to be used as a scaffolding material in tendon tissue engineering applications.

**Institution**: Case Western Reserve University
**Title**: Development of Hollow Gold Nanoparticle-Modified Nanoconstructs for Potential Applications in Phototriggerable Drug Delivery Systems
**Authors**: M.J. Dzuricky, C.L. Modery, T. Navran, and G. Kaur
**Objective**: In design of targeted drug delivery vehicles, significant challenges still remain regarding achieving 'site-specific controlled release' of payload. To this end, we are investigating the potential of Hollow Gold Nanoparticles (HGNs) to refine existing vehicle design to achieve Near Infra Red (NIR)-triggered photothermal destabilization for rapid drug delivery on cue.
**Methods**: HGNs were synthesized using galvanic replacement reaction from silver seed templates. HGN size and shell thickness was characterized and correlated with their absorption spectra, to find the optimum dimension conditions for NIR-sensitivity. The optimized HGNs were tethered to the surface of model compound-loaded liposomes and NIR-triggered release kinetics was characterized.
**Results**: HGN shell thickness and overall diameter can be controlled by varying reaction condition parameters such as overall reaction time, stoichiometric ratios of reactants and differential control of reaction rate. Optimum reaction condition parameters were established to achieve HGN dimensions that allow NIR-sensitivity. HGN dimension and morphology were characterized by Dynamic Light Scattering and Transmission Electron Microscopy, and optical properties were characterized by UV-Vis Spectroscopy. HGNs ~30 nm in diameter with a shell thickness of ~2 nm showed optimal NIR sensitivity. Tethering these optimized HGNs to model compound-loaded liposome surface allowed for NIR-induced liposomal destabilization and rapid payload release.
**Conclusions**: We have established optimal reaction conditions to yield HGNs of NIR-sensitive optimal diameter and shell thickness, reproducibly. We have also established a novel strategy to decorate liposomal surface with these HGNs via lipoic acid spacers. The HGN-decorated liposomes allowed for NIR-triggered rapid photothermal destabilization and release of model payload. Optimization of this strategy can lead to an effective way of ensuring site-specific controlled release of bioactive agents from such liposomes on cue, for a variety of target diseases where the liposomes can be first targeted to disease site via cell-selective ligand-receptor interactions, followed by inducing NIR-triggered release of drug payload.

**Institution**: Purdue University
**Title**: A Metal-Assembled Collagen Scaffold for the Delivery of Stem Cells
**Authors**: V. Hernandez-Gordillo, D. Przybyla, and J.A. Chmielewski
**Objective**: We have previously reported the design of a peptide-mimetic that assembly into a mesh-like three-dimensional (3D) structure upon the addition of metals that can be used for the culture of human cells. Thus, we wanted to incorporate human stem cells that have the potential to use in tissue regeneration.
**Methods**: We have encapsulated human stem cells in a collagen-based scaffold that assembles into a 3D structure upon the addition of ZnCl2. We have evaluated cell survival via MTS assay and cell differentiation via RT-PCR.
**Results**: An important aspect in the design of our bioscaffold is the possibility of incorporating biologically important His-tagged molecules within the 3D scaffold. Here, we report the incorporation of RGDS-His7, GFP-His8 and EGF-His6 within the 3D scaffold. We show that the addition of His-tagged molecules do not interfere with the assembly of the 3D scaffold. We also show by real time PCR (RT-PCR) that some genes important for osteogenic and chondrogenic differentiation are over-expressed in the absence of stimulating factors, when the cells are encapsulated in the 3D scaffold. Moreover, human stem cells are viable for up to 24 days.
**Conclusions**: There is currently a focus in designing biomaterials not only for the delivery of cells but also for the delivery of growth factors. It is believed that such growth factors will be released within the cellular microenvironment and aid in the differentiation of stem cells We have been successful in incorporating his-tagged molecules, such as GFP-His8, and EGF-His6 into the collagen scaffold. Moreover we have demonstrated that the cells encapsulated within the scaffold are viable for up to 24 days.

**Institution**: Indiana University-Purdue University Indianapolis
**Title**: Thiol-ene Hydrogels for Generation and Facile Recovery of Beta-Cell Spheroids
**Authors**: A. Raza, H. Shih, and C.C. Lin
**Objective**: To characterize the formation of pancreatic beta-cell spheroids in PEG-norbornene hydrogels synthesized by step-growth thiol-ene photopolymerizations, as well as to investigate chymotryspin mediated gel erosion kinetics for rapid recovery of these beta-cell spheroids.
**Methods**: MIN6 beta-cells were encapsulated in hydrogels formed by thiol-ene photopolymerization using 4-arm PEG-norbornene and dithiolthreitol or a peptide CGGYC. Cell survival was characterized using Alamar Blue reagent and Live/Dead staining. Hydrogel swelling ratio was measured for calculating mesh size. Enzyme-mediated gel erosion was quantified by gel mass loss as time.
**Results**: Increasing polymer (PEG4NB) concentration decreased hydrogel swelling and mesh size. Beta-cells encapsulated in thiol-ene hydrogels had high viability and formed spherical clusters naturally. The quantity of cell spheroids generated declined at higher polymer contents, potentially due to restricted cell proliferation in denser polymer networks. The average diameter of cell spheroids decreased with increasing cell packing density in gels. On the other hand, the compositions of thiol-ene hydrogels and enzyme concentrations affected enzymatic gel erosion kinetics. PEG4NB/CGGYC gels with 4wt% PEG4NB exhibited a fast erosion profile at low chymotryspsin concentration (1 mg/mL), which is important in rapid recovery of cell spheroids.
**Conclusions**: In conclusion, thiol-ene hydrogels provide a cytocompatible environment for beta-cell encapsulation. The formation and recovery of beta-cell spheroids depend largely on hydrogel compositions. Furthermore, enzymatic degradation rate depends on hydrogel composition and enzyme concentration. Our results suggested that thiol-ene hydrogels crosslinked by 4wt% PEG4NB and CGGYC peptide provide a suitable gel platform for rapid recovery of cell spheroids. This hydrogel system may serve as a cell culture platform for generation and recovery of tissue-engineered cell constructs for regenerative medicine applications.

**Institution**: Purdue University
**Title**: Development of Collagen-PEG Hydrogels for Repair of Vocal Folds
**Authors**: B.K. Chan and G. Schmidt
**Objective**: Natural polymer such as collagen has been widely used in medical applications due to its tunable biodegradability, weak antigenecity and superior biocompatibility. Difficulties persisting with using collagen-based biomaterials however include weak mechanical properties and poor processing abilities. Some of these processing and mechanical deficiencies can be overcome by incorporating synthetic polymer such as poly(ethylene) glycol PEG. In this contribution, we investigate the effect of double cross-linkings of PEG and collagen on the physical properties of collagen hydrogels.
**Methods**: Hydrogels were formed by mixing Type-1 Collagen and PEG (MW: 12,000) and then expose the pre-cursor solution to UV, following by incubation at 37°C for overnight. Mechanical properties of the nanocomposite hydrogels were determined using an AR2000 and ARES rheometers.
**Results**: Rheology results show that double-crosslinked hydrogel samples exhibit viscoelasticity plateau within testing shear stresses (0.1 - 1000Pa) and frequencies (1 - 20Hz). Elastic moduli (G') are more than 10-fold greater than loss moduli (G") for all hydrogel samples. Tensile testing results show that all samples display elastomeric properties. Double cross-linked samples show increased stretching compared to pure PEG and photo cross-linked only hydrogel samples. Also, reduced swelling is observed in the double cross-linked samples.
**Conclusions**: All hydrogel samples show structural stability by wide linear viscoelasticity limits. The incorporation of collagen and double cross-linking of hydrogels exhibited increased elongation and reduced swelling. Future plans include structural characterization, stress relaxation experiments, and cell culture to study cell adhesion, proliferation, and spreading. This system can potentially replace the current materials by extending longevity and providing mechanical properties that better match the natural tissue of the vocal folds.

