# 2023 McGowan Retreat
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# Investigating the role of the mSWI/SNF (BAF) complex during neural differentiation

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Stem cells are characterized by their inherent property to either self-renew or acquire a specialized cell fate. Differentiation of stem cells into specialized cell types requires re-organization of the genome to establish accessibility at the necessary protein-coding and regulatory loci for appropriate function of the new cell. This process is governed by an orchestra of regulatory components including nucleosome remodeling complexes, transcription factors (TFs), and histone posttranslational modification deposition. However, the role of the non-protein-coding transcriptome in regulating cell fate decisions is not well understood. The mSWI/SNF (BAF) complex is the only nucleosome remodeling complex that is essential at every major stage of development and is combinatorially assembled from 15-17 protein subunits to give rise to cell type specific BAF complex compositions, which are essential for both maintenance of self-renewal in embryonic stem (ES) cells and cell fate commitment in neurogenesis and cardiomyocyte differentiation. Interestingly, the BAF complex is mutated in approximately 20% of all cancers and is also highly mutated in neurological disorders, including psychiatric and genetically inherited disorders.

We previously established that the composition of the essential and highly conserved BAF nucleosome remodeling complex present in ES cells, esBAF, functions as a global repressor of non-coding (ncRNA) expression genome-wide, regulates nucleosome positioning to facilitate open chromatin at pluripotency TF binding sites, and is essential for maintenance of pluripotency. Here, I am investigating how the BAF complex regulates differentiation along the neuroectoderm lineage. I am utilizing novel synthetic biology approaches to understand how the non-coding transcriptome, genome-wide accessibility, genome-wide TF localization, and histone modification deposition are impacted when the ATPase activity that confers functionality to the BAF complex is abolished, either via chemical degradation or pharmacological inhibition. This work will uncover novel regulatory functions of cell-type specific BAF complex assemblies and provide insight into the role of the BAF complex in both developmental and disease contexts, ultimately informing potential therapies.

# Investigating the Role of Alternative Splicing in Reproductive Aging

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While all physiological processes decline with age, the female reproductive system is particularly sensitive to age-related decline (reproductive aging). Beyond the well-known loss of fertility, reproductive aging has profound consequences for women's overall health. Menopause increases the risk of multiple age-related diseases and may even accelerate the aging process. Genetic studies have shown both that women who undergo menopause later in life tend to be healthier and live longer.

Studies into the molecular and genetic basis of how reproductive health is maintained throughout life are thus crucial both to improving women's health and to the broader understanding of aging in general.

Pre-mRNA splicing (splicing) is a core biological process that produces variant proteins from a single gene. Splicing patterns are crucial for controlling both gene expression and protein diversity. We have long understood that dysregulated splicing is both a signature and a driver of the aging process. However, we do not understand the role of splicing in reproductive aging.

Using data from genetic studies in humans and laboratory studies of animals, I am investigating how splicing influences reproductive health and longevity. My research is an attempt to determine how splicing patterns change with age, how the loss of splicing regulators may cause accelerated reproductive aging, and what genes and biological processes are affected. The end goal of this research is to discover ways in which reproductive health can be maintained throughout women's lives, thus helping women lead longer and healthier lives.

# Analysis of Connexin Recycling as an Alternative Method for Gap Junction Plaque Formation During Wound Repair

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Gap junction channels, which are found in plaques at cell-cell contact sites, have been suggested to play a pivotal role in migration and wound healing. However when cells in a monolayer culture lose contact, gap junction plaques internalize to result in annular gap junction vesicle release into one of the two separating cells. This internalization process is thought to serve as a degradative method for eliminating old gap junction channels. During wound healing, as new cell-cell contacts form, gap junction plaques are rapidly established at rates that are inconsistent with the time needed for synthesis and delivery of new gap junction proteins to the plasma membrane. We hypothesize an alternative method of gap junction plaque formation that rely on the delivery of old gap junction protein from annular gap junction vesicles to the plasma membrane. Information on gap junction protein recycling is needed if we are to fully understand gap junction formation and its role in wound healing. To characterize gap junction protein recycling, we used biotin pulse-chase methods and time-lapse imaging to measure the cellular dynamics of connexin-43 (Cx43), the most ubiquitously expressed member of the gap junction protein. The SW-13 adrenal cell line that forms large Cx43 gap junction plaques was used in this study and for time-lapse imaging they were transfected to express fluorescent mEos3.2 tagged Cx43. Both Cx43 internalization and recycling were demonstrating with the pulse-chase methods. With time-lapse imaging, annular gap junction vesicles were shown to dock with the plasma membrane for short periods (less than 3 min). These docked annular gap junction vesicles however then disassociated from the plasma membrane and re-entered the cytoplasm. It is tempting to suggest that annular gap junction vesicles membrane may transfer their Cx43 to gap junction plaques in a dock-and-run-delivery movement pattern. We are in the process of using 3D confocal computer-assisted reconstruction to better characterize the docking process and we are looking for an appropriate wound model system in which to evaluate the dock and run during wound closure. Here we report the recycling of Cx43 and suggest a mechanism which involves the delivery of old Cx43 to the plasma membrane from annular gap junction vesicles. Such a mechanism would be a rapid and economical method for gap junction plaque formation, particularly as cells reestablish contact. Understanding this alternative method for gap junction plaque formation and cell-cell communication potentially could lead to the development of new targets to promote wound repair. NSF MCB #2011577

