

In Vitro Co-Culture Model to Study the Antifibrotic Effect of Porcine Derived Lamina Propria Extracellular Matrix Hydrogel

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Voice disorders represent a significant healthcare challenge that affect approximately 20 million Americans each year [1]. Due to their anatomical location, vocal folds (VFs) are susceptible to various injurious stimuli that can cause damage leading to irreversible changes in structure and ultimately in vibratory function. VF fibrosis represents a challenging therapeutic scenario since it is associated with significant changes in composition and mechanical properties of the extracellular matrix (ECM). This outcome is likely due to interactions between macrophages within the most superficial layer and resident fibroblasts in the VFs. Naturally derived ECM-based biomaterials have been explored as bioactive scaffolds that promote tissue remodeling, reduce fibrosis, and are safe for clinical use. We have previously derived a porcine vocal fold lamina propria ECM (VFLP-ECM) scaffold [2] and have developed a hydrogel form of the ECM scaffold (VFLP-ECMh) that can be injected into the site of injury using minimally invasive methods. Our current study focuses on determining how VFLP-ECMh modulates the fibrotic response by assessing the paracrine effects of the VFLP-ECMh on macrophage polarization and fibroblast activation under static and vibratory conditions. Macrophages and human vocal fold fibroblasts (hVFFs) were co-cultured for 48 hours at 37°C. TGFβ1 cytokine was used to induce fibrosis in hVFFs. Real time quantitative polymerase chain reaction (RT-qPCR) was used to evaluate macrophage polarization and to determine *ACTA2* expression in hVFFs. Our results show that VFLP-ECMh modulates the expression of *ACTA2* in hVFFs. In addition, co-culturing hVFFs with macrophages resulted in changes in gene expression patterns typically associated with tissue fibrosis, highlighting the potential effects of macrophage polarization on resident fibroblasts.

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Design and development of stent coated with novel cardiac stromal cell-mimicking regenerative factors

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Introduction:

Stent angioplasty is a common procedure for patients with myocardial infarction due to acute coronary syndrome. The current drug eluting stents delay re-endothelialization and result in impaired healing due to the use of a drug coating on the metal stent that can lead to a cytotoxic and/or antiproliferative behavior. To reinstate the endothelial layer at the site of injury/ implantation and reduce late stent thrombosis, coating with stromal cell secretory regenerative factors is an effective and novel therapy. Novel stem cell-like synthetic cell-mimicking nanoparticles (CMNP) act as “synthetic stem cells” and mimic the paracrine and biointerfacing activities of natural stem cells. They are superior compared to cardiac stromal cells due to their stability during storage and their inability to stimulate a T-cell immune reaction [1]. Our spray coating process mimics the current drug coating process onto the stent which will lead to an easy commercial translation of our technology.

Materials & Methods:

CMNP particles containing various growth or regenerative factors (RF) secreted by human cardiac stromal cells (CSCs) were loaded into PLGA particles using a water/oil/water (w/o/w) emulsion and water/oil (w/o) technique as described by Tang J. et al [1,2]. Dichloro methane (DCM) was used as the oil phase. These CMNP particles were then coated using our custom designed spray coating machine (Sanco Engineering, Surat, India) (Figure 1A) onto platinum chromium bare metal alloy stents (Boston Scientific Cooperation). Drug loading was optimized using different ratios of regenerative factors to the PLGA polymer, with different concentrations of the water and oil phase. The total loading was characterized by bicinchoninic acid (BCA) protein assay using a BioTek Synergy HT multi-detection microplate reader.

Results & Discussion:

Table 1: Loading Optimization Trials

No.	Spray Coating Method	RF: PLGA Ratio	Loading (µg)
01	w/o/w emulsion method	3:100	<5
02	w/o/w emulsion method	5:100	<5
03	w/o emulsion method	20:100	<10
04	w/o emulsion method	35:100	101
05	w/o emulsion method	65:100	150
06	w/o emulsion method	85:100	124
07	w/o emulsion method	100:100	74

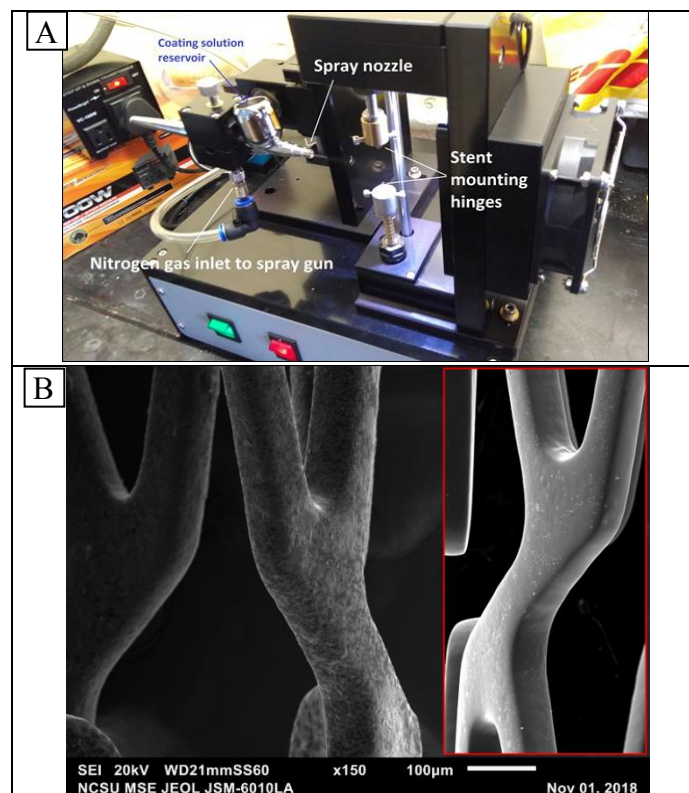


Figure 1A Spray Coating machine; 1B. SEM image of coated (left) and uncoated (right) stent strut.

Different ratios of regenerative factors (RF) to PLGA polymer in w/o/w and w/o emulsions were prepared and coated in triplicate on the stents, and total loading and surface smoothness were optimized as shown in Table 1 and Figure 1B. The stability of the coated stents was confirmed after two months of storage at -20 °C.

Conclusion:

This technique enables the loading of at least 100 µg of RF onto the stent, which achieves our target loading. These novel CMNPs along with a water in oil emulsion spray coating method mimics the current drug spray coating process and will lead to easy commercial translation of our technology. The unique features of this coating, such as mimicking the stem cell properties, avoiding a T-cell immune reaction and providing storage stability, make this novel coated stent approach as an easy commercial and practical alternative to stem cell therapy for cardiac patients.

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Cellular Backpacks for Macrophage Immunotherapy

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Adoptive cell therapies are a potent, clinically proven approach to treat diseases in a targeted and specific manner. However, living cells are subject to environmental cues that can change their function. For example, macrophages are one of the most plastic immune cells and change function rapidly based on environmental factors. Macrophages are a first defense to many diseases, including cancers. Despite this, the development of adoptive macrophage therapies has been hindered by macrophage's tendency to switch to a protumor phenotype upon entering the body. Tumor microenvironments cause macrophages to shift their phenotype from pro-inflammatory (M1) to anti-inflammatory (M2), leading to enhanced tumor growth and metastasis. We report a new strategy to overcome this harmful polarization using an engineered particle referred to as a “backpack”. Backpacks are discoidal microparticles that adhere to macrophage's surface and regulate their phenotype through the release of polarizing cytokines. These backpacks enhance the pro-inflammatory M1 polarization and lead to a durable anti-tumor function despite the highly immunosuppressive nature of the used murine breast cancer model. Overall, this study highlights a new pathway to control and maintain phenotypes of adoptive cellular immunotherapies