**Institution**: Purdue University
**Title**: Mechanically Tough Pluronic F127/Laponite Nanocomposite Hydrogels
**Authors**: C.J. Wu, A.K. Gaharwar, B.K. Chan, and G.Schmidt
**Objective**: Mechanical properties of polymer hydrogels are critical to their performance as tissue engineering scaffolds especially in load bearing tissues and wound sealants. In this study, we aim to synthesize mechanically tough nanocomposite hydrogels by photo-cross-linking PEO-PPO-PEO triblock copolymer diacrylates (Pluronic F127 diacrylate) in the presence of silicate nanoparticles, Laponite.
**Methods**: Precursor solutions of nanocomposite hydrogels were prepared by dissolving first Pluronic F127 diacrylate (PluDA) and then Laponite in de-ionized water containing 0.1-w/v% initiator I2959. The resulting hydrogels are subjected to tensile tests, rheological measurements and cryo-SEM to determine their mechanical properties and network structures.
**Results**: The resulting nanocomposite hydrogels have high elongations and improved toughness when compared to their polymer hydrogel counterparts. Oscillatory shear and creep experiments suggest that the silicate nanoparticles physically interact with the covalently cross-linked polymer networks and impart viscoelasticity to the hydrogels. Imaging the structures of deformed nanocomposite hydrogels with cryo-scanning electron microscopy (cryo-SEM) leads us to believe that stretched hydrogels have finer network structures with smaller pore sizes when compared to the unstretched ones.
**Conclusions**: Our results suggested the successful synthesis of nanocomposite hydrogels with high elongations and improved toughness compared to their polymer hydrogel counterparts. Cryo-SEM images showed that the network of nanocomposite hydrogels becomes finer with elongation. These structural transitions combined with the viscoelastic properties measured suggest that physical interactions between Pluronic F127 and Laponite may contribute to structural rearrangements during deformation, whereas covalent cross-linking of polymer chains forms elastic networks to maintain the integrity of hydrogels. Overall we expect our findings will provide new synthetic designs of mechanically robust hydrogels for their applications in load bearing tissues.

**Institution**: Purdue University
**Title**: Physically and Chemically Cross-linked Hydrogels from Poly(ethylene glycol) and Silicate Nanoparticles
**Authors**: C.P. Rivera, A.K. Gaharwar, C.J. Wu, and G. Schmidt
**Objective**: The structures and mechanical properties of both physically and covalently cross-linked nanocomposite hydrogels made from poly (ethylene glycol) (PEG), and silicate nanoparticles (Laponite RD) are investigated.
**Methods**: The injectable nanocomposite precursor solutions can be covalently cross-linked via photopolymerization. The resulting hydrogels have interconnected pores, high elongation and toughness. These properties depend on the hydrogel composition, polymer-nanoparticle interactions and degree of cross-linking (both physical and covalent).
**Results**: Covalent cross-linking of polymer chains leads to the formation of an elastic network, whereas physical cross-linking between nanoparticles and polymer chains induces viscoelastic properties. At high deformations covalent bonds may be broken but reversible and physical bonds rebuild and somewhat self-heal the overall network structure. Addition of silicate also enhances bioactivity and adhesiveness of the hydrogel as these materials stick to soft tissue as well as to hard surfaces. In addition, MC3T3-E1 mouse preosteoblast cells readily adhere and spread on the nanocomposite hydrogel surfaces.
**Conclusions**: Collectively, the property combinations such as elasticity, stiffness, interconnected network, and adhesiveness and cell adhesion provide inspiration and opportunities to engineer mechanically strong and elastic tissue matrixes for a variety of tissues such as vocal folds, ligaments, cartilage and orthopedic interfaces.

**Institution**: Indiana University-Purdue University Indianapolis
**Title**: Dual-mode Degradable Hydrogels Formed by Thiol-ene Photo-Click Reactions
**Authors**: H. Shih, A. Raza, and C.C. Lin
**Objective**: To characterize hydrolytic and enzymatic degradation of poly(ethylene glycol) (PEG)-peptide hydrogels formed by thiol-ene photo-click reactions. Hydrogels crosslinked by step-growth photopolymerization of 4-arm PEG-norbornene and bis-cysteine containing peptides were used to study hydrolytic degradation, while a chymotrypsin sensitive sequence was used to explore user-controlled gel erosion.
**Methods**: 4-arm PEG-norbornene (PEG4NB) and bis-cysteine peptide crosslinkers were used to form hydrogels via a step-growth photopolymerization mechanism. The hydrolytic degradation of hydrogels was characterized by gel swelling and oscillatory rheometry, while the enzymatic degradation of thiol-ene hydrogels crosslinked by peptide CGGYC was quantified by mass loss after chymotrypsin treatment.
**Results**: We found that PEG4NB hydrogels were hydrolytically degradable in physiological (pH 7.4) and basic (pH 9) conditions but appeared to be stable in acidic environment (pH 6). In addition, hydrolytic hydrogel degradation could be tuned by using peptide crosslinker with single amino acid substitution (next to cysteine) and the degradation rates could be tuned from 2 to 7 weeks. PEG4NB-CGGYC gels were used for chymotrypsin-mediated erosion, which was achieved in 5 to 25 minutes. Furthermore, hydrogels crosslinked by a mixture of enzymatic cleavable and non-cleavable peptides displayed a unique 'dual-mode' (both hydrolytic and enzymatic) degradation behavior.
**Conclusions**: While ester hydrolysis is both acid and base-catalyzed, we found that hydrolytic degradation of PEGNB hydrogels is only base-catalyzed. The same gel formulation (PEG4NB-CGGYC) that undergoes hydrolytic degradation could also be degraded via enzymatic surface erosion, demonstrated by a linear gel mass loss as time. This rapid erosion provides an approach to recover tissue engineered constructs from gels for characterization or applications. In summary, we have developed a PEG hydrogel system with dual-mode and tunable degradation profiles. This diverse gel platform has great potential as a dynamic matrix for promoting tissue morphogenesis.

**Institution**: Purdue University
**Title**: Production of Adhesive Elastin-Based Proteins
**Authors**: M.J. Brennan, R.S.C. Su, J.J. Wilker, and J.C. Liu
**Objective**: The objective is to clone, express, and characterize recombinant proteins to produce a novel surgical adhesive, cell attachment coating, or tissue engineering scaffold. A repetitive elastin domain (VPGXG) will be combined with a tyrosine-rich pre-adhesive domain. Tyrosine residues will be converted to DOPA (3,4-dihydroxyphenylalanine) to confer adhesive properties.
**Methods**: Standard cloning methods were used to combine and concatemerize a repetitive elastin domain with a tyrosine-rich domain. The protein was expressed in Escherichia coli, identified by Western blot, and purified using temperature cycling and nickel affinity chromatography.
**Results**: Construction of the DNA sequences was successful, as verified by sequencing. A protein with 6% tyrosine was overexpressed in BL21(DE3). Increasing the percentage of tyrosine to 17% dramatically reduced expression. This phenomenon may be due to the density of tyrosine residues and will be investigated further. A protocol for purification is currently being developed.
**Conclusions**: In this study, a protein containing an elastin region and a pre-adhesive region was successfully overexpressed in E. coli. Following purification, the tyrosine residues in the pre-adhesive region will be converted to DOPA in order to imitate the properties of natural mussel adhesive proteins, which contain up to 30% DOPA. The protein will then be tested for its ability to coat surfaces and act as an adhesive for dry, wet, and biological substrates.