# Profilin-1/CCL2 is a novel signaling axis of tumor cell-directed migration of immune cells

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Profilin-1 (Pfn1) is an actin-binding protein that is downregulated in human breast cancer (BC). Pfn1 has been previously demonstrated to have tumor-intrinsic roles as a suppressor of tumorigenicity and dissemination in BC; however, whether Pfn1 has extrinsic immunological effects on the tumor microenvironment (TME) is unknown. We sought to investigate the effect of Pfn1 expression on the immune composition of the TME and the chemotaxis of immune cells. We performed multiplexed quantitative immunohistochemistry on a tissue microarray of clinical BC samples which revealed a significant positive correlation between tumor cell-specific Pfn1 expression and the percent of CD8+ T cells in the TME, more prominently in triple-negative breast cancer (TNBC) samples. Bioinformatics analyses of the METABRIC transcriptome dataset confirmed Pfn1 expression to be positively correlated with the CD8+ T cell fraction, in addition to, the pro-inflammatory IFN-gamma gene signature and the M1 to M2 macrophage ratio in TNBC. Co-culture studies demonstrated that elevating Pfn1 expression in TNBC cells enhances chemotactic migration of monocytes in a paracrine fashion. To investigate the underlying mechanism, we performed luminex analyses of the conditioned media of TNBC cells, and identified CCL2, a major chemoattractant of monocytes, to be dramatically upregulated and downregulated upon overexpression and knockdown of Pfn1, respectively. These data were further supported by real-time quantitative PCR-based confirmation of Pfn1-dependent changes in CCL2 transcription in TNBC cells as well as a significant positive association between Pfn1 and CCL2 mRNA expression in the clinical specimens of TNBC. Silencing CCL2 expression in TNBC cells abolished Pfn1-dependent changes in the chemotactic migration of monocytes, suggesting that CCL2 is a key mediator of Pfn1-stimulated migration of monocytes. Collectively, these findings provide the first evidence for extrinsic immunological effects of Pfn1 in BC. Since tumor infiltration of CD8+ T cells is associated with improved prognosis in BC and is a key determinant of immunotherapy success in TNBC, our work lays the conceptual foundation for future studies to explore whether Pfn1 modulation could be a novel strategy to enhance immunotherapy efficacy in BC.

# scRNA-seq reveals TET-dependent gene expression events that occur during retinogenesis

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Purpose: 5hmC is an epigenetic modification required for normal retinogenesis. It is known that tet2-/-tet3-/- (5hmC-null, DMUT) zebrafish show loss of terminal differentiation markers in several retinal cell types, but the extent of developmental impairment and the gene expression events that underly that impairment in each retinal cell type are not well known. The goals of this study are (1) to probe the extent to which developmental phenotypes occur in each cell type in DMUT retinae, and (2) to identify gene expression events required for cell type-specific development that are lost in DMUT retinae.

Methods: We generated 18 scRNA-seq libraries, including sibling control (CTL) and DMUT cells at four timepoints that span retinogenesis (36, 48,72, 120 hpf). Preprocessing (Bioconductor), batch correction (Batchelor), and cell type identification (AUCell) were done. Computational lineage trajectories were built (URD, Principal Curve), and genes differentially expressed between related CTL and DMUT trajectories were determined (Tradeseq). Genes of interest were tested by injecting target-specific F0 loss-of-function CRISPR complexes into embryos of the Sofa1 transgenic line, in which all major retinal neuron subtypes are labeled. Morphological differences and proportions of different retinal cell types were quantified in F0 crispants.

Results: 146,571 high quality retinal cells were profiled. KS tests performed on lineage-specific pseudotime analyses showed that CTL and DMUT pseudotimes are drawn from significantly different distributions ($p<0.05$), and 120 hpf CTL cells show significantly older pseudotimes in 7 of 8 retinal lineages ($p< 2.2e-16$, Wilcoxon rank sum). These data suggest significant developmental divergence in timing of CTL and DMUT retinal differentiation, and also suggest that differentiated CTL cells are more mature than DMUT cells of the same lineages. Gene expression alignment of transcriptomic lineage trajectories predicted testable lineage-specific targets that show differential expression between CTL and DMUT retinae.

Conclusions: Our results suggest that tet2-/-tet3-/- retinae are transcriptionally divergent across all major cell lineages. Tet/5hmC LOF studies that assess divergences in lineage-specific gene expression enable us to further understand the gene expression events that facilitate normal retinogenesis.

# Immune Infiltration and Vasa Vasorum Remodeling of Pulmonary Artery in SARS-CoV-2 Infection

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Objective: To study and understand the vasa vasorum remodeling in main pulmonary artery in patients who underwent bilateral lung transplant due to severe COVID-19.

Methods: We collected main pulmonary artery specimens and clinical data from patients who were diagnosed with COVID-19 induced respiratory failure and underwent bilateral lung transplantation with IRB-approval and an informed consent process. Specimens of main pulmonary artery were also collected from lung transplant donors as the control group. We conducted Hematoxylin and Eosin (H&E) staining and immunohistochemistry analysis on paraformaldehyde-fixed and paraffin-embedded pulmonary artery samples. Some samples underwent IHC were embedded in optimal cutting temperature (OCT) compound and cryosectioned. We identified vasa vasorum with αSMA and/or CD31, macrophages with CD68, neutrophils and monocytes with CD15, platelets with CD61, T cells with CD44, and leukocytes with CD45 and CD11b.