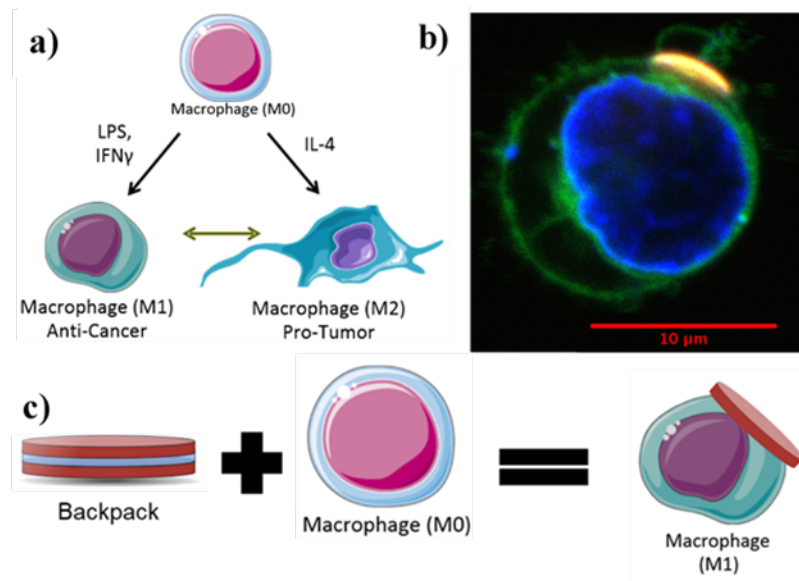


Figure 1. (a) Macrophage polarization based on environmental cytokines. (b) Backpack (green) attached to macrophage surface. (c) Schematic of attaching backpacks to macrophages.

Title: Engineering anisotropic tissue using ultrasound-induced patterning of cells within biomaterials

Abstract: Current efforts to manufacture engineered human tissues are aimed at mimicking the cellular and extra-cellular matrix (ECM) alignment (anisotropy), which is necessary to achieve a biomechanical form and function similar to the native tissues. Current biofabrication approaches such as bioprinting are limited in their potential to render this anisotropy due to the intrinsic homogeneity of cellular bioinks. To achieve this, we propose a novel Ultrasound-assisted Bioprinting (UAB) method that uses ultrasonic standing waves to align cells within bioprinted constructs. In this work, we describe the process of fabricating single or multi-layered constructs with viable human adipose-derived stem cells controllably aligned along distinct strands. First, using a COMSOL multiphysics computational model, we describe the process physics and the expected cellular patterning within the 3DUAB as a function of process parameters - ultrasound frequency (0.71, 1, 1.5, 2 MHz) and voltage amplitude (50 mV and 100 mV). Then, using three experimental studies on single layered constructs, we investigate the effect of frequency and amplitude on the product quality attributes - cellular viability, spacing between adjacent parallel cell strands (strand spacing) and strand width. Finally, we demonstrate the bioprinting of a multi-layered human knee meniscus with a tissue-specific circumferential cellular alignment within each layer. Results show that the experimental results corroborated with the computational model, wherein the cells were aligned in linear strands. The cell viability and strand width were found to be dependent on the interaction of frequency and voltage ($p < 0.01$), while the strand spacing was found to be dependent only on the frequency ($p < 0.0001$). Lastly, the multi-layered knee meniscus fabricated through the novel 3DUAB process depicted patient-specific macro-geometry and tissue-specific circumferential cellular organization throughout the entire thickness of the construct. The findings of this work represent an advancement towards scalable biomimetic tissue manufacturing.

Effects of Covalent Nanosilver Incorporation on Platelet-Like Particle Properties

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Abstract: Platelets are important to stopping bleeding and promoting healing following injury. In traumatic injury and chronic wounds, platelets can be depleted and healing can be complicated by bacterial infection. Our group has previously developed synthetic platelets (SPs), consisting of highly deformable microgels coupled to fibrin targeting antibodies¹ that augment hemostasis and mimic native platelet clot retraction, which is a feature that we hypothesize to promote healing by promoting durotaxis and enhancing cell infiltration. The purpose of this study is to enhance this design and develop antimicrobial synthetic platelets by incorporating nanosilver into our SP design. Nanosilver SPs (AgSPs) were fabricated by in situ reduction of silver ions by sodium borohydride³. Deformability was characterized by size and height data collected atomic force microscopy (AFM). AgSP nanometal distribution and stability was characterized by transmission electron microscopy. Clot retraction was characterized by incorporating AgSPs into fibrin clots and imaging via confocal. Antimicrobial ability was characterized by a colony forming assay. In this assay, we also compared activity to nanogold composite ULCs. Murine liver laceration and full dermal thickness injury models (uninfected and infected) were used to evaluate *in vivo* performance. AgSPs were similar to unloaded microgels in size, height, and clot retraction ability. Antimicrobial activity in AgSPs was comparable to ampicillin while antimicrobial activity in nanogold composite ULCs was minimal. Liver laceration and uninfected dermal injury models confirm retained *in vivo* performance. Additionally, infected full dermal thickness wound model showed improved wound healing outcomes when treated with AgSPs. In conclusion, we have successfully synthesized AgSPs. Nanosilver incorporation does not influence SP clot retraction ability and bestows significant antimicrobial effects both *in vitro* and *in vivo*.

In particular, AgSPs are able to significantly improve wound healing outcomes in infected injuries. In the future, long-term safety and efficacy of these Ag SPs will be investigated.

Image

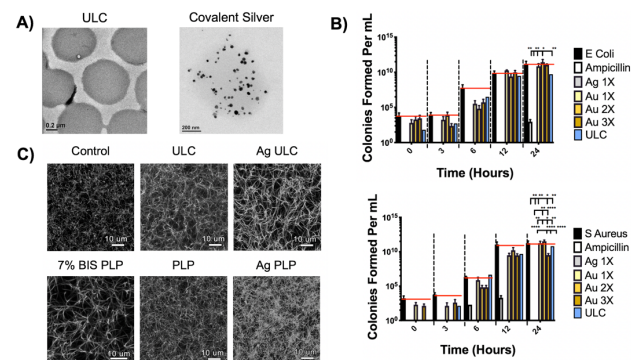


Figure: Section A – Representative TEM images of nanometal composites. Section B – Results of the antimicrobial assay for all covalent nanometal composites. Section C – Representative confocal images of clots incorporated with synthetic platelets.

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Ultrasonic Printing of Microgel Bio-Inks for the Creation of Patterned Substrates

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Introduction: The recent emergence of colloidal based hydrogel materials with highly tunable chemical and mechanical properties has allowed for the creation of scaffolds capable of interacting with cells on multiple length scales. These scaffolds retain the beneficial characteristics of bulk hydrogels, such as being biocompatible, biodegradable, and having controllable elastic moduli, but have the added benefit of having a higher degree of control over those properties. Also, since the material characteristics are specific to each individual particle, multiple particle types can be used to create scaffolds with distinct properties confined to specific areas. To this end, microgel particles with highly controllable viscoelastic properties were used to create uniform 4-layer thin films where it was found that as particle crosslinking density increased, film viscoelasticity, as measured as loss tangent, decreased which, in turn, directed how fibroblasts adhered, spread, and migrated. However, the current layer-by-layer (LBL) method of microgel thin film fabrication is limited by the fact that there is no spatial control over microgel deposition making the creation of more complex scaffolds difficult. We hypothesize that ultrasonic microplotting, a printing technique with a high degree of spatial control over bio-ink deposition, could be used to overcome this limitation to print microgel bio-inks in highly defined patterns. Here we evaluated various printing parameters to optimize bio-ink formulation and analyze cell adhesion and spreading responses on patterned surfaces.