**Institution**: Indiana University-Purdue University Indianapolis
**Title**: Residual Stress of Ceramic Coatings on Biocompatible Magnesium Alloys
**Authors**: J. Zhang
**Objective**: The objective of this study is to evaluate the residual stresses of the ceramic coatings on biocompatible AZ31 magnesium alloys. The coatings improve the corrosion resistance of the magnesium alloys and are produced by microarc oxidation (MAO) method. The effect of applied voltage on residual stress is also studied.
**Methods**: An integrated experimental and modeling approach has been employed. Residual stresses attributed to the MgO constituent of the coatings at oxidation voltages between 250 V to 350 V have been evaluated by X-ray diffraction (XRD) using $\sin^2\psi$ method. An analytic model is also used to compute the stress distributions in the coatings.
**Results**: The measured stresses using $\sin^2\psi$ XRD method in the MgO constituent of the MAO coatings are tensile in nature. The residual stresses decreased with the increase of the applied voltage. The predicated stresses from the model are in good agreement with the experimental measurements.
**Conclusions**: At 350V, coatings have a uniform surface morphology and the lowest residual stress. This is the optimal voltage in the MAO process to produce the high-quality corrosion resistant coatings. The voltage dependence of the residual stress is attributed to the micropores and cracks during the microarc discharge process which release the residual stresses in the coatings.

**Institution**: Purdue University
**Title**: Expression of Resilin-Based Proteins and Purification by Selective Precipitation
**Authors**: K.M. Cherry, J.N. Renner, Y. Kim, and J.C. Liu
**Objective**: We have designed a resilin-based protein for use as a scaffold in tissue engineering of cartilage replacements. The objective is to identify a protocol for expression and purification of this protein in a repeatable, efficient, and cost-effective manner. Doing so will facilitate further experimentation with the protein.
**Methods**: The resilin-based protein was expressed in various E. coli hosts by IPTG induction. Denatured bacterial lysates were prepared and purification was performed by selectively precipitating proteins using ammonium sulfate. The remaining undesired proteins were removed through exploitation of resilin's heat stability by heating the solution to 80°C.
**Results**: Four different bacterial hosts were tested using IPTG induction. The cell line BL21(DE3)RIPL displayed the highest expression levels of the desired recombinant protein. Proteins were salted out using increasing concentrations of salt. A 10% ammonium sulfate solution precipitated undesired proteins. Increasing the ammonium sulfate concentration to 20% precipitated the desired resilin-based protein. The pellet was resuspended and heated to 80°C. Because of resilin's heat stability, the desired protein remained in solution whereas other proteins precipitated. The remaining supernatant was ~95% pure as assessed by densitometry analysis of SDS-PAGE gels.
**Conclusions**: An expression protocol and salt-and-heat purification method were established for recombinant resilin-based proteins. By screening hosts and investigating the effect of salt concentration, resilin-based proteins were manufactured and recovered to 95% purity. This method is a cost-effective, quick, and efficient way to purify a robust protein that demonstrates high heat stability. The isolated resilin-based protein will be used for characterization of intrinsic protein properties, mechanical properties, and cell response. Evaluation of these results will determine the potential of the protein-based material as a scaffold for chondrogenic tissue engineering.

**Institution**: Indiana University-Purdue University Indianapolis
**Title**: PEG-DTT-Albumin Hybrid Hydrogels for Controlled Release Applications
**Authors**: K.J. Lowery and C.C. Lin
**Objective**: The purpose of this research was to synthesize and characterize hybrid hydrogels formed by step-growth photopolymerization of norbornene-functionalized poly(ethylene glycol) (PEGNB), dithiothreitol (DTT), and thiolated bovine serum albumin (BSA), as well as to use the PEG-DTT-albumin hydrogels for controlled release of basic fibroblast growth factors (bFGF).
**Methods**: Sulfhydryl groups were introduced on the BSA surface using 2-iminothiolane (Traut's reagent) at various reaction conditions. Hydrogels were formed via photopolymerization of PEGNB, dithiothreitol (DTT), and thiolated BSA (BSA-SH). Hydrogel properties were characterized by *in situ* rheometry, BSA release, gel fraction, and gel swelling. bFGF release was quantified by ELISA.
**Results**: *In situ* rheomotry results revealed that the hydrogel elastic moduli increased with increasing [BSA-SH], likely due to multiple crosslinking points on BSA-SH (degree of thiolation = 6). Control experiments using PEG-DTT hydrogels incorporated with unmodified BSA showed decreased moduli at higher [BSA] because non-thiolated BSA interfered with hydrogel crosslinking. BSA release showed more albumin was crosslinked/retained in PEG-DTT-BSA(SH) hydrogels due to covalent crosslinking of BSA-SH to the gel. At a higher BSA concentration, gel swelling decreased when BSA-SH was used. This was due to the formation of a tighter network. Increased BSA-SH in the gels also reduced bFGF release rate.
**Conclusions**: We have successfully synthesized PEG-DTT-albumin hybrid hydrogels with tunable biophysical properties. The covalent incorporation of BSA in hydrogels improved hydrogel mechanical properties but only slightly decreased hydrogel swelling. More importantly, growth factor release from these hybrid hydrogels can be easily tuned by incorporating small amount of thiolated BSA. This hydrogel platform provides an easy way of controlling material properties, as well as offers a means of achieving sustained growth factor delivery that may be useful in facilitating tissue regeneration.

**Institution**: The University of Akron
**Title**: Degradability of PEG-Collagen Modular Scaffolds
**Authors**: M.J. Majcher and R.K. Willits
**Objective**: Overall, the purpose of the experiment is to analyze the structure of a modular polyethylene glycol (PEG) scaffold formed via compaction of PEG microgels and collagen. The main goal is to produce a modular gel in which stiffness remains constant as the scaffold degrades.
**Methods**: PEG microgels were formed via salt precipitation of PEG-diacrylate and UV crosslinking as previously described. The microgels, which were activated to bind to amine groups, were then compacted with collagen and 4-arm PEG-amine to form a scaffold. To examine the stiffness, oscillatory shear rheometry was performed after exposure to collagenase.
**Results**: The microgels were characterized by swelling and sizing. The average diameter of the microgels was 1.6 microns, which was consistent with previous reports. The swelling ratio ($M_{wet}/M_{dry}$) was calculated to be approximately 15. FITC-collagen degraded completely within 72 hours in solution and the standard curve is linear from 4.125 μg to 41.25 μg of collagen. Degradation amounts and stiffness values are still to be collected at time points of 2, 4, 6, 8, and 12 days.
**Conclusions**: These modugels were previously used to demonstrate changes in 3D PC12 cell behavior when collagen concentration increased, but gel stiffness remained constant. While the changes in cell behavior were indicative of increases in collagen, this study seeks to confirm that the collagen does not have a significant impact on the stiffness by degrading the collagen and re-examining the stiffness. However, the correlation between degradation and stiffness has yet to be determined. These results will allow future investigations on how varying protein content in 3D, without the resulting typical changes in stiffness, alters cell behavior.

**Institution**: Purdue University
**Title**: Modular Protein-Based Materials with Tunable Mechanical Properties for Cartilage Engineering
**Authors**: R.S.C. Su and J.C. Liu
**Objective**: A novel recombinant protein composed of abductin structural repeats will be manufactured to create a matrix with mechanical properties similar to those of natural articular cartilage. The crosslinking density of the matrices will be varied, and the resultant physical and mechanical properties will be measured.
**Methods**: Abductin oligo-nucleotide sequences were designed. The final protein design incorporates mechanical domains consisting of 36 repeats of an abductin decapeptide sequence. A recursive cloning method was developed to achieve this number of repeats. The Studier auto-induction method was used to express the desired protein. Results were analyzed by SDS-PAGE and Western blot.
**Results**: We have successfully developed a modular design for recombinant proteins to be used in cartilage engineering. The proteins consist of structural repeats, bioactive domains, and cross-linking sites. To date, we have successfully cloned the final abductin sequence containing 36 repeats. Studier auto-induction and nickel-based affinity purification show promise for expressing and purifying the abductin-based protein in a small scale. We are currently working on scaling up expression and purification.
**Conclusions**: Abductin has attractive compressive properties for cartilage tissue engineering. Varying the cross-linking density of abductin-based materials will allow a wide range of mechanical properties to be achieved. The physical and mechanical properties of these materials will be measured, and the response of cells of these material properties will be characterized.