Results: A total of 6 COVID-19 patients (median age = 44.2 years) were included in this study. Four patients had a history of pulmonary artery hypertension and had undergone veno-venous extracorporeal membrane oxygenation during their illness. We found that COVID-19 patients exhibited more vasa vasorum in the adventitia of the main pulmonary artery, and vasa vasorum were compressed. We also identified more immune cell infiltration within and around vasa vasorum of pulmonary artery in COVID-19 patients by observing more CD15, CD44, CD45, and CD68 positive cells. We also observed abundant CD15+ cells within the lumen of vasa vasorum in COVID-19 patients. In addition, we observed an even distribution of CD44+ cells in the adventitia, whereas accumulation of CD45+ and CD68+ cells were noted in the periphery of vasa vasorum in specimens from COVID-19 patients. However, no cells were found to be positive for CD61 in all specimens examined.

Conclusions : We identified compressed and increased number of vasa vasorum in patients undergoing lung transplant due to severe SARS-CoV-2 infection, which may result from contraction of endothelial cells and/or pericytes. The CD15+ cells within the lumen may reflect the starting stage of immunothrombosis. The more immune cells infiltrating the periphery of vasa vasorum of COVID-19 patients, like macrophages and leukocytes may be recruited via paracrine factor secretion by pericytes and/or endothelial cells. Future studies on endothelial cells and pericytes, two main residential cells of vasa vasorum, would help to further understanding of the COVID-19 pathophysiology.

# Modulating mesothelial cell motility and contractility alters ovarian cancer metastatic potential

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Ovarian cancer is the leading cause of death among gynecological cancers and metastasis to the peritoneum occurs in over 60% of patients. Cancer cells in the peritoneum attach and invade through a layer of mesothelial cells that form the peritoneal membrane, leading to extensive metastasis in the peritoneal organs. The signaling pathways in mesothelial cells that are necessary for effective clearance are poorly understood. The goal of this study is to explore how modulation of the mesothelial barrier alters clearance dynamics of ovarian cancer spheroids to ultimately slow down metastatic spread. We discovered that treatment with Forskolin, a CAMP activator, significantly reduced the number of clearing spheroids compared to the control untreated condition. Treating mesothelial cells with protein kinase inhibitor peptide (PKI), a protein kinase A (PKA) inhibitor, in the presence of Forskolin restored the high clearance rate. Staining with phalloidin revealed a reduction in actin stress fibers in the Forskolin treated cells when compared to control. We saw reduction in both the traction and wound healing abilities of the mesothelial cells with Forskolin treatment, suggesting that it is reducing the motility of mesothelial cells. Lastly, we found that treating the mesothelial cells with Calyculin A, a potent phosphatase inhibitor, increased spheroid clearance. This suggests a link between mesothelial cell migration and contractility and spheroid clearance events. Understanding the signaling pathways in mesothelial cells that enable fast clearance will provide us with new insights on how ovarian cancer causes distant metastasis and potentially identify targets for improved treatments.

# Hepatocyte Gdf15 protects against CCL4-induced liver injury independent of feeding and body weight

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Recent estimates suggest that about 1 in 400 adults in the United States has cirrhosis. Liver disease is a global health problem driving the development of chronic diseases. Thus, it is important to understand biology in the context of chronic liver disease. Growth differentiation factor 15 (GDF15) is a stress-induced secreted protein whose circulating levels are increased in the context of fibrotic liver disease. Recombinant GDF15 and GDF15 overexpression reduces body weight and improves glucose homeostasis and insulin sensitivity in obese models, which is largely attributed to the central action of GDF15 to suppress feeding and reduce body weight. Our previous work has demonstrated that liver hepatocytes are the primary source of circulating GDF15 in obesity and suggest the potential for local effects of GDF15 secretion on insulin sensitivity. Here, we demonstrate that hepatocyte Gdf15 protects against CCL4-induced fibrotic liver injury independent of feeding and body weight. We performed CCL4 studies in WT and GDF15 knockout first mice treated with AAV8-GFP or -FLP and found restoring hepatocyte GDF15 expression reduced fibrosis to levels similar to WT CCL4-treated mice. This contrasted with no difference in feeding between CCL4-treated groups despite marked differences in circulating GDF15 levels, inflammation markers, and body weight loss. These data support that GDF15 has local effects within the liver to remodel the extracellular matrix (ECM) and suggests a novel mechanism independent from the central action within the brain.

# β-Catenin Activation Promotes B-cell Exclusion in the Hepatocellular Carcinoma Microenvironment

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Background: Current immunotherapeutic approaches for hepatocellular carcinoma (HCC) are focused on T-cell specific immune checkpoint inhibitors (ICIs). Work in other cancer models have linked ICI response to B-cell signaling in the tumor microenvironment. Our group and others have demonstrated that β-catenin-mutated HCCs promote resistance to ICIs. Here, we investigated if β-catenin-mutation in HCCs may play a role in B-cell exclusion and impact subsequent response to ICIs.

Methods: Public HCC datasets were assessed for mutations in the β-catenin gene, CTNNB1, and B-cell related gene signatures. Our clinically relevant mouse HCC models of T41A-CTNNB1/G31A-NFE2L2, S45Y-CTNNB1/hMET, and MYC/hMet treated with and without anti-PD-1 therapy, were assessed for the presence or absence of B-cells by immunohistochemistry (IHC). Next, the influence of β-catenin suppression on B-cells was explored using antisense technology in HCC models.