Materials and Methods: Microgel particles were created using 91% poly(N-isopropylacrylamide) (poly-NIPam), 4% N,N'-methylenebis(acrylamide) (BIS), and 5% Acrylic Acid (AAc). Bio-inks were then created at a 2, 0.2, or 0.02 mg/mL microgel concentration with the addition of either 5%, 10%, or 20% by volume of low molecular weight polyethylene glycol (PEG). The microgel bio-inks were printed onto clean glass slides using a SonoPlot GXII ultrasonic microplotter using a 50 μ m glass capillary print head. After printing, the glass slides were imaged using an EVOS microscope and the patterns were analyzed in ImageJ. To test cell responses on printed microgel films, microgel particles were coupled to the RGD integrin binding motif using EDC-NHS crosslinking.

Results and Discussion: ImageJ analysis of the printed microgel bio-inks on clean glass surfaces, as seen in **Figure 1**, showed that as microgel and PEG concentrations increased, the ability of the microgel bio-inks to print completely also increased. Only the bio-inks with a 2 mg/mL microgel concentration and PEG concentrations of either 10% or 20%, were able to print 100% of the pattern. However, the 2 mg/mL and 20% PEG bio-ink printed at a greater resolution when compared to the 2 mg/mL 10% PEG bio-ink making it the better bio-ink candidate for moving forward. When microgels were coupled to the RGD integrin binding peptide and printed onto a glass surface, it was seen that fibroblasts were capable of adhering and spreading on the printed microgel films.

Conclusions: Results of printing varying microgel bio-ink compositions showed that a bio-ink composed of 2 mg/mL microgels and 20% PEG by volume is able to print a complex pattern in its entirety while also having the best resolution. It was also seen that cells are able to attach and spread to the printed microgel bio-inks when coupled with RGD. Future studies will evaluate the efficacy of printing multiple bio-inks with different microgel compositions on the same pattern as well as creating complex patterns for the study of cell migration on substrates with varying loss tangent properties.

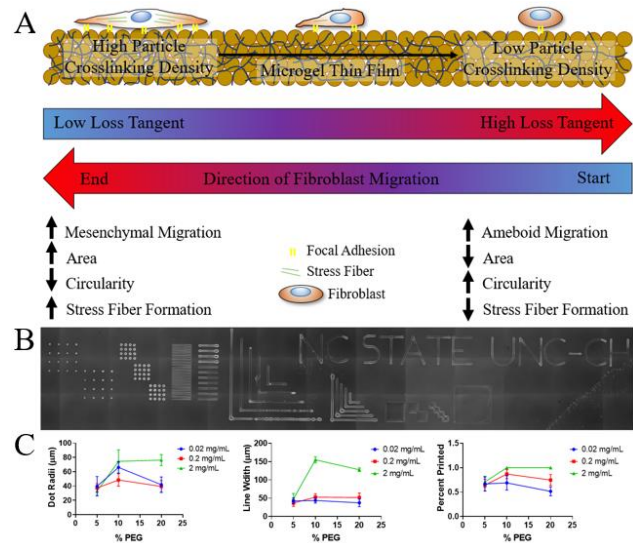


Figure 1 A) Previous results using uniform microgel thin films showed that loss tangent decreased as particle crosslinking density increased and that loss tangent can direct cellular responses. When patterned into a gradient, it is hypothesized that fibroblasts will migrate from areas of high to low loss tangent. B) Using the ultrasonic microplotting technique, microgel bio-inks can be printed into complex patterns. C) Bio-inks composed of varying microgel and PEG concentrations were evaluated. It was found that a bio-ink containing 2 mg/mL of microgel and 20% PEG by volume resulted in the most complete prints.

Fabrication and Evaluation of Auxetic Knitted Textile Scaffolds for Craniofacial Tissue Regeneration

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Hemifacial microsomia is the second most frequent congenital craniofacial malformation which was observed in 1 in about every 600 newborns according to the recent WHO study [1]. Hemifacial microsomia is characterized by underdevelopment of head and neck tissues on one side of the skull causing facial asymmetry. This results in a number of functional and psychological difficulties such as problems in chewing, breathing, hearing, expression and smiling. The current treatment includes removal of defective tissue and realignment and correction of jaw bones, followed by filling the defects with biological tissue grafts to restore normal function. Current surgical treatment which is mostly carried out on infants and children, either uses of autografts, which increases donor site morbidity, or the use of allografts which are associated with a higher risk of rejection. This study provides an alternative approach by developing a synthetic, biocompatible and biodegradable textile scaffold with the ability for *in vitro* regeneration of facial skeletal muscle and brain tissue using autologous primary cells. The study will include fabrication of novel auxetic textile scaffolds using elastomeric biodegradable poly-ε-caprolactone (PCL) multifilament yarn and weft knitting technology. Characterization of their physical and mechanical properties will include comparison with the mechanical properties native facial skeletal muscle tissue. These properties mainly include total porosity, pore size distribution, surface characteristics, tensile strength and elasticity. To improve the hydrophilicity and cell attachment, it is proposed to modify the scaffolds surface by surface activation using radio frequency plasma followed by crosslinking with genipin and grafting with Type I collagen. Further biological evaluation will include measuring the biocompatibility in terms of the extent of proliferation and cell metabolic activity.

References:

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“Feeling Out” the Niche for Hair Follicle Development in Bioengineered Skin Grafts

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Skin plays an essential role in protection, insulation, temperature regulation, and prevention of water loss among other functions. For this reason, severe skin injuries such as burns and deep wounds can be debilitating and life-threatening. While biomaterials are promising substitutes for skin grafts, they lack hair follicles, a skin appendage that plays a major role in the physical appearance and function of skin. The purpose of this research is to measure if the mechanobiological and growth properties of dermal cells change with age, and determine whether this leads to an inappropriate environment for folliculogenesis in biomimetic skin grafts seeded with adult cells. Using decellularization, a process of removing all cellular material except for the extracellular matrix, porcine skin was processed into a hydrogel, which poses as a 3D environment to support cells. Due to the similarities in porcine and human skin, porcine dermal and epidermal cells from adult, neonatal, and fetal animals were isolated and seeded in these bioengineered skin grafts. In this research, we have studied contractile force generation and growth differences between fetal, neonatal, and adult dermal cells using three assays: collagen-based cell contractility assay, cell proliferation assay, and micro-post assay. Future assays will compare the self-organizing and aggregate-forming properties of adult, neonatal, and fetal dermal and epidermal cells when co-cultured in 3D, incorporating structural pores and basement membrane in the form of decellularized urinary bladder matrix. This research helps to address a larger, long-term goal to promote natural skin healing by inducing folliculogenesis in biomaterial skin grafts.

Effect of Extracellular Matrix Components Isolated From Decellularized Porcine Vocal Fold Lamina Propria on Activation of Vocal Fold Fibroblasts

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Vocal fold (VF) tissue is highly susceptible to extensive scarring following trauma, which commonly leads to permanent loss of functionality. Scar tissue formation involves a disordered remodeling of the extracellular matrix (ECM) around the injury site by activated local vocal fold fibroblasts (HVOX). This remains a challenge for VF wound healing, as current biomaterial and pharmacological solutions are thus far inadequate for modulating scar formation. Our study sought to determine the effect of exposing HVOX to matrix-bound vesicles and macromolecules (MBVsM) isolated from decellularized ECM derived from porcine vocal fold lamina propria (VFLP) on the activation of fibrotic phenotype. To determine the effects of ECM components on gene expression and confirm the bioactivity of the ECM, 1000 cells/ μ L of HVOX were seeded in hydrogels composed of Collagen 1, Collagen 1 loaded with MBVsM, VFLP-ECMh or urinary bladder matrix-ECMh. The resulting 3-D spheroid cultures were activated with TGF- β 1 and observed for contraction. The study found that the fibroblasts seeded in ECM hydrogels showed significant contraction activity, while those exposed only to Collagen gel did not. Moreover, expression of the recognized fibrosis marker α -Smooth Muscle Actin (α -SMA) was lower in fibroblasts exposed to ECM components within Collagen 1 matrix than those seeded in plain Collagen 1 hydrogel. Evidence suggests that exposing HVFF to ECM promotes fibroblast activation while modulating the scarring phenotype.