**Institution**: Purdue University
**Title**: VEGF Peptide Promotes Endothelial Differentiation of Mesenchymal Stem Cells
**Authors**: R.J. Galas and J.C. Liu
**Objective**: he objective of this study is to evaluate the use of the VEGF peptide "QK" in directing the endothelial differentiation of mesenchymal stem cells (MSCs) for future use in recombinant scaffolds and hydrogels
**Methods**: MSCs were cultured at confluence in the presence of $1x10^{-6}$ M QK for up to three weeks. Gene and protein expression of endothelial markers was quantified by qPCR and thresholding of immunofluorescent images. Capillary formation on Matrigel was evaluated by measuring tube length and the number of branch points.
**Results**: This study demonstrated that the peptide QK resulted in an increase in endothelial markers after one week. After two weeks of differentiation on tissue culture polystyrene, cells formed capillary-like networks when seeded on Matrigel. Treating cells with QK increased path length and number of branch-points in the networks. Studies to evaluate gene expression levels are ongoing.
**Conclusions**: Soluble QK increased endothelial characteristics of differentiating MSCs. The increase indicates that QK is a promising peptide for inclusion into tissue engineering scaffolds because bound QK should also direct endothelial differentiation of MSCs. Spatially controlling the inclusion of QK could control the pattern of the resulting vasculature. This patterned vasculature could be able to support other tissues derived from the MSC lineage, such as bone. Thus, the aim is to derive a vascularized tissue from a single stem cell source.

**Institution**: Vanderbilt University
**Title**: Stimuli-Responsive Microspheres for Sustained Protein Delivery to Ischemic Environments
**Authors**: R.V. Joshi and C.L. Duvall
**Objective**: To design a new pH and temperature responsive microparticle system for delivery to the low pH environment of ischemic wounds (5.2-7.2). These microspheres will gradually release their payload at slightly acidic pH and then undergo dissolution and removal from the site of injection as the tissue returns to physiological pH.
**Methods**: FITC-BSA encapsulated microparticles were fabricated from a copolymer composed NIPAAm, PAA and BA formulated by RAFT polymerization and characterized for molecular weight, polydispersity, composition and LCST. Microsphere size and morphology was determined by SEM and fluorescence microscopy. *In vitro* release was quantified in PBS at pH 5.5, 6.5, and 7.4.
**Results**: The polymer of molar composition of 57.21% NIPAAm, 17.78% PAA, and 25.01% BA by NMR had a molecular weight (Mn) of 27.98 kDa with a polydispersity of 1.089. The LCST study indicated insolubility of the polymer at 37°C and ischemic pH but solubility at 37°C at higher pH (7.0 to 7.5). SEM showed that microspheres ~2 microns in diameter were formed, and fluorescence microscopy confirmed the incorporation of FITC-BSA. Release experiments indicated a burst release due to microsphere solubilization at pH 7.4, while a more sustained release was observed at pH 5.5 and 6.5 over the span of 23 days.
**Conclusions**: This study illustrates the ability of pH- and temperature-responsive, "intelligent" microspheres fabricated from poly(NIPAAm-co-PAA-co-BA) to release encapsulated protein in a sustained manner to ischemic (acidic) environments and be subsequently cleared upon restoration of physiologic pH. We have shown that at 37°C, this injectable system solubilizes at pH 7.4 but forms microgel spheres in conditions with slight acidity (pH 5-7). Future studies will pursue therapeutic application of this delivery system to release pro-angiogenic proteins to ischemic tissues.

**Institution**: Purdue University
**Title**: Bio-Inspired Design of a Cellular, Interlocking Material
**Authors**: S. Khandelwal, R.J. Cipra, S.J. Bolton, and T.H. Siegmund
**Objective**: Shape optimization, structural design and element arrangement are the main methods used by nature to optimize mechanical properties of toughness, stiffness and strength. The spine, foot, shark tesserae and nacre serve as examples. We investigate if a bio-inspired engineering material could replicate such structures, and interrogate the resulting material in regards to its mechanical properties.
**Methods**: Cellular tetrahedra shaped unit elements with a range of relative densities were arranged in an interlocked pattern without the use of adhesives. Constraints are provided by external boundaries. Materials were tested in an impact tower to characterize their mechanical properties. Numerical and theoretical models were developed to predict the observed results.
**Results**: Material assemblies made out of brittle constituents possess a quasi-ductile response. Strength, stiffness and toughness exhibit a linear dependency on the relative density. The analytical and numerical models qualitatively predict the observed response.
**Conclusions**: A bio-inspired design of cellular materials is demonstrated. The design is based on the principle of topologically interlocking whereby individual solid elements interact with each other by contact. Tension is carried by constraining tensile members. The resulting materials are shown to be light weight and damage tolerant. In the cellular materials the Voigt upper bound is realized for the material stiffness. The material has a structure and function similar to that of the spine and the foot, or shark tesserae in that the stiffness is adaptively changeable, and we demonstrate that such change in stiffness does occur without the loss of toughness.

**Institution**: Purdue University
**Title**: Chiral Mechanical Characteristics of a Continuum Helicoids Dissection
**Authors**: S. Varanasi and T.H. Siegmund
**Objective**: DNA has been shown to possess chiral characteristics. It is of interest to understand the elastic deformation characteristics of structures with chiral architecture. In order to study chiral elasticity we employ solids dissected into two identical continuum helicoid parts.
**Methods**: We studied the deformation response of the chiral elastic structure under various loading conditions and determined the role of the material properties of the individual phases in the chiral structure (such as elastic moduli and Poisson's ratios), and hypothesized that chirality depends on the contrast in mechanical properties of the two phases, and the interaction between the phases. A CAD model of the helicoid dissection was created. Finite Element based numerical models were developed using the CAD model geometry. Numerical simulations were conducted for various loading and boundary conditions seeking quasi-static thermoelastic response. A compressive displacement load was applied on the opposite edge in the same direction while the nodes on this edge were free to move in the other two directions.
**Results**: It was found in the cases of varying elastic moduli and Poisson's ratios that the chiral structure was predicted to rotate about the helicoids axis when a compressive or tensile displacement load was applied. Expectedly, no rotation was observed in the degenerate case. It was also found that the relation between the magnitude of the angle of rotation and the displacement load is nearly linear. The direction of rotation is only dependent on the handedness of the dissections but not on the material properties of the dissections. The conditions of bonding between the dissections will also be discussed.
**Conclusions**: The effect of contrasting material properties in a chiral continuum structure formed by assembling two helicoids in producing non-intuitive deformation response was studied. Most of the observations made in this structure can be generically extended to other continuum structures such as a cylinder or a sphere formed by the assembly of helicoids dissections. This study will be useful in gaining insights into the mechanics of DNA and other chiral biological structures such as bone.

**Institution**: Purdue University
**Title**: Modified Expression Conditions for Resilin-Based Proteins
**Authors**: Y. Kim, J.N. Renner, K. Cherry, and J.C. Liu
**Objective**: Resilin has emerged as a potential material for tissue engineering applications because of its attractive properties. We are developing an artificial protein containing bioactive domains and resilin repeats from Anopheles gambiae. Modifications to the Studier auto-induction protein expression were employed to increase the yield of the target protein.
**Methods**: The Studier auto-induction method was utilized to express the proteins in E. coli BL21(DE3)pLysS. Samples were taken during expression to monitor bacterial growth and analyze the expression by Western blot. The band intensity was quantified by Image J software. The heating and salting out method was used to purify proteins.
**Results**: The Studier auto-induction method resulted in leaky expression and degraded or truncated proteins. Western blot analysis demonstrated that only 50% of expressed proteins were the desired resilin protein of 50 kDa. Adding 0.5% glucose to the overnight culture medium suppressed leaky expression. The percent of desired protein was increased by lowering the culture temperature from 25°C to 20°C and by harvesting cells in the exponential phase. Scale-up experiments confirmed that these modifications resulted in a high percent of desired protein. The resilin-based proteins at 50kDa were purified by the precipitation with 20% ammonium sulfate followed by heating at 80°C.
**Conclusions**: In order to improve the yield of the target protein, the standard Studier auto-induction protein expression method was modified for resilin-based proteins. Adding glucose to the overnight culture, lowering the culture temperature, and harvesting cells in the exponential phase are recommended to avoid protein degradation or truncation when expressing proteins with a large number of repetitive resilin sequences. The resilin-based proteins were purified via a heating and salting out method. The structure, mechanical properties, and biological activities of the resilin-based proteins will be characterized to assess their potential as a tissue engineering matrix.