Results: Overall, 26% of HCC cases in The Cancer Genome Atlas (TCGA) showed CTNNB1 mutations. Ingenuity pathway analysis of TCGA RNA-sequencing data demonstrated that multiple pathways in B-cells were significantly altered in CTNNB1 mutated vs non-mutated cases. Similarly, comparing these two cohorts, differentially expressed genes were overlapped with a publicly available B-cell signature which revealed 130 overlapping genes, of which 101 were downregulated, including MS4A1 (which encodes for CD20). In our murine HCC models, we also noticed decreases in CD20+ immune cells on IHC in both β-catenin-driven models compared to MYC/hMET model. Additionally, we noted no differences in B-cell numbers following anti-PD-1 treatment in β-catenin-driven models. Moreover, using an antisense oligonucleotide to suppress β-catenin in HCC models increased CD20+ immune cell infiltration in the tumor microenvironment and simultaneously significantly reduced tumor burden.

Conclusion: β-Catenin-driven HCC may drive a B-cell exclusionary phenotype. Future directed studies aim to elucidate the mechanism of B-cell signaling in ameliorating tumor burden following β-Catenin inhibition in conjunction with anti-PD-1 therapy in CTNNB1-mutated HCC.

# A controlled and reproducible mouse model of corneal anterior stromal injury caused by excimer laser-mediated photoablation

Mithun Santra (1), Moira L. Geary (1), Sabrina Mukhtar (1), Vishal Jhanji (1,2), Deepinder K. Dhaliwal (1), and Gary Yam (1,2)

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Corneal opacities and scarring are leading causes of global blindness. The standard of care is donor cornea transplantation; however, the global shortage of donor tissue restricts the treatment outcomes. The emergence of cell-based and cell-free strategies using corneal stromal stem cells, stromal keratocytes, secretome, and extracellular vesicles could serve as alternatives for corneal scar management. The therapeutic efficacy of these approaches has to be validated by in vivo corneal injury models. Most studies employ mechanical ablation (e.g. by Algerbrush burring) or chemical burns to injure corneas, but these damages result in inconsistent wound size and depth and have poor reproducibility, hence requiring extra use of animals in order to draw significant conclusions.

Using an excimer laser platform (NAVEX Quest EC5000, NIDEK) to anterior stromal injury on Balb/c mouse corneas (N=50), we examined different options: i) the ablation depth with dioptric (D) adjustment based on the Munnerlyn formula, and ii) with or without corneal epithelial removal. To examine the wound creation and scar manifestation, we performed anterior segment optical coherent tomography (AS-OCT) at pre- and post-injury and at time intervals (7 and 10 days). Serial OCT images were processed for 3D reconstruction with Fiji, and scar volume and density were measured with Metamorph II. Corneas were harvested for marker expression analysis of fibrosis (αSMA, Col3a1, tenascin C, and fibronectin) and inflammatory genes (MCP1 and iNOS) using qPCR and immunostaining. An OCT examination was done to evaluate the stromal ablation. Animals were takedown at different day intervals (7, 10, and 14 days). Corneas were isolated and imaged to assess scar formation. Data were compared with the Algerbrush-injured corneas.

Consistent anterior stromal injury at about 1/3 stromal depth was obtained with prior removal of corneal epithelium with 1 mm trephine, followed by the laser ablation at 140 mJ and -10 D for 11 sec in a mesh off/on the procedure to cause an ablation depth of 27 μm (out of ~90 μm full stromal depth) and 1 mm wound diameter. After 10 to 14 days, the injured corneas (N=12) developed fibrosis and scarring with defined size and depth and had upregulated fibrosis and inflammatory gene markers.

In conclusion, we developed a reproducible and consistent corneal injury model with anterior stromal wounds in mice. This is a suitable in vivo model for the future testing of drug treatments and cell-based and cell-free therapies.

# Beneficial influence of synthetic cannabinoid agonist on human chondrocytes in inflammatory environment

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INTRODUCTION: Recently, many states in the US legalized the use of medical marijuana in clinical use . Importantly, studies in animal models of arthritis showed that cannabinoids might attenuate joint damage[1]. However, the underlying mechanism has not been completely understood. Interleukin-1β (IL-1β), is known to be associated with the pathogenesis of osteoarthritis.

METHODS: Human chondrocytes were isolated from healthy articular cartilage. To generate cartilage in vitro, chondrocytes were pelleted and subjected to 14 days chondrogenic culture (Fig. 1A). After that, pellets were treated with different concentrations of Win( Win-55,212-2,a synthetic cannabinoid agonist) for 2 days . [Study 2] To simulate cartilage degradation observed in OA, we first treated pellets with IL-1β (10ng/ml) for 2 days and then applied Win for another 2 days.