Analysis of Force Application by Synthetic Platelet Mimics for Improving Wound Healing in Models of Hemophilia

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After an injury, platelets play an important role in hemostasis by supporting clot formation to stem bleeding and by augmenting subsequent tissue repair. Typically, thrombin is generated on platelet surfaces during the clotting process. This supports the formation of a stable hemostatic plug. However, this is not the case for patients with hemophilia. Hemophilic patients have a deficiency in coagulation factors VIII or IX, impairing proper clotting. Consequently, patients with hemophilia experience reoccurring bleeding and lack robust fibrin formation. This study aims to address the poor clot formation present in hemophilic conditions by applying synthetic platelet-like particles (PLPs) to an *in vitro* model of hemophilia. PLPs are microscale colloidal hydrogels made of polyNIPAm, coupled to a fibrin binding antibody. These PLPs possess the capability to mimic the clot contraction abilities of native platelets due to their high deformability and high fibrin affinity; this allows PLPs to induce strain within a fibrin network and bring about clot contraction. In this project, we aim to determine whether PLPs incorporated into a hemophilia-like fibrin clot would be able to generate forces within the clot in a manner reminiscent of native platelets under healthy healing conditions.

Methods: Normal and hemophilia-like plasma clots +/- PLPs were seeded around PDMS microposts and the deflection of the microposts was tracked over time in order to quantify clot formation. The generated force was calculated by correlating force with deflection distance. Posts were imaged over a 24 hour period in order to observe post-to-post distances at Day 0 and Day 1 after initial clot formation.

Results and Conclusions: PLPs were shown to enhance force generation within hemophilic clot networks (**Figure 1**), indicating that PLPs are likely able to augment clot contraction within a model of hemophilic clotting.

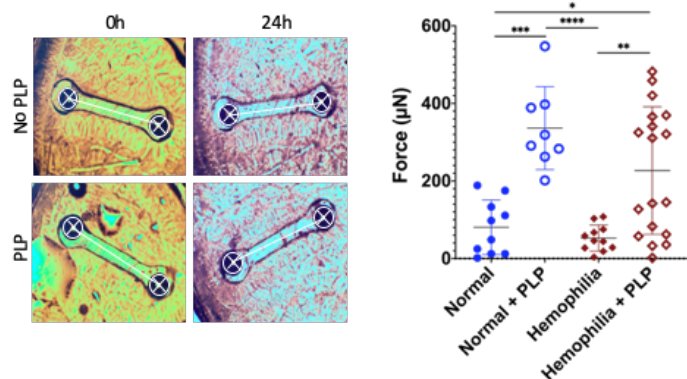


Figure 1: Comparison of force generation within clots without PLPs and with PLPs for both non-hemophilic and hemophilic plasma at 24 hours. n = 9/group; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

The Development of a Three-Dimensional *In Vitro* Model of Canine and Human Mastocytosis
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Mastocytosis is the proliferation and accumulation of mast cells in one or more organs. This often occurs in the skin but can also be found in the gastrointestinal tract and bone marrow, and can eventually develop into tumors or aggressive systemic diseases. Preclinical drug development for mastocytosis is difficult due to the biological and clinical diversity of this disease. Currently, *in vitro* and murine models do not accurately represent the highly variable tumor cell growth that naturally occurs in canine and human disease, making it difficult to develop new therapeutic strategies. We have developed a 3D *in vitro* model to study mastocytosis using extracellular matrix (ECM) hydrogels derived from decellularized porcine dermis. ECM hydrogels are reported to mimic the native ECM, providing tissue-specific signals. We hypothesize that culturing canine and human mast cells within a tissue-specific 3D structure, as shown in Figure 1, will influence cell phenotype and behavior leading to a more accurate representation of tumor cell growth *in vivo*. LIVE/DEAD staining successfully demonstrated the ability of the cells to survive in the hydrogels. The comparison of metabolic activity indicated a significantly higher rate of metabolic activity in the ECM hydrogels as compared to the collagen and tissue culture plastic (TCP) controls for canine and human mast cell lines. Altogether, this suggests an increase in proliferation in the ECM hydrogel environment. Assessment of pathological features showed that cells in ECM hydrogels consistently achieved high grade tumor classification based on the number of multinucleated cells, bizarre nuclei, and mitotic figures. RT-qPCR and flow cytometry indicated downregulation of IgE receptors associated with mature mast cell growth. Our results indicate an overall difference in cell behavior in the dermis ECM hydrogel as compared to controls, providing important information in determining if this model is representative of the highly variable *in vivo* conditions.

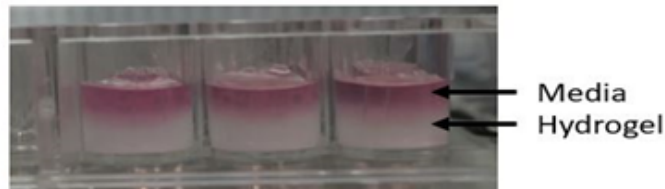


Figure 1: Dermis ECM hydrogels in a 96-well plate.

Muscle and Tendon Derived Extracellular Matrix Promotes Expression of MTJ specific Integrins in Myoblast Cell Culture.

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Introduction: Musculoskeletal disorders are well characterized with respect to one cell type, however the interaction between cells within tendon and muscle at the muscle tendon junction (MTJ) are not characterized and may be an important consideration for regenerative therapies. The overlap of the two tissues may provide cross-talk crucial to the expression of MTJ specific proteins. Currently *in vitro* models are often composed of one tissue and do not re-capitulate the complexity, especially at the interface of the muscle and tendon.

Materials and Methods: Porcine muscle and tendon tissue was decellularized, lyophilized and digested according to previous methods to yield tissue specific hydrogels. C2C12 myoblast cells were cultured on tissue culture plastic in either regular growth media (RGM) or muscle differentiation media (mdiff). To determine effects of ECM conditioned media, both medias were supplemented with 400ug/ml of either type I collagen, mECM or tECM. To determine effects of culturing cells within tissue specific matrix, C2C12s were suspended in the hydrogels before self-assembly and cultured in either RGM or mdiff media. RNA from cells was extracted and used to run q-PCR to determine relative gene expression for the MTJ specific integrin Paxillin.

Results: In monolayer culture with ECM conditioned media, culture with media supplemented with tissue specific ECM resulted in higher relative expression of Paxillin normalized to monolayer culture in RGM. Paxillin expression also increased in 3D tissue specific environments normalized to C2C12s cultured in type I collagen. In both monolayer and 3D environments containing tECM, Paxillin expression in the muscle cells was highest compared to mECM.

Conclusions: Tissue specific derived matrix had an effect on paxillin expression in muscle cells, suggesting that mECM or tECM contained signals that helped promote MTJ expression in myoblast cells. Interestingly, exposure to tECM increased expression of the integrin, suggesting a gradient of microenvironment may be important in MTJ signaling *in vivo*.