**Institution**: Illinois Institute of Technology
**Title**: Phase Contrast X-Ray Imaging of Biomaterials for Tissue Engineering Applications
**Authors**: A.A. Appel, J.C. Larson, S.I. Somo, M.A. Anastasio, and E.M. Brey
**Objective**: The objective of this research is to evaluate mechanisms of X-ray contrast produced by model polymer scaffolds and determine the extent to which X-ray signatures can be used to identify these scaffolds *in vitro* and in tissue with X-ray phase contrast (PC) imaging technique Multiple Image Radiography (MIR).
**Methods**: CT scans of samples were performed at X-15A beamline at National Synchrotron Light Source at Brookhaven National Laboratory using X-ray PC technique known MIR. A silicon analyzer crystal employing (3,3,3) reflection and an X-ray energy of 20-keV were used. Data were acquired at 11 angular analyzer positions over 8 μradians.
**Results**: MIR produces three separate images depicting X-ray absorption, refraction, and ultra-small-angle-scatter (USAXS) properties of samples. Hydrogels imaged prior to implantation were invisible in absorption-based images, but could be discerned from culture media in refraction images. Refraction images of porous hydrogels have a speckle pattern that is not present in nonporous gels. X-ray refraction CT allowed for 3D imaging of the interface between hydrogels and fibrovascular tissue in explanted samples. Porous structure of poly(lactic-co-glycolic acid) (PLGA) scaffolds could been seen in all three MIR property images. Refraction and scatter volume data allowed quantitative analysis of PLGA volume fraction over time.
**Conclusions**: X-ray PC imaging techniques can be used to image polymer scaffolds commonly used in tissues engineering. The ability to image structures based on differences in X-ray refractive index allowed identification of hydrogels in both cell culture conditions and embedded in tissues. X-ray PC allowed imaging of the structure of polyester scaffolds and quantification of percent scaffold volume over time. The technique was also effective in visualizing various types of tissue invasion into these scaffolds. These samples give a glimpse into potential use of PC X-ray techniques to quantify and characterize biomaterials and engineered tissues *in vitro* and *in vivo*.

**Institution**: University of Kentucky
**Title**: Temperature Effect on PLGA Scaffold Properties and IGF Bioactivity
**Authors**: A. Clark, T.A. Milbrandt, J.Z. Hilt, and D.A. Puleo
**Objective**: To determine if the processing temperature needed to obtain higher compressive modulus will adversely affect bioactivity of IGF-I after its encapsulation in PGLA scaffolds.
**Methods**: Two types of PLGA microspheres were prepared using a double emulsion technique, mixed with NaCl, consolidated, sintered and leached. To study the effect of temperatures on growth factor activity, an IGF-I solution was incubated at various temperatures and the bioactivity was determined by measuring DNA contents with a Hoechst assay.
**Results**: Compression tests showed that there was a significant difference, up to a 10-fold increase in strength, by increasing the temperature up to or above the Tg. This mechanical property is important for maintaining the microarchitecture for cell ingrowth and matrix synthesis without collapsing. After IGF incubation at elevated temperatures the DNA assay results suggest that the elevated temperatures did not adversely affect bioactivity of IGF-I based on the statistically ($p<.001$) similar DNA contents compared to normal 37°C incubation; IGF-I stimulated about a 140% increase in proliferation compared to cultures without growth factor.
**Conclusions**: Higher compressive strength of the scaffold is necessary to withstand physiologic conditions, requiring the use of higher temperatures during fabrication. These elevated temperatures, however, did not affect the bioactivity of the IGF-I solution. A drug-containing, porous, polymeric scaffold with controllable properties, such as release profile, degradation rate, and compressive modulus, allows for the tunability to meet a wide range of site-specific needs in helping to regenerate damaged tissues.

**Institution**: University of Kentucky
**Title**: Development of Biodegradable Hydrogels for Controlled Release of Antimicrobial/Antioxidant Agents
**Authors**: A.L. Vasilakes, J.P. Byarski, D. Biswal, R. Peyyala, D.A. Puleo, J.Z. Hilt, and T.D. Dziubla
**Objective**: A biodegradable hydrogel, co-loaded with vancomycin and catalase, is developed to interfere with the ability of Staphylococcus aureus to develop resistance against vancomycin. To inhibit bacterial resistance emergence, the antioxidant-enzyme catalase is used to break down hydrogen peroxide, a signaling molecule used to induce genetic mutation.
**Methods**: Co-loaded poly(β-amino ester) hydrogels are formed through free radical polymerization. Vancomycin release is tested on S. aureus seeded agar plates as well as via sink condition degradation in PBS solution. Catalase release in PBS is tested through $^{125}I$ radiolabeling and activity is tested with an ODP/HRP assay.
**Results**: Extended release is shown for both vancomycin and catalase under sink conditions, and vancomycin release on bacteria shows zone of inhibition areas comparable to that of Kirby-Bauer controls. Importantly, after analyzing the activity of the drugs, the presence of catalase did not reduce the inhibitory effects of loaded vancomycin, and vancomycin did not interfere with catalase activity.
**Conclusions**: By acting upon the bacteria with two agents which function through independent mechanisms, it is possible to suppress the ability of bacteria to propagate via the antibiotic vancomycin, as well as evolve resistance via hydrogen peroxide signaling in formed biofilms via the antioxidant enzyme catalase. Sustained release of the drugs is achieved and is imperative for this hydrogel system to be effective *in vivo* as an infection reducing and preventing medical device.

**Institution**: University of Kentucky
**Title**: Investigation of Composite Biodegradable Hydrogel Systems as 3D Porous Scaffolds
**Authors**: A.M. Hawkins, T.A. Milbrandt, D.A. Puleo, and J.Z. Hilt
**Objective**: To facilitate the formation of a 3D porous scaffold, we propose using fast degrading hydrogel particles entrapped in a slower degrading outer matrix hydrogel. The introduction of the scaffold to an aqueous environment will begin the porogen degradation process, thereby opening the porous network and potentially releasing drug molecules.
**Methods**: In this work, composite poly(β-amino ester) biodegradable hydrogel systems were prepared through free radical polymerization. System properties were studied (i.e., degradation, compressive moduli, 3D structure, etc.). A procedure for creating hydrogel particles of controlled particle size was developed, and the fast-degrading particles were entrapped in a slower-degrading outer matrix.
**Results**: The hydrogel selected to make the fast-degrading porogen particles degraded in a period of approximately 7 hours. These particles were entrapped in a system that degraded over 4 months. Composite systems with particle loadings of 25%, 35%, and 35% (by mass) were studied. The degradation profile indicated, as expected, that systems with higher porogen loading had a greater percentage of mass loss in the first day of degradation. MicroCT analysis showed pore formation in all systems with varying degrees porosity dependent on the porogen loading.
**Conclusions**: These systems have the potential to be used as biodegradable tissue engineering scaffolds that would allow cell ingrowth into the porous structure. The use of the biodegradable porogen particles and outer matrix can allow for a drug to be released rapidly upon implantation of the porogen or slowly through degradation of the outer matrix. The use of PBAE biodegradable systems enables precise control of the hydrogel degradation rate, mechanical integrity, swelling, and cytotoxicity used for each portion of the scaffold.