RESULTS: [Study 1] PCR results of SOX9 was increased in the 1μM group(Fig. 1B). There were no significant changes in all other tested genes and GAG results (Fig. 1D). However, results from IL-6 ELISA showed that 0.01 and 0.1 μM groups contained significantly more IL-6 (Fig. 1C). [Study 2] IL-1β treatment suppressed the expression of AGG, COL2 and SOX9, and promoted the expression of IL6 and NF-κB, when compared to control groups( Fig. 2A) . A significantly increased IL-6 expression (Fig. 2B) and loss of GAG (Fig. 2C) in all IL-1β-treated groups. Results from PCR showed that Win was not able to suppress the expression of IL6 and NF-κB, or restore the expression of chondrogenic genes. However, Win at 1 μM significantly reduced the accumulation of IL6 in the medium(Fig. 2B). The GAG assayed showed that Win treatment might attenuate the loss of GAG due to IL-1β treatment. However, no statistical difference was observed (Fig. 2C). The preliminary data showed that Win at 0.01 and 0.1μM might reduce P-P65 protein level, which needs to be further validated in the future(Fig. 2D).

DISCUSSION: In normal engineer cartilage, whether cannabinoids can be beneficial or detrimental to osteoarthritic cartilage is uncertain with current evidence. For IL-1β insulted cartilage, the IL-6 ELISA results showed evidence of downregulated inflammation with a relative higher dose of Win. However, low dose of Win showed a beneficial influence on the phenotype of IL1-β-insulted cartilage, indicated by suppressing cartilage degradation (GAG loss) and NF-κB pathway. Therefore, the mixed results were found in this study. Whether cannabinoids can be a druggable target to treat OA requires further investigation.

# Diploid hepatocytes resist acetaminophen-induced acute liver injury and drive compensatory regeneration

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The liver contains diploid and polyploid hepatocytes, with polyploids comprising nearly 50% of human and 90% of mouse hepatocytes. The functional differences between diploid and polyploid hepatocytes are poorly understood, but emerging data suggest that each ploidy population promotes regeneration in an injury-specific manner. We hypothesize that diploid hepatocytes drive rapid regeneration in the context of acute liver injury. To study ploidy populations in vivo, we utilized mice with a lifelong liver-specific knockout of E2f7 and E2f8 (LKO) that are functionally normal but are depleted of polyploid hepatocytes (LKO livers are >70% diploid). Acute liver injury was induced by acetaminophen (APAP), a common analgesic that causes liver injury and failure when taken in excess. LKO and control mice were injected intraperitoneally with 300 mg/kg APAP and livers were harvested over 0-96 hours. Elevated liver enzymes, necrosis, and apoptotic hepatocytes revealed that while APAP damaged both treatment groups, control livers were significantly more damaged than LKO livers. Reduced damage and accelerated liver healing in the LKO model could be caused by gene expression effects associated with E2f7/8 loss or by enrichment of diploid hepatocytes. To discriminate between these possibilities, we first analyzed gene expression by RNA-sequencing in LKO and control mice after APAP injury. Differential gene expression was observed over the time course, which could contribute to varied sensitivity to APAP. Second, to focus on gene expression differences only, we knocked out E2f7/8 in adult livers where ploidy was equivalent in each group. Both groups responded to APAP equivalently. Finally, to evaluate ploidy effects in a wild-type model, the response to APAP by diploid and polyploid hepatocytes was investigated in vitro. Both populations were equally damaged, but diploid hepatocytes showed enhanced proliferation. Together, the data suggest that the response to APAP overdose in the LKO model is controlled by variations in gene expression and the enrichment of diploid hepatocytes. In conclusion, consistent with previous observations during liver regeneration, diploid hepatocytes are a driver of rapid compensatory regeneration after drug-induced acute liver injury, which underscores a novel role for hepatic ploidy populations.

# Exploring monocyte and trophoblastic signaling and their role in pre-eclampsia

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Maternal monocytes adapt during pregnancy to maintain immune protection while providing immunotolerance to fetal cells. Much of this behavioral adaptation occurs from the influence of the syncytiotrophoblast cells on the monocytes as they circulate through the maternal fetal interface (Mincheva-Nilsson & Baranov, 2014). Preeclampsia (PE) affects 2-8% of pregnancies and is a leading cause of maternal morbidity and mortality. Limited diagnostic measures exist, and delivery is the only “cure”(Ives et al., 2020). Researchers believe preeclampsia stems from placental malperfusion caused by syncytiotrophoblast stress (Redman et al., 2021). It remains unanswered whether this stress derives from prolonged placental hypoxia or via a hypoxia-reperfusion type injury (Hung et al., 2002; Soleymanlou et al., 2005). Syncytiotrophoblast stress alters cellular signaling to circulating monocytes (Göhner et al., 2017), and the latter become more pro-inflammatory in PE (Alahakoon et al., 2018; Al-ofi et al., 2012). Literature is unclear how these signaling changes in preeclampsia affect monocyte trafficking to the maternal-fetal interface. As circulating monocytes are easily accessible, understanding their behavioral changes in PE provides insight into novel diagnostic methods or therapeutic targets.

The aim of this study was threefold: to determine how monocyte recruitment changes in PE, which factor predominantly influences migration, and which placental stress model most accurately models these results in vitro. Monocyte recruitment was assessed via a Boyden Chamber Assay. Naïve or TGF-β1 conditioned THP-1 monocytes (ATCC) were placed in the upper well of the chamber. Placental-explant media from PE and non-PE patients (Magee-Womens Research Institute, Pittsburgh, PA) was placed in the bottom well of the chamber. For in vitro studies, conditioned media from BeWo B-30 cells (a placental choriocarcinoma line) cultured under hypoxia or hypoxia-reperfusion was placed in the bottom well. In vitro models of hypoxia and hypoxia-reperfusion injury induced levels of migration similar to the healthy control, mimicking monocyte trafficking in late-onset PE. However, it remains unclear how to effectively model the monocyte recruitment patterns observed in early-onset PE. Furthermore, our results indicate placental factors secreted in early-onset PE decrease monocyte migration to the maternal-fetal interface. It remains unanswered whether this change is a protective mechanism or a potential therapeutic target. The difference in migration results between early-onset and late-onset PE groups further confirms these disease subsets possess different etiologies. Monocyte inflammation more strongly influenced migration than placental disease state, implying the onset of PE can be predicted by assaying monocyte behavior.