Surface property modification of $\text{Al}_x\text{Ga}_{1-x}\text{N}$ substrates with gradient Al composition for biointerface applications using wet beaker chemistry

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Biomolecular gradients, which are widely known to be important for controlling cellular dynamics, are not fully understood because there is a lack of *in vivo* platforms to study the details of the chemical concentrations and gradient slopes that comprise them. Inorganic materials, such as III-nitride semiconductors, are an ideal platform to study biomolecular gradients because the surfaces can be modified using chemical etching to modulate the topography, surface charge, and surface chemistry of the substrates. Furthermore, compositional monotonic gradients can be formed during III-nitride growth that allow for selective *in situ* surface modification using wet chemical etching to vary surface properties along the compositional gradient. We utilized four wet beaker chemistries to modify the surface of an $\text{Al}_x\text{Ga}_{1-x}\text{N}$ film ($0.173 \leq x \leq 0.220$) to demonstrate that the surface properties can be tuned according to chemical treatment and compositional gradient. A variety of techniques were used to characterize variations in the surface properties including the topography, surface charge, hydrophobicity, nanostructure formation, and surface chemistry. We found that there was spontaneous, defect-dependent oxide nanostructure formation on the surface because of the chemical treatments. Therefore, while the surface properties were depend on chemical treatment, we could not predictably tune the surface properties according to the Al:Ga gradient because of the oxide nanostructures. This work can be used for future studies that use wet beaker chemistry to functionalize inorganic materials for biointerface applications.

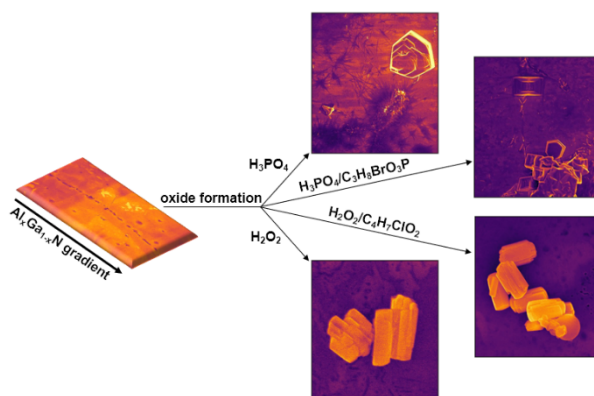


Figure 1. $\text{Al}_x\text{Ga}_{1-x}\text{N}$ gradient substrates were functionalized using four wet chemical treatments to modify the surface properties. Oxides nanostructures were formed as a result as seen in scanning electron microscopy images.

Reference: Gleco, S., et al. *ACS Omega* **2019**, 4 (7), 11760–11769.

Doped Semiconductor Materials Properties Can Alter the Biofilm Behavior

Presenting author: Alexey V. Gulyuk

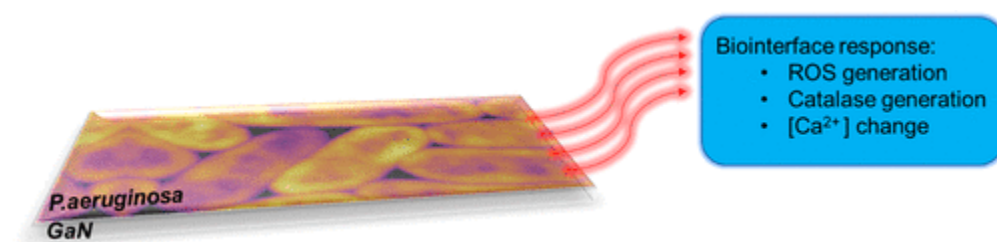
Other authors: Dennis R. LaJeunesse, Pramod Reddy, Ronny Kirste, Ramon Collazo, Alben Ivanisevic.

North Carolina State University

Abstract:

A treatment of GaN samples with UV light introduces alterations in surface charge and chemistry. The behavior of *Pseudomonas aeruginosa* films also changes in response to substrate property modification. The GaN surfaces were characterized by atomic force microscopy, Kelvin probe force microscopy, and X-ray photoelectron spectroscopy. The *Pseudomonas aeruginosa* film responses were quantified by measuring amount of catalase, reactive oxygen species, and intracellular Ca²⁺ concentrations. Performed studies support the hypothesis that certain *P. aeruginosa* biofilm characteristics can be tuned by the interfacial properties and the length of the biofilm-substrate interaction.

Image:



References:

Interfacial Properties of Doped Semiconductor Materials Can Alter the Behavior of *Pseudomonas aeruginosa* Films. Alexey V. Gulyuk, Dennis R. LaJeunesse, Pramod Reddy, Ronny Kirste, Ramon Collazo, and Alben Ivanisevic. *ACS Applied Electronic Materials* **2019** 1 (8), 1641-1652.
DOI:10.1021/acsaelm.9b00347

Preparation and Characterization of Chitosan Based Hydrogels Containing Cyclodextrin-Inclusion Compounds or Nanoemulsions of Thyme oil

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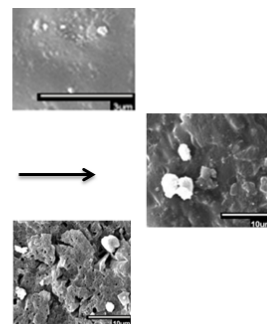
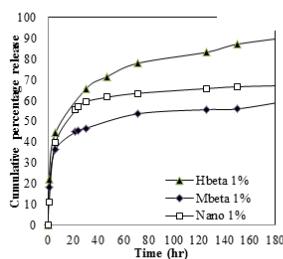
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Abstract

Hydrogels derived from natural polysaccharides are ideal scaffolds to be used in biomedical applications. The pH sensitive polyvinyl alcohol and chitosan hydrogels containing inclusion compounds of thyme oil (TM) with host Methyl- β - (M β -) and Hydroxypropyl- β -cyclodextrins (H β CD-ICs) and thyme oil nanoemulsions were prepared via controlled, biocompatible and low cost freeze-thaw method. The structure of the hydrogels was characterized by FTIR, and optical and scanning electron microscopy. The physicochemical properties of the hydrogels such as gel fractions, swelling ratio and tensile properties were measured. The water vapor transmission rate of the hydrogels indicated that they can maintain a moist environment over wound bed. Encapsulation and release of antibacterial thyme oil from the hydrogels were determined by UV spectroscopy. In all cases, hydrogels with lower amounts of thyme oil evidenced slower and more controlled release. Different kinetic models were applied for evaluating drug release mechanism. The antibacterial activity of the samples was studied by counting the number of surviving both gram-negative and gram-positive bacteria in a broth medium and the results proved the antibacterial activity of all prepared hydrogels. The results of MTT assay indicated more cell viability of TM-nano hydrogels in comparison with that of TM- β CD-ICs. Cell attachment observations also showed great biocompatibility of TM-nano hydrogels. Prepared hydrogels, especially those containing TM-nano, might be used as potential wound dressings to improve wound healing process.

Image



Engineering a Nanocarrier Drug Delivery Model to Target Mast Cells in an Extracellular Matrix Hydrogel

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Mast cells (MC) are innate and adaptive immune cells that function during the pro-inflammatory response by releasing histamine and other mediators attracting immune cells. The release of histamine from MCs leads to tissue inflammation and vessel leakage. Mast cell tumors (MCTs) are a blood cell cancer caused by a mutation to the c-kit gene that causes MCs to grow rapidly and uncontrollably. Currently, there are several drawbacks that limit the clinical application of approved MCT therapeutics on the market today. In this study, we are determining the optimal nanocarriers properties, including size, cross-linking density, and charge, in drug delivery to develop an *in vitro* model for MCs using cell culture inserts seeded with endothelial cells in extracellular matrix (ECM) hydrogels. ECM decellularization is the process of removing the DNA from native tissue and digesting with a series of detergents to obtain an ECM hydrogel. Native ECM hydrogels better represent the three-dimensional structure surrounding living tissue that creates a complex extracellular environment. Unlike synthetic hydrogels, ECM hydrogels retain biological information from the original tissue. We aim to determine the optimal properties of nanocarriers to facilitate delivery of therapeutics to mast cells and inflammation using a three-dimensional environment. We hypothesize that nanocarrier properties will determine the amount of nanocarrier uptake by mast cells. Nanocarriers were fabricated using a precipitation polymerization reaction of NIPAm monomer, BIS cross-linker, and acrylic acid, initiated with ammonium persulfate. Nanocarrier extravasation properties such as size, cross-linking density, and charge were controlled during fabrication of nanocarriers. Also, nanocarriers were observed through fluorescence in native ECM hydrogel after 72 hours. Through these experiments, we will determine the optimal properties of nanocarriers for uptake by mast cells and the endothelial membrane interactions with nanoparticles.