**Institution**: University of Kentucky
**Title**: Drug Delivery from Biodegradable Space-Making Calcium Sulfate/Poly(β-amino ester) Hydrogel Composites
**Authors**: B.R. Orellana, M.V. Thomas, J.Z. Hilt, and D.A. Puleo
**Objective**: For oral prosthetics to be successful, sufficient amount of alveolar bone to anchor an implant is needed. Calcium sulfate (CS) composites will be developed to act as a 'tenting' barrier to soft tissue infiltration, while allowing the delivery of osteogenic agents from biodegradable hydrogel particles to promote vertical ridge regeneration.
**Methods**: Blank, 1%wt. and 10%wt. hydrogel composite samples were fabricated. Destructive degradation testing was performed to understand degradation profiles of the composites. Micro CT imaging was used to determine distribution trends of hydrogel particles throughout the CS matrix. Release of model lysozyme protein from composites was tested for controlled delivery kinetics.
**Results**: Samples degraded very consistently to one another via surface erosion, suggesting the amount of hydrogel particles did not have a significant effect on the dissolution rate. The hydrogel loading-independence of composite degradation rate may allow for tuning to provide a sustained delivery of drug-loaded particles to stimulate bone regeneration. MicroCT images showed a uniform distribution of particles within the CS matrix. This provides confidence for reproduction, and the consistent release of particles as the composites degrade. The 10%wt. [286mg (0.37% of total mass)] and the 1%wt. [30.7mg (0.037% of total mass)] loaded hydrogel composites demonstrated a zero-order release.
**Conclusions**: Calcium sulfate composites containing biodegradable hydrogel particles for delivering osteogenic biomolecules can be useful for vertical bone augmentation. The incorporation of hydrogel particles in calcium sulfate had little effect on degradation, thus providing a suitable composite for promoting controlled release of drug. Composite release studies showed promising results for sustained release of protein. Further release studies will investigate altering the drug loading in hydrogel particles needed to optimize the delivery capabilities of the composites.

**Institution**: University of Kentucky
**Title**: The Effect of Plasticizers and Drug on Drug Delivery Films
**Authors**: C.L. Rabek, T.D. Dziubla, and D.A. Puleo
**Objective**: Chronic inflammation can cause contracted fibrotic scar tissue with functional and aesthetic consequences. Well timed release of drugs may prevent excessive scarring. Cellulose acetate phthalate with Pluronic F-127 is an erosion-controlled system but films are rigid. To impart flexibility, plasticizers were added. The effect of plasticizers in combination with drug on the mechanical properties and degradation were explored.
**Methods**: CAP, Pluronic F-127, Ketoprofen, and plasticizer were combined to make films. Films were cut into discs for degradation studies or into dog bones for mechanical testing. Discs were incubated on a shaker in PBS. At different time periods, discs were removed and mass loss was determined. Mechanical testing was done using BOSE ELF 3300 in tensile mode.
**Results**: Degradation increased with increasing contents of both plasticizer and ketoprofen. Films plasticized with triethyl citrate (TEC) had lower moduli and ultimate tensile strengths but larger % elongations than films plasticized with tributyl citrate (TBC). Addition of ketoprofen to films caused a lower elastic modulus, lower ultimate tensile strength, and higher % elongation. For the same weight %, TEC had greater mechanical effects (i.e., lower modulus and higher % elongation) than TBC. More TEC molecules were present in films causing a higher degree of plasticization (TEC: 0.74 mol per film for 10 wt%, TBC: 0.58 mol per film for 10 wt%). Ketoprofen acted as a plasticizer; plasticizer (TBC or TEC) and drug have synergistic effects.
**Conclusions**: Degradation and mechanical properties of CAP-Pluronic films can be varied by type and amount of plasticizer and drug, thereby making this system tunable in its behavior for different applications.

**Institution**: Indiana University-Purdue University Indianapolis
**Title**: Novel Carbon Nanotubes/45S5BioglassC for Orthopedic Applications
**Authors**: J. Zhang
**Objective**: The objective of this study is to synthesizing a novel carbon nanotube/bioglass composite for orthopedic applications. The mechanical properties of the composite are characterized. The effect of carbon nanotube content on the mechanical properties is investigated, thus identify the optimal processing parameters.
**Methods**: The composite powders are consolidated into multi-wall carbon nanotubes MWCNTs/45S5Bioglass composites by spark plasma sintering (SPS) technique. A homogeneous dispersion of MWCNTs in the 45S5Bioglass powders is achieved using wet mixing followed a dry mixing process. The mechanical properties, including hardness, flexural strength and fracture toughness are measured.
**Results**: Mixing process of wet 10 minutes followed dry 10 minutes can prevent the agglomeration of MWCNTs and obtain microstructurally uniform mixers. The optimal condition of sintering is temperature 850$^{o}$C, pressure 40 MPa and holding time 10 minutes. The optimal content of MWCNTs adding in the composites is 5wt%. Compared with the 45S5Bioglass matrix, the flexural strength and fracture toughness were increased 159% (up to 106 MPa) and 105% (up to 1.17 MPa), respectively.
**Conclusions**: Multi-wall carbon nanotubes/45S5Bioglass composites have been successfully synthesized by means of a mechanical alloying process followed by the spark plasma sintering (SPS) technique. The optimal content of MWCNTs adding in the composites is 5wt%. With this content, the MWCNTs are homogeneously dispersed in the bioglass matrix without agglomerations. The MWCNTs in the path of crack propagation bridge the two crack surfaces, which strongly support the crack bridging effect during crack propagation. It substantially improves both the strength and the toughness of the composite. The present study suggests that the MWCNTs/45S5Bioglass composites have potential orthopedic applications.

**Institution**: University of Kentucky
**Title**: Biocompatibility of Adsorbed Recombinant Protein rTp0483 and Human Serum Fibronectin
**Authors**: M. Dickerson, M. Knecht, and K.W. Anderson
**Objective**: The bacterium T. pallidum spreads in the host by binding ECM proteins using outer membrane proteins (OMPs) on the surface of the bacteria while minimizing immune responses. The OMP Tp0483 also binds serum fibronectin (FN), which may contribute to the immunoevasiveness of the bacteria. Here potential biocompatibility applications are investigated.
**Methods**: Carboxyl (COO-) polystyrene microspheres (PSMs), plain PSMs, or PSMs coated with rTp0483, FN, rTp0483+FN, or BSA were added to CRL-2449 macrophages and phagocytosis, cytotoxicity, and macrophage activation analyzed. Identical coatings were applied to COO- surfaces and RAW 264.7 macrophages used to compare cell adsorption, cytotoxicity, and macrophage activation.
**Results**: Addition of FN to adsorbed rTp0483 decreased phagocytosis of both types of PSMs by CRL-2449 compared to rTp0483 alone and reduced FN modulated RAW 264.7 adhesion compared to FN alone. rTp0483 adsorbed on COO- surfaces was moderately cytotoxic to RAW 264.7. Addition of FN to adsorbed rTp0483 on COO- PSMs had no effect on TNF-α or NO generation of CRL-2449 but resulted in a significant decrease in TNF-α generation for plain PSMs compared to rTp0483 alone. Addition of FN to adsorbed rTp0483 on COO- surfaces led to reduced TNF-α and NO generation on a per cell basis.
**Conclusions**: Activation of macrophages exposed to rTp0483 indicates that it is an antigen. Decrease in phagocytosis and FN modulated cell adhesion when FN was added to adsorbed rTp0483 suggest that it may play a role in immunoevasiveness. Attenuation of TNF-α when FN was added to rTp0483 on plain PSMs and TNF-α + NO on COO- surfaces support this. Lack of inhibition for COO- PSMs may indicate that the suppressive effect is sensitive to differences in surface chemistry and curvature. Cytotoxicity of rTp0483 without FN on COO- surfaces may also play a role by preventing interactions with phagocytic cells.