# In vitro study to identify ligand-independent function of estrogen receptor-α in suppressing DNA damage-induced chondrocyte senescence

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INTRODUCTION: Osteoarthritis (OA) is the most common joint disease affecting over 500 million people worldwide. During OA progression, chondrocytes undergo many pathological alternations that are linked with cellular senescence. Cellular senescence can be induced by different factors, such as the persistent DNA damage, which can activate DNA damage response signaling (DDR) as well as the DNA repairing system in cells. However, the full map from DNA damage to the generation of OA/senescent phenotype in chondrocytes has not been established. Recently, we discovered that estrogen receptor α (ERα), plays a key role in maintaining the chondrocyte phenotype (Wang N, 2022). In this study, we hypothesized that DNA damage would result in the reduction of ERα levels in chondrocytes, which in turn lead to the generation of the senescence phenotype.

METHODS: With CORID approval, human chondrocytes were isolated from healthy knee joint cartilage. Animal study was approved by IACUC. To create OA models, destabilization of the medial meniscus (DMM) surgery was performed on C57BL/6 mice. After surgery, both DMM and sham surgery groups were housed for 8 weeks. The knee joints were fixed and embedded in paraffin. For immunofluorescence staining (IF), samples were first penetrated by 0.02% Triton X-100 (Sigma-Aldrich) for 10 minutes. After being blocked with 5% BSA, slides were exposed to primary antibodies overnight at 4°C. Alexa Fluor® 488-conjugated Secondary antibody was used. 4′ ,6-diamidino-2-phenylindole (DAPI)-containing antifade medium was utilized to mount the slides. To assess the function of ERα, normal human chondrocytes were pretreated with doxorubicin (DOX, 100nM) for 3 days, transfected with the lentiviral vector carrying ESR1 gene or the control lentivirus carrying mCherry. Cell phenotypes were examined by real time-PCR, Western blot, and Senescence associated β-Galactosidase staining (SA-β-Gal staining). Comet assay Kit was utilized to assess the level of DNA damage.

RESULTS: The OA chondrocytes contained DNA damage and displayed senescence features, which were accompanied by significantly reduced ERα levels. Overexpression of ERα reduced the levels of DNA damage and senescence in DOX-treated normal chondrocytes and OA chondrocytes. Moreover, DOX-induced the activation of NF-κB pathway, which was partially reversed by overexpressing ERα.

DISCUSSION:Our results demonstrated the critical role of ERα in maintaining the health of chondrocytes by inhibiting DNA damage and senescence. This study also suggests that maintaining the ERα level may represent a new avenue to prevent and treat OA.

# Development of responsive element luciferase probes to measure real-time macrophage polarization

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Alternatively activated macrophage or M2 macrophage, in response to inflammatory stimuli, plays a pivotal role in wound healing, immunosuppression and tumor progression. STAT6-mediated signaling pathway, activated by interleukin 4(IL-4)/IL-13, drives the alternative macrophage polarization. STAT6 functions by forming a phosphorylated homodimer and binding onto STAT6-responsive element within promoters to regulate anti-inflammatory associated genes. Measuring STAT6 activity can be a proxy for measuring macrophage polarization. However, cellular probes that enable real-time measurement of macrophage polarization are needed. Specifically, we developed a STAT6-RE THP1 reporter that allows us to access STAT6 activation in response to inflammatory stimuli. Combined with bioluminescence temporal spectroscopy (BTS) we can quantitively measure macrophage polarization. In this study, THP-1 monocytes were lentivirally transduced to express the STAT6-RE-FLuc-GFP. We demonstrate that reporter is activated by only by phosphorylated STAT6 via immunohistochemistry and ELISA. Importantly, the STAT6-RE can accurately report the dynamics of endogenous STAT6 activity. The results indicated a novel probe that can be used to assess macrophage alternative polarization. These experiments allow for a better understanding of the association between molecular mechanisms and macrophage polarization, facilitating the development of therapeutic strategies on manipulating macrophages for attenuating inflammation and tumor progression as well as promoting wound healing.

# Age- and Race-Related Differences in Normal Clitoral Shape Across the Adult Lifespan

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OBJECTIVE: Sexual dysfunction—disorders of sexual desire, arousal, orgasm, and pain—is a common underreported problem impacting ~43% of women in the US, where younger women and black women are more likely to report sexual problems. There is little research on how sexual dysfunction relates to sexual anatomy, especially among diverse populations. The clitoris is the primary sexual organ involved in sexual arousal/orgasm. Quantitative/inclusive data on clitoral anatomy is essential for understanding its relationship with the physiology/pathophysiology of sexual function/dysfunction. As a first step, this study aimed to identify age-related and racial differences in normal clitoral shape in adult women.