A Novel Thermo-responsive Hydrogel Scaffold to Deliver Neural Stem Cells for Post-surgical Treatment of Glioblastoma Multiforme

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Introduction:

Glioblastoma multiforme (GBM) is the most aggressive form of brain cancer and 90% of GBM patients die within 24 months after diagnosis. Treatment options for GBM include surgery and chemoradiation however, recurrence is common, and the disease is universally fatal. New approaches have emerged using induced neural stem cells (iNSCs) as a treatment modality for GBM therapy. Tumorcidal iNSCs have shown to induce apoptosis and extend survival after surgery. However, rapid stem cell clearance and poor retention and persistence of stem cells in the post-surgical cavity remain a major challenge that limits efficacy of engineered stem cell-based therapy. To address these challenges, we developed a novel biodegradable, biocompatible, thermo-responsive hydrogel as a scaffold for the delivery and prolonged retention of neural stem cells.

Materials and Methods: C17 neural stem cells (1×10^6 cells/mL) were encapsulated in 1mL of hydrogel and seeded directly into an 8 well chamber glass slide to investigate stem cell persistence. An in vitro stem cell persistence study was conducted by using LIVE/DEAD[®] Viability/Cytotoxicity kit and analysis was performed using confocal imaging to quantify cell persistence at days 1 and 7. Persistence was determined by comparing the number of cells encapsulated in the hydrogel on day 7 with respect to day 1. Subsequently, migration of neural stem cells from the hydrogel scaffold was performed using confocal imaging to analyze the migratory properties of neural stem cells.

Results and Discussion: Stem cells were encapsulated at an optimal density of 400-500 live cells/144mm² and stem persistence in the three-dimensional hydrogel scaffold 420 live cells/144mm² and 400 live cells/144 mm² on day 1 and 7, respectively. C17 stem cell migration was analyzed using confocal microscopy. The confocal imaging analysis revealed that neural stem cells were able to migrate from the biodegradable hydrogel scaffold within 14 days. These data demonstrate that the innovative combined hydrogel/iNSCs technology could be a promising delivery system for the treatment of glioblastoma multiforme.

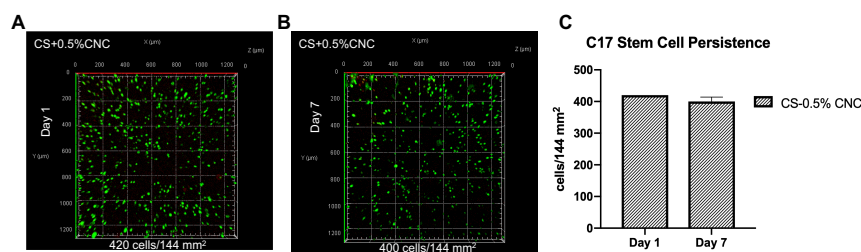


Figure 1. Persistence of C17 stem cells within hydrogel matrices. Representative confocal fluorescent images of encapsulated stem cells within the hydrogel matrix on day 1 (A) and day 7 (B). Fluorescent images are captured at 5x to demonstrate overall cell growth. (C) Summary data showing the graphical representation of C17 stem cell persistence.

Conclusions: The substantial impact of this innovative hydrogel/iNSCs technology may be immense for a disease that currently has no cure. This technology has the potential to change clinical practice and improve the standard of care for GBM and other solid organ tumors. Continued efforts to aid in the development of delivery systems is beneficial to improve the treatment of GBM.

Fibrin-modulating Nanogels for Treatment of Disseminated Intravascular Coagulation

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Abstract: Disseminated intravascular coagulation (DIC) is a pathological process causing systemic coagulopathy through excessive thrombin generation. While over-activation of clotting causes microthrombi throughout the body and multi-organ failure, DIC also contributes to bleeding as clotting factors are consumed. Since patients can present with bleeding or thrombosis, this creates therapeutic dilemmas in managing. We hypothesize fibrin-specific nanogels (FSNs) loaded with tissue plasminogen activator (tPA) can treat the multifaceted complications of DIC. Core-shell poly(N-isopropylacrylamide) nanogels with 10% N,N'-Methylenebis(acrylamide) (BIS) cross-linked core and 2% BIS cross-linked shell were synthesized in precipitation polymerization reactions, including acrylic acid for coupling of an anti-fibrin fragment E antibody. tPA was loaded into FSN particles through a rehydration technique. Release studies were conducted for kinetics and tPA functionality. tPA-FSNs were examined for modulating clotting dynamics *in vitro* using a PDMS fluidics chamber. *In vivo*, tPA-FSNs were administered in a lipopolysaccharide-induced DIC rodent model and examined for microthrombi presentation, clotting capability, and bleeding outcomes. Core-shell particles demonstrated a burst release profile of functional tPA. Unloaded FSNs exhibit fibrin-binding at wall shear rates of 1s^{-1} and augment the clotting process *in vitro*. Conversely, tPA-loaded FSNs bind to fibrin and facilitate targeted clot dissolution. *In vivo*, DIC animals exhibit darkened organ color and significantly more fibrin deposition, which is diminished with tPA-FSN treatment. Elevated fibrin degradation products in DIC animals validate microthrombi formation and fibrin turnover, while platelet counts demonstrate recovery to normal levels only with tPA-FSN treatment. In addition, clot structure shows fibrin network structure is severely compromised in DIC. However, tPA-FSNs restore clot structure. Upon injury, animals with DIC had significantly higher blood loss than healthy animals, but tPA-FSN treatment did somewhat reduce blood loss. Together, these data demonstrate that a fibrinolytic agent loaded into fibrin-targeting microgels can act as a targeted therapeutic strategy in DIC to improve outcomes.

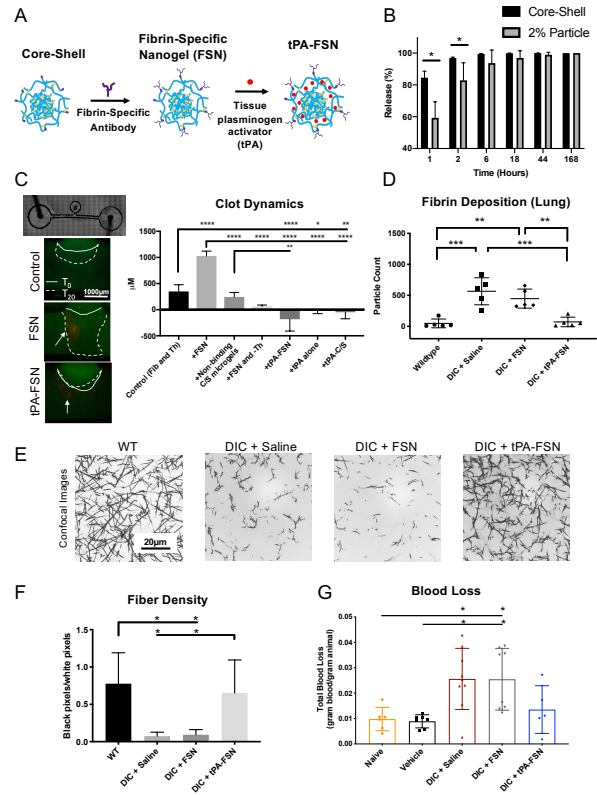


Figure 1: Core-shell fibrin-specific microgels release tPA (A-B) to modulate clotting dynamics (C) and diminish fibrin deposition (D), restore clot structure (E-F), and combat bleeding risks (G) associated with disseminated intravascular coagulation.