**Institution**: University of Kentucky
**Title**: Modification of Bone Tissue Engineering Scaffolds for Osteogenic Biomolecule Delivery
**Authors**: R.M. Rudd and D.A. Puleo
**Objective**: The objective of this project is to investigate and develop a novel delivery system for osteogenic biomolecules to stem-like cells via bone tissue engineering scaffolds.
**Methods**: Porous PLGA scaffolds are fabricated by fusing PLGA microspheres made by a double emulsion method. The scaffolds are then mineralized by submerging them in simulated body fluid five times more concentrated than physiological blood plasma. Following mineralization, bisphosphonate molecules were bound to the mineralized scaffolds for future drug release.
**Results**: X-ray diffraction was used to analyze the mineralized scaffolds. After comparing the mineralized scaffolds' diffractograms with hydroxyapatite and carbonated apatite diffractograms found in literature, it is evident that the mineral formed on the scaffolds has a similar crystalline structure to hydroxyapatite. SEM images were also taken to confirm mineral formation on the surface of the scaffolds. To quantify bisphosphonate binding, a TNBS assay was performed. Qualitatively, it is evident that the bisphosphonate is binding to the scaffolds in a concentration-dependent manner, so work is currently being done to quantify this interaction.
**Conclusions**: Once bisphosphonate binding is quantified, the mineralized scaffolds will serve as a platform for controlled release of biomolecules that induce osteogenic differentiation of mesenchymal stem cells. The R groups attached to the geminal carbon of the bisphosphonate molecules can be changed to alter the stability of the bonds between bisphosphonates and osteogenic biomolecules that can either be retained on the surface of the scaffolds with stable bonds or released as a function of changes in pH within a wound site. Once the release of these biomolecules is controllable, it will be possible to induce mesenchymal stem cell differentiation.

**Institution**: University of Kentucky
**Title**: PEG-Iron Oxide Core-Shell Nanoparticles for Dual Cancer Therapy
**Authors**: R.J. Wydra, S.E. Seger, A.M. Kruse, Y. Bae, K.W. Anderson, and J.Z. Hilt
**Objective**: Hyperthermia, the heating of tissue in the 41-45°C range, can induce cellular death on its own or work in conjunction with chemotherapy for improved cancer therapy. In this study, core-shell nanoparticles were prepared with the intent of co-delivery of a chemotherapeutic (17-N-allylamino-17-demethoxygeldanamycin (17-AAG)) and heat.
**Methods**: The core-shell nanoparticles were prepared using atomic transfer radical polymerization (ATRP) to coat iron oxide ($Fe_3O_4$) nanoparticles with a poly(ethylene glycol) (PEG) based polymer shell. Combinational therapy of chemotherapeutic and hyperthermia (particle mediated and elevated temperature incubator) on A549 was investigated to demonstrate an enhanced therapeutic effect.
**Results**: FT-IR measurements verified the PEG coating by identifying peaks at 1715$cm^{-1}$ and 1105$cm^{-1}$, which represent the carbonyl group (C=O) and ether group (C-O-C), respectively. TGA indicated different mass loss profiles and slight differences in overall mass loss between the core citrate coated particles and the polymer coated particles. When the particles were exposed to an alternating magnetic field and heated to the thermoablation range, there was complete cellular death as a result of the heat generated by the nanoparticles. Cytotoxicity studies were used to screen the therapeutic concentrations GA or 17-AAG effective in sensitizing A549 lung carcinoma cells to hyperthermia.
**Conclusions**: ATRP was successfully utilized to coat iron oxide nanoparticles with a PEG based polymer shell. Thermoablation of A549 demonstrates the potential use of polymer coated particles for thermal therapy. Initial results suggest that particle mediated hyperthermia in conjunction with 17-AAG shows potential as a dual cancer treatment. Future work involves the refinement of the dual therapy study to demonstrate a synergistic effect of a co-delivery of chemotherapeutics and hyperthermia from magnetic nanoparticles.

**Institution**: University of Kentucky
**Title**: Mechanical Properties and Loading Techniques of Mucoadhesive Films for Treatment of Oral Dysplasia
**Authors**: S.K. Ramineni, L.L. Cunningham, T.D. Dziubla, and D.A. Puleo
**Objective**: Improve imiquimod loaded mucoadhesive drug delivery system by exploring various ways of loading imiquimod. In addition, tensile and shear adhesion properties of films with differing ratios of film-forming and mucoadhesive components were compared to identify a composition for better handling and drug release properties.
**Methods**: Patches were made from a blend of film-forming polymer, polyvinylpyrrolidone (PVP), and mucoadhesive polymer, carboxymethylcellulose (CMC). Solubility of imiquimod in mucoadhesive polymer solutions was improved by two methods, complexation with amphiphilic hydroxypropyl-β-cyclodextrin (HPβCD) and solubilization in 3:7 [methanol:acetate buffer (100 mM, pH 4.0). Mechanical properties of films were determined by uniaxial tensile testing with displacement rate of 3 mm/sec. Shear adhesion properties were determined on mucin-coated membranes with testing at rate of 0.1 mm/sec.
**Results**: Both HPβCD and acetate buffer improved the solubility of imiquimod and its uniformity of distribution in mucoadhesive films. DSC studies confirmed the formation of complexes resulting in an aqueous solubility of 1:1 (HPβCD:imiquimod) of 100 μg/ml. By comparison, solubility of imquimod in acetate buffer was 2.2 mg/mL. The 2:3 PVP:CMC films had the highest elastic modulus and ultimate tensile strength compared to other films, which may be attributed to better alignment of both polymers (PVP and CMC) and lower moisture content of films. Shear adhesion of the films increased with increasing PVP content which likely is a result of the hygroscopic nature of PVP.
**Conclusions**: Although cyclodextrin can complex with imiquimod and increase its solubility, it was not sufficient to achieve clinically required doses in films, but this could be achieved by using acetate buffer. The 2:3 PVP:CMC films are a good choice with a compromise between obtaining sustained imiquimod release and robust mechanical and shear adhesive properties. Treatment of precancerous lesions by invasive mucoadhesive films can help in halting progression to malignant cancer, decrease incidence, metastasis, and significantly improve survival periods.

**Institution**: University of Kentucky
**Title**: Multiple Drug Delivery System Based on CAP-Pluronic Association Polymer
**Authors**: S.C. Sundararaj, M.V. Thomas, T.D. Dziubla, and D.A. Puleo
**Objective**: To develop a drug delivery system capable of delivering multiple drugs in the required temporal sequence using cellulose acetate phthalate (CAP) and Pluronic F127 (P) association polymer system (CAPP). This particular system will be aimed at releasing four drugs in the appropriate sequence for the treatment of periodontitis.
**Methods**: Drug loaded and blank CAPP films were made using solvent evaporation technique. Multiple drug delivery device was fabricated by attaching blank and drug loaded CAPP films in the appropriate order with a polystyrene backing layer. This was followed by *in vitro* drug release and bioactivity studies.
**Results**: The *in vitro* studies showed that the drug release from the CAPP films occurred in a zero-order manner. The degradation of multiple drug delivery device with polystyrene backing layer resulted in successful intermittent release of a single drug and sequential release of more than one drug. Release of four drugs (antibiotic, anti-inflammatory, anti-bone resorptive and osteogenic drug) in the required temporal sequence was achieved for the treatment of periodontitis. The bioactivity studies that were conducted on the released drugs also showed that the drug released from the CAPP device was in bioactive form.
**Conclusions**: The CAPP device fabricated was capable of surface erosion based release of same drug in intermittent manner and sequential release of multiple drugs in the appropriate order. The release of antibiotic, anti-inflammatory, anti bone resorptive and osteogenic drug can be used to treat the bacterial infection, inflammation, bone resorption stages of periodontitis and help in regeneration of the alveolar bone. This device can be used as a template for treatment of complex disease condition and tissue engineering purpose which requires administration of more than one drug or growth factors.