METHODS: Retrospective, noncontrast axial rest pelvic MRIs (1.5T/3T) of adult women were collected and grouped by race (Black, White) and age (Young Adult (YA) [18-34], Early Midlife (EM) [35-49], Older Adult (OA) [50+]). Clitoral anatomy was segmented and reconstructed as 3D models. After establishing corresponding points, a principal component analysis was performed to identify principal components (PCs) that described/quantified significant morphological variation of the clitoris. A Two-Way MANCOVA analyzed the interaction and main effects of age and race on clitoral anatomy while controlling for parity.

RESULTS: 121 women, 60 Black (26 YA, 20 EM, 14 OA) and 61 White (29 YA, 14 EM, 18 OA), were evaluated. There were 11 significant PCs that collectively described ~93% of the total variance in clitoral shape in the study population. There was a significant main effect of race ($p<0.001$) on overall clitoral shape. (i.e., PCs 1-11 combined). Particularly, there was a significant main effect of race for PC1 ($p=0.003$), PC2 ($p<0.001$), PC7 ($p=0.04$), and PC9 ($p=0.03$). In addition, there was a significant interaction effect of age*race ($p=0.006$) for PC3 where among Black women, clitoral shape significantly differed between YA and EM ($p=0.001$) whereas among White women, clitoral shape significantly differed between (1) YA and OA ($p=0.002$) and (2) EM and OA ($p=0.001$).

CONCLUSIONS: Racial differences, more so than age differences, were observed in clitoral anatomy. Shape differences were concentrated in the glans, vestibular bulbs, and crura. These sites are associated with the primary erogenous zones and perineal nerves involved in clitoral erection/sensation during sexual arousal/activity. They also coincide with clitoral supportive structures involved in the sexual response. These racial and age-related anatomical differences may in some way account for race/age disparities in sexual dysfunction. Future work should explore the role of the clitoris and its supportive structures in sexual dysfunction in diverse/underrepresented populations.

# Large Scale Biomimicry Impact of Three-Dimensional Annulus Shape and Leaflet Lateral Profile on Engineered Mitral Valve Mechanics, a Numerical Analysis

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One of the leading causes of morbidity and mortality in the Western industrialised countries is Heart Valve Disease. The incidence of these valve diseases in the USA is estimated as 2.5%. The American Heart Association show that over 4 million people in the USA suffer from Mitral Valve (MV) disease with an annual increase of about 350.000 new cases. To date, the valve repair and replacement market are estimated to be worth $2.8 billion, with a projected compound annual growth rate of 9.1%.

Several prosthetic devices are used for the repair of the mitral valve, which is characterized by different anchoring mechanisms, coaptation and limited perivalvular leak. However, all the commercial devices are characterized by three leaflets and this is associated with an alteration of the blood flow. In addition, mitral valve dynamism is neglected by a flat annulus configuration. Due to the complexity of the MV, only a limited number of groups are developing numerical methods that are including the chordal apparatus. None of them has standardized the chordal apparatus.

The MV has a complex and dynamic anatomical structure, consisting of; two asymmetric leaflets, a 3D saddle shape annulus, chordae tendineae (CT), and papillary muscles (PM). Damage of any of these components can lead to mitral regurgitation, which consists of mitral valve incontinence, leading to backward blood flow during ventricular systole.

This study aims to analyse the effect of the dynamic 3D saddle shape of the annulus and physiological-inspired lateral profile on the leaflets during the ventricular systole.

The simulations were performed on Abaqus. The Holzapfel-Gasser-Ogden model was chosen to describe the material behaviour and connector elements (axial) were used for simulating the CT that connected the PM and the leaflet. The ventricular systolic phase was simulated by applying 120 mmHg on the outer surface of the valve for 0.3 seconds.

As aspected the option of the 3D annulus and the lateral profile of the leaflet physiologically inspired affected the von Mises distributions, reducing the maximum von Mises stress and the $\Delta\sigma$ between the maximum and minimum von Mises. However, the simulation performed with both models showed that the most stressed region is the commissural zone and the area around the chordae insertion.

We reached the conclusion that the 3D saddle shape annulus and the physiological-inspired lateral profile provided a more homogenous stress distribution on the leaflet, biomimetic configuration influenced the results.

# Mathematical Model of Influenza Infection in Juvenile Mice Suggests Increased Production of Type 1 Interferon by Infected Cells is Associated with Severe Infection

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Each year, influenza infection can cause anywhere between 290,000 and 650,000 deaths worldwide. Children are uniquely susceptible to severe influenza infection which can result in pneumonia, a leading cause of death in children globally. Studies have shown that the immune response in children to influenza infection is vastly different than those of adults, with children relying more heavily on their innate immune responses. Additionally, murine studies have revealed that while viral burden is similar between juvenile and adult mice, juvenile mice experience higher levels of inflammation, lung injury, and mortality. Thus, an improved understanding of the underlying mechanisms that drive severe disease in pediatric cases can reveal pathways critical to inflammation induced lung injury, allowing for improved therapeutics.