Decellularized Vocal Fold Lamina Propria-ECM And Associated Matrix-bound Vesicles: Proteomic And In Vitro Characterization Of The TGF- β 1 Mediated Fibrotic Response In Human Fibroblasts

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Voice disorders affect about 20 million people in the U.S. and there is currently no clinically available biomaterial with significant satisfactory wound healing outcomes. Vocal fold lamina propria (VFLP) is highly susceptible to injury. During injury, vocal fold fibroblasts (HVOX) respond by overproducing extracellular matrix (ECM) proteins increasing the tissue stiffness and leading to diminished function. Decellularization of porcine tissues can yield scaffolds with low risk of immunogenicity, while allowing tissue regeneration^{1,2}. However, the factor(s) in the acellular-ECM scaffold responsible for the phenotypic response remains unknown. Recently, Matrix-Bound Vesicles (MBVs) were isolated from decellularized-ECMs and it recapitulates the ECM-phenotype response in macrophages³. We hypothesize that decellularized VFLP-ECM scaffold has a unique proteinic composition and modulate the TGF- β 1 mediated fibrotic response in HVOX. Also, we hypothesize that MBVs and macromolecules isolated from the VFLP-ECM scaffold will mediate the HVOX activation. VFLP and urinary bladder matrix (UBM) were obtained via established methods and processed according to a discovery proteomics workflow. VFLP-ECM hydrogel (VFLP-ECMh) was used for the in vitro testing of HVOX upon TGF- β 1 stimulation. MBVs and macromolecules larger than 100 KDa were isolated via ultra-filtration of digested VFLP-ECM. The proteomic discovery analysis revealed a unique composition in the VFLP-ECM scaffold when compared to UBM-ECM. Significant differences were found in collagens, elastin, aggrecans, proteoglycans, and other ECM proteins. Gene correlation analysis identified proteins (e.g., LTBP4 and Col18A1) that might play a role in the observed HVOX response. The downregulation of ACTA2 and Col1A1 genes suggests a modulation of the HVOX phenotype seeded on VFLP-ECMh. In addition, α -SMA staining showed a reduced expression in HVOX seeded on VFLP-ECM in support of the gene expression results. By supplementing MBVs and macromolecules to culture media, we were able to recapitulate the downregulation of ACTA2, which is a critical marker for initiation of scar formation. VFLP-ECMh has a specific composition when compared to UBM-ECM. In addition, VFLP-ECMh is able to downregulate α -SMA as well as affect ECM-related genes when stimulated by TGF- β 1.

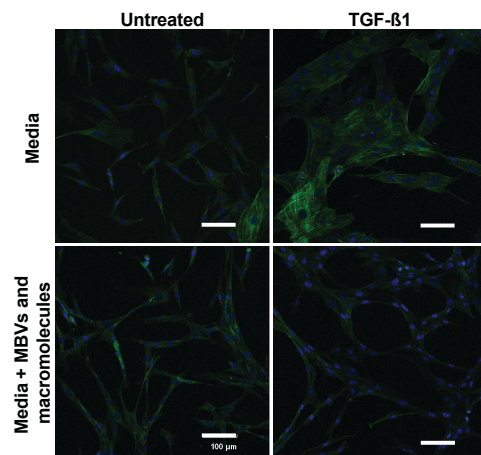


Figure 1. α -SMA staining in HVOX grown either in regular media or media supplemented with MBVs and macromolecules isolated from VFLP-ECM. Cells were stimulated to myofibroblast phenotype using 10 ng/mL of TGF- β 1. Green represents α -SMA. Blue is Dapi staining.

References:

1. Freytes DO, et al. Preparation and rheological characterization of a gel form of the porcine urinary bladder matrix. *Biomaterials*. 2008 Apr;29(11):1630-7.
2. Wrona EA, et al. Derivation and characterization of porcine vocal fold extracellular matrix scaffold. *Laryngoscope*. 2016 Apr;126(4):928-35.
3. Hussey GS, et al. Matrix bound nanovesicle-associated IL-33 activates a pro-remodeling macrophage phenotype via a non-canonical, ST2- independent pathway. *Journal of Immunology and Regenerative Medicine*. 2019;3:26-35.

Neonatal vs. Adult Fibrin Matrix Properties in Wound Healing

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When early wound healing processes are disrupted and result in a deficient provisional fibrin matrix, chronic wounds can occur. Fibrin sealants have been explored as matrices to treat non-healing wounds. However, commercially available fibrin sealants utilize very high concentrations of thrombin and fibrinogen to effectively produce a polymerized matrix, which can inhibit cell migration due to the formation of a thick, dense fibrin network. The overarching goal of this project is to develop a novel fibrin scaffold derived from neonatal fibrin, as opposed to adult fibrin, to enhance outcomes of fibrin sealants in the treatment of chronic wounds. To date, extensive differences have been identified in fibrin network properties between adults and neonates. Wound healing outcomes have been linked to fibrin matrix structure, such as porosity and permeability, which can affect the binding and migration of cells. **We hypothesize that fibrin scaffolds produced from neonatal fibrin will promote enhanced cell migration and wound healing outcomes compared to adult fibrin scaffolds due to inherent biochemical and structural differences in neonatal vs. adult fibrin networks.** Results:

Initial characterization of adult and neonatal networks revealed distinct structural, mechanical, and fibrinolytic properties. Cell assays indicated significantly higher fibroblast attachment and migration on neonatal fibrin matrices compared to adults. In our characterization of porcine specimens, we identified similar age-dependent distinctions in fibrin clot structure, stiffness, degradation, and functionality between porcine and human samples. Conclusions: Neonatal fibrin clots have distinct structural, mechanical, and functional properties compared to adults. On-going experiments include in vivo wound healing studies in murine model. Additionally, we found neonatal porcine fibrin matrix properties mirrored those of neonatal humans. Therefore, it is possible that subsequent studies and materials may utilize neonatal porcine fibrin in place of human derived protein.

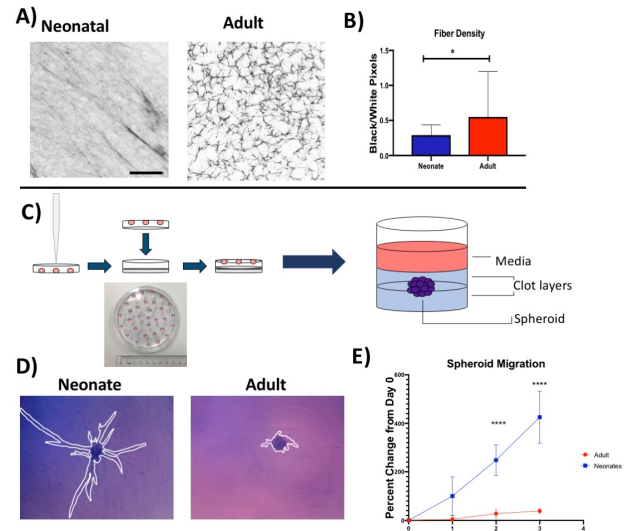


Figure 1: A) Neonatal and adult fibrin matrix structure analysis with confocal microscopy and corresponding fiber density quantified via ImageJ. B) Fiber Density in adult networks is significantly higher than neonatal networks. Scale = 10 μ m, N=4, $p < .05$. C) Human dermal fibroblasts are cultured into spheroids via hanging drop culture and sandwiched between two fibrin layers to simulate a three dimensional wound environment D) Fibroblast migration is quantified by tracing the spheroid body boundary using imageJ. E) Fibroblast migration is significantly increased in neonatal fibrin networks. N=3-4, $p < .0001$.