**Institution**: Illinois Institute of Technology
**Title**: Decoupled Degradation and Stiffness in PEG Hydrogels Enhances Fibroblast Invasion
**Authors**: S. Sokic and G. Papavasiliou
**Objective**: A key factor controlling tissue regeneration is the degradability of the engineered construct. Here, a novel polymerization approach was developed to engineer PEG-peptide hydrogels with varying numbers of enzymatically-degradable cleavage sites independent of variations in the mechanical properties for controlled study and enhanced 3D fibroblast invasion.
**Methods**: Peptides containing either single (SSite) or three (TriSite) collagenase-sensitive (GGL¯GPAGGK) cleavage sites were synthesized with solid phase peptide synthesis. PEGDA hydrogels with degradable crosslinks were polymerized using free-radical photopolymerization. Compressive modulus (E) and degradation rates were quantified to confirm independent tuning of biomaterial properties. Fibroblast invasion within hydrogels was monitored over 2 weeks.
**Results**: Degradation kinetics showed that TriSite hydrogels degraded faster than SSite hydrogels of similar compressive modulus. TriSite hydrogels completely degraded while SSite hydrogels degraded to only 25% of the initial wet weight within 5 hours. Fibroblast invasion only occurred at the 0.25 min polymerization condition with E=~250 Pa in both hydrogel types after 2 weeks. Invasion in TriSite hydrogels was enhanced ~10 fold compared to SSite hydrogels. Fibroblast sprout formation in SSite hydrogels was more clustered compared to spindle sprout formation in TriSite hydrogels. Fibroblasts failed to invade hydrogels at a modulus of ~1600 Pa regardless of the PEG type investigated.
**Conclusions**: In this study, a novel method for decoupling PEG matrix mechanical properties from the degradation kinetics was developed. Hydrogel degradation and subsequently fibroblast invasion was enhanced through the incorporation of multiple collagenase-sensitive cleavage sites independent of the matrix mechanical properties. This approach will allow for controlled study of cell-substrate interactions and shows promise in enhancing cell invasion and degradation in 3D for numerous tissue engineering applications.

**Institution**: Illinois Institute of Technology
**Title**: Effects of Covalent Crosslinking on Dermis-Derived Hydrogel Stiffness and Degradation
**Authors**: S. Pilipchuk, M. Vaicik, J.Larson, E. Gazyakan, M.H. Cheng, and E.M. Brey
**Objective**: A technique previously developed for extracting and assembling extracellular matrix (ECM)-rich hydrogels from soft tissues makes them promising for soft tissue reconstruction applications. This study investigates the effect of glutaraldehyde (GA) as a crosslinking agent on the mechanical properties, biological activity, and *in vitro* and *in vivo* degradation of dermis-derived hydrogels.
**Methods**: Dermal tissue extracts were assembled into hydrogels and crosslinked in 0.625% GA for 0.5, 1, 12, and 24hrs. Mechanical properties were determined using compression testing and resistance to degradation tested using pepsin. Hydrogels were implanted into subcutaneous space of rats and the area of hydrogel remaining quantified at post-implantation intervals.
**Results**: The elastic moduli and yield stress of gels increased with GA incubation time. Non-crosslinked gels were rapidly degraded by pepsin while crosslinked gels were resistant to pepsin-induced degradation. Hydrogel extraction in buffer removed excess GA and gels supported cell attachment and growth. After subcutaneous implantation, non-crosslinked hydrogels completely degraded by three weeks as compared to crosslinked hydrogels. Little tissue was observed in crosslinked gels at week 1, but vascularized tissue invasion was present at weeks 3 and 6. Masson's trichrome and hematoxylin and eosin (H&E) staining images show normal wound healing response to hydrogel implantation with minimal inflammation.
**Conclusions**: GA-induced crosslinking shows a significant increase in the elastic modulus and yield stress of dermis-derived hydrogels with exposure time, suggesting that crosslinking can be used to successfully improve their mechanical properties. Results also indicate resistance to degradation *in vitro* and *in vivo*. The presence of only a mild inflammatory response, absence of multinucleated giant cells, and no noticeable fibrous encapsulation of the biomaterial in a subcutaneous implant model are indicative of hydrogel biocompatibility. Crosslinking of tissue derived hydrogels may improve their potential for future applications in wound-healing models and other applications in three-dimensional tissue reconstruction and regeneration.

**Institution**: University of Kentucky
**Title**: Stability of Multilayered Polymeric Self-assemblies for Oral Wound Applications
**Authors**: S.P. Authimoolam, D.A. Puleo, and T.D. Dziubla
**Objective**: To develop a regenerative treatment strategy for oral wounds with an ability to form robust long lasting soft tissue barrier. In accomplishing this, an affinity based layer-by-layer (LBL) polymeric assemblies administered through series of mouth rinses were developed. These films were then evaluated for their mechanical and chemical stability.
**Methods**: LBL assemblies were formed using alternating layers of biotinylated polymer and streptavidin. Proteolytic stability tests on developed assemblies were studied using radiolabeling method. From results of proteolytic stability, multivariate component analysis was performed. Mechanical stability on *ex vivo* LBL was also studied; through a repeat contact adhesion test using porcine skin.
**Results**: From biotinylated poly(acrylic acid) (PAA) synthesized for various molecular weights (MW) and biotin conjugation, the higher MW PAA's demonstrated significant LBL growth using biotin-streptavidin affinity linkages. Chemical stability tests performed using whole saliva and in bacterial enzyme (pronase) demonstrated significant LBL stability from assemblies of conjugated PAA with high MW. From statistical analysis, an effect of multifactorial dependence controlling proteolytic LBL stability was noticed. In adhesion tests through barrier fatigue contact models, physical barrier effects of LBL in preventing surrounding tissue adhesion and its wear resistance was demonstrated using biotinylated PAA of high MW and layers.
**Conclusions**: Stability tests on LBL assemblies in evaluating its proteolytic and mechanical wear effects demonstrated a robust barrier tendency. This barrier effect resulted from LBL factors includes assembly layers, polymeric MW and affinity based linkages from biotin conjugation. Statistical analysis of proteolytic stability showed a multifactorial dependence of barrier stability with LBL factors, whose understanding aided in designing tunable LBL assemblies. Thus, tunable, affinity-based LBL polymeric self-assemblies with durable barrier properties offer an exciting potential treatment approach for oral mucosal injuries.

**Institution**: University of Kentucky
**Title**: Synthesis and Characterization of a Bioactive Polymeric Pro-Drug for Bone Regeneration
**Authors**: T.A. Asafo-Adjei, D.A. Puleo, and T.D. Dzuibla
**Objective**: Biodegradable polymers used drug delivery include poly(lactic-co-glycolic acid) (PLGA) and poly(lactide) due to biocompatibility, adjustable degradation kinetics, and mechanical strength. However, they are passive in promoting healing and residual particles still induce some degree of inflammation. Developing a bioactive poly(simvastain) diblock copolymer has been proposed for bone tissue healing.
**Methods**: An oligomeric poly(simvastatin) block was synthesized by ring opening polymerization. Characterization was done using Fourier Transform Infrared Spectroscopy (FTIR), gel permeation chromatography (GPC), nuclear magnetic resonance (NMR), and diffraction light scattering (DLS). Control reactions using lactone monomers were conducted to help predict likely behavior of simvastatin's lactone ring during polymerization.
**Results**: The IR spectrum of the diblock copolymer show characteristics from both poly(ethylene glycol) methyl ether (mPEG) and simvastatin monomer components, while controls of both components mixed in comparable molar ratios to the copolymer showed spectra more reflective of simvastatin. GPC revealed MWs of the crude copolymer ranging from 8,000 to 10,500 Da, with an mPEG block of 5,000 Da. NMR integration showed supporting evidence of the formation of an oligomeric simvastatin block. Micelle formation has been evident showing the amphiphilic nature of the copolymer and bonding between both blocks.
**Conclusions**: Characterization methods have shown evidence that that oligomeric blocks of poly(simvastatin) were synthesized. Simvastatin is a well known hypolipidemic prodrug, but the purposes of the present copolymer utilize other desirable properties of the monomer in its active form. These properties include being osteogenic by up-regulating bone morphogenic protein-2 and possessing anti-inflammatory and angiogenic properties. Together, these attributes make poly(simvastatin) desirable as a bioactive micellar and/or polymeric drug delivery system for therapeutic applications in bone regeneration.