Incorporation of Inflammatory Cells in the Design of Tissue-Engineered Constructs and Drug Delivery Models Using Extracellular Matrices

Emily W. Ozpinar^{1,2}, Ariana Frey^{1,2}, Andreea Badileanu^{1,2}, Camilo Mora-Navarro^{1,2}, Glenn Cruse^{2,3} and Donald O. Freytes^{1,2}

UNC-Chapel Hill/NCSU Joint Department of Biomedical Engineering, Raleigh, NC¹

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Extracellular matrix (ECM) provides structural support to the cells in our body as a network of proteins, sugars, and biochemicals. These frameworks form the organs and tissues in our body, while playing important roles in their function, and behavior. ECM can be obtained from porcine tissues through a process known as decellularization, in which a series of washes, specific to the tissue's origin, remove any cellular components, avoiding an immune response. As they possess tissue-specific mechanical properties and biochemical composition, ECM-derived biomaterials make for ideal scaffolds for tissue implants and for *in vitro* drug models. However, as any tissue-engineered construct will interact with the inflammatory response at the site of implantation, it is crucial to understand how cells of the immune response interact with ECM. In this presentation, we describe two studies using ECM-based hydrogels to control the behavior and gene expression patterns of immune cells *in vitro* (**Figure 1**). The first study uses ECM to downregulate the effect of lipopolysaccharide, an inflammatory signal for human peripheral blood-derived macrophages. The second study demonstrates how ECM affects the metabolic activity and gene expression of human mast cells in three-dimensional culture. These studies demonstrate how ECM-based hydrogels can regulate these two immune cell types and highlight the importance of incorporating the potential response in the design of tissue-engineered constructs.

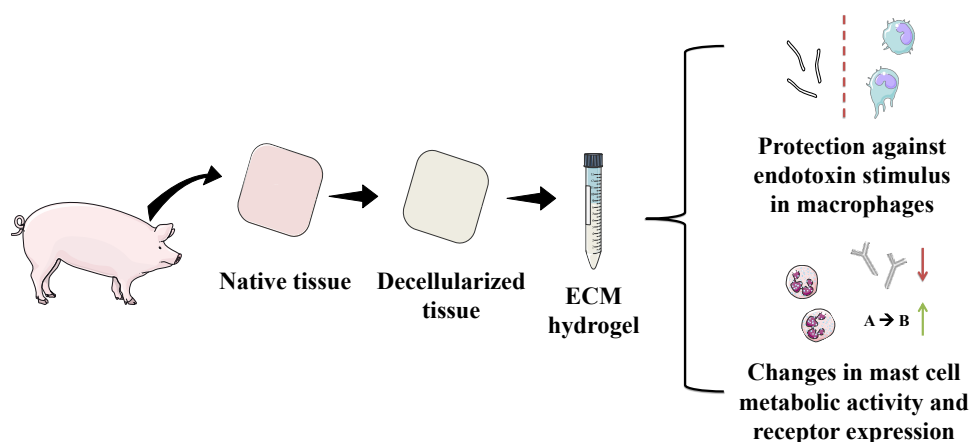


Figure 1: Graphical abstract summarizing the process of obtaining extracellular matrix (ECM) hydrogels and how ECM hydrogels affect immune cell behavior in two separate studies.

Acknowledgements: We thank our funding sources: National Institutes of Health (R01DC017139), American Heart Association Predoctoral Fellowship (19PRE3430006) to E.W.O., UNC/NCSU Joint Department of Biomedical Engineering, NCSU Comparative Medicine Institute (CMI) and the NCSU Center for Human Health and the Environment (P30 ES025128).

Anisotropic Scaffold Fabrication using High-Throughput 3D-Melt Blowing

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Tissue engineering scaffolds should recapitulate the 3D micro-architecture and macro-geometry of the native matrix they replace¹. This study characterizes 3D-Melt Blowing (3DMB), a patent-pending scalable process for manufacturing biomimetic fibrous scaffolds by synergistically uniting nonwoven melt-blowing fiber formation with a multi-axis temporo-spatially controlled collector². Polycaprolactone (43 kDa) was used to fabricate scaffolds wherein three critical 3DMB parameters were varied— collector surface speed (SS = 150, 700 m/min), die-collector-distance (DCD = 150, 200 mm), and collector location relative to the fiber nozzle (CAO = 0, 80 mm) – and their effects on fibrous, mechanical, and cellular critical quality attributes were investigated. SS and CAO had significant effects on angular coherency and mechanical properties wherein scaffolds with high CAO and SS had the greatest angular coherency and porosity while demonstrating the greatest stiffness and strength relative to their weight. DCD had limited effects. Scaffolds of contrasting CQA were selected for cell culture: (150 mm DCD, 0 mm CAO, 150 m/min SS) and (150 mm DCD, 80 mm CAO, 700 m/min SS). Scaffolds (ø 8 x 3 mm) were seeded with NIH-3T3 fibroblasts (5 x 10⁵ cells / scaffold) after mild alkaline hydrolysis (2.5 M NaOH, 1h, 50° C) to enhance cell adhesion and cultured over 7 days³. Cellular viability (Day 1, 7, n=1) and metabolic responses (Day 2, 4, 7, n=3) were assessed by Live/Dead and alamarBlue, respectively. Scaffold design and time point had a significant interaction effect on cellular CQA (p < 0.05). The high SS and CAO group showed greater metabolic activity over time, despite a slightly lower cell viability upon seeding. This is likely due to improved media perfusion and growth surface area through increased porosity. This demonstrates that 3DMB process parameters modulate scaffold CQA which then play key roles in supporting the viability and metabolic activity of cells.

Refs: [1] Mauck RL. 2009 Tissue Eng. Part B Rev. 15.2, 171-193. [2] Schuchard K. IISE Annu. Conf. Proc. 2019. [3] Bosworth L. 2019 J. Nanomater. Vol. 2019.

A hybrid vascular graft harnessing the superior mechanical properties of synthetic fibers and the bioactivity of collagen yarns

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Introduction:

At the present time, there is no successful off-the-shelf small-caliber vascular graft (< 6 mm) for the repair or bypass of the coronary or carotid arteries. Collagen has long been considered as a viable material to encourage tissue regeneration and revascularization, but its use has been limited by its inferior mechanical properties. In this study, a hybrid vascular graft was fabricated using novel electrochemically aligned collagen (ELAC) yarns combined with traditional poly(lactic acid) (PLA) multifilament yarns. The collagen component is able to promote the recruitment and proliferation of endothelial cells. At the same time, PLA is able to provide sufficient mechanical properties and structural stability to facilitate the manipulation, the production scale-up and clinical application of the vascular graft.

Experimental Methods:

An optimization trial involved knitting tubular scaffolds from ELAC thread plied with one or two ends of PLA multifilament yarn. The scaffold was knitted on a small diameter circular knitting machine which gave comparable compliance to a native vessel. Its mechanical properties were characterized according to ISO 7198: 2017. The rate of degradation was measured in terms of losses in weight and tensile strength, and its biocompatibility to human umbilical vein endothelial cells (ECs) was evaluated using alamar Blue® assay and live/dead assay.

Results and Discussion:

The collagen/PLA hybrid vascular graft showed significantly improved mechanical properties compared to a pure collagen vascular graft. The cell adhesion and proliferation assay showed that there was a significantly larger number of HUVECs adhering to the collagen/PLA yarns compared to the PLA control.

Conclusions:

The circular-knitted collagen/PLA vascular scaffold showed tunable mechanical properties that fulfill the requirement of clinical translation. The incorporation of novel electrochemically aligned collagen (ELAC) threads, significantly improved initial cell recruitment as well as subsequent cell attachment and proliferation on the scaffold.