Non-linear ordinary differential equation (ODE) models are highly effective tools for identifying how differences in immune responses emerge. ODE models have been used to describe the dynamics of innate immune responses to influenza. However, ODE models have not been used to consider differences in immune regulation between juveniles and adults. To understand the underlying mechanisms driving severe pediatric infection, we developed a mathematical model consisting of six ODEs. Our ODE model focuses on the innate immune response to influenza infection and incorporates neutrophils, monocytes, and type 1 interferons (IFN), of which the latter two are observed to be significantly elevated in influenza infected juvenile mice. Using our model, we capture the observed juvenile immune response to influenza and identify parameters responsible for disparities in immune responses between juvenile and adult mice. The model suggests that while recruitment of inflammatory monocytes cannot explain differences in pathologies between juvenile and adult mice, variations in IFN production by infected cells, and to a lesser extent viral sensitivity to IFN, are key drivers of severe infection. In the future, we aim to use knockout studies of CCR2-/- juvenile mice for validation of our model and to expand the model to consider additional immune cells and cytokines. Development of an accurate model of influenza-induced immune responses in juveniles will enable in silico treatment design and guide future drug discovery.

# Identifying the mechanisms linking sex and influenza infection using computational modeling

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In humans, differences in the immune response between males and females greatly influence influenza virus infection outcomes. During the 2009 H1N1 pandemic, females made up 53.2% of the total hospitalizations and adult females specifically were at greater risk than their male, age-matched counterparts for hospitalization and death. The innate immune response has been implicated as a factor of these sex differences in influenza pathogenesis. Sex hormones have multiple, specific effects on the innate immune response. Treatment with estradiol can reduce excess inflammation due to influenza and reduce mortality in mice through stimulation of interferon production pathways that result in promotion of anti-inflammatory activity. Experiments also show that influenza infection causes decreased sex organ function and persistently lowered estradiol concentration, which promotes pro-inflammatory pathways. The feedback between estradiol, influenza, and interferon during infection has not been considered in any immune model of influenza infection to our knowledge. We modified an existing mathematical model published by our group and fit the model to data from male and female mice infected with influenza in order to identify difference in male and female immunoregulation. The model was fit to sex-specific murine data. Several parameterization exercises were performed to determine which immune processes are differently regulated in males and females. Using MCMC, a large parameter space was sampled, and parameter distributions were compared to identify specific rates leading to sex-specific immune responses to influenza. Local and global sensitivity analysis revealed that the male and female models are sensitive to different parameters. These specific mechanisms could be targeted by novel or existing drugs to treat and prevent severe influenza infection. Currently, our collaborators are generating high-quality, comprehensive, sex-specific murine data that we plan on using to validate immune models incorporating additional immune cells that have been identified as significant for influenza infection clearance. We also plan to use this data to validate our conclusions from the current model we have developed. We also aim to include hormone concentrations in our future models to analyze hormone-specific impacts on the immune response in males and females.

# Mathematical Modeling of Fibroblast-Mediated Drug Resistance in HER2+ Breast Cancer

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The tumor microenvironment can mediate tumor development and drug resistance through a myriad of growth factor/cytokine signals that can activate pro-survival pathways in tumor cells. Drug resistance is a major challenge in patients with HER2 overexpressing (HER2+) breast cancer, which accounts for ~20% of all breast cancer cases. Many of these patients (38-75%) do not respond to HER2 targeted therapies. Fibroblasts are a prominent cell type found in the tumor microenvironment that is linked to poor patient prognosis and drug resistance. Recent studies have found that fibroblasts co-cultured with HER2+ tumor cells prevent tumor cell death and increase tumor cell proliferation in the presence of a HER2-kinase inhibitor (Lapatinib). Fibroblasts confer lapatinib resistance in part through increased anti-apoptotic protein expression and PI3K/Akt/mTOR pathway activation in tumor cells; however, this resistance can be modulated by altering the number of tumor cells, number of fibroblasts, and drug concentration in vitro. An ordinary differential equation (ODE) model is used to infer types of cell-cell interactions between cancer cells and fibroblasts (bidirectional vs. unidirectional signaling) as well as predict conditions (lapatinib dose and number of fibroblasts) at which cancer cells are fully protected from lapatinib.

# Investigating the interplay between plaque growth dynamics and interferon receptor availability via Multicellular Spatial Model using CompuCell3D

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Interferons are a group of proteins that play a critical role in the immune response to viral infections. Their antiviral properties have led to the popularity of interferon inhalers among frontline workers as a preventive measure during the SARS-CoV2 pandemic. However, it is essential to regulate interferons as prolonged, excessive levels of interferon in the bloodstream, along with an elevated inflammatory state can lead to tissue damage and hinder clinical recovery. Therefore, it is crucial to understand the regulation of interferons during an infection to develop effective treatment strategies for viral infections.

The advances in clinical immunology over the last decade has prompted the development of new mathematical models to research unanswered questions in immunology Most mathematical models used to simulate immune responses are based on ordinary differential equations (ODEs). Such models, although useful in mapping the overall immune response, typically ignore the diffusion of virus, local cytokine signaling and the stochastic nature of individual cell responses. To address these shortcomings, our lab has developed a novel spatial model called the multicellular spatial interferon signaling (MSIS) model. The model is designed to include multiple cell states and external diffusion of virus and was created using CompuCell3D software, which enables spatial and agent-based modeling of biological systems. However, the MSIS model fails to account for a recently discovered mechanism, where interferon loses its impact on viral load beyond a certain concentration . We believe that understanding the cellular mechanisms involved in regulating interferon is critical to modeling this phenomenon accurately.

The primary objective of this project is to enhance the MSIS model by incorporating the interaction of interferon receptors (IFNRs) and its depletion, building on the detailed work done by Korwek et al. Our goal is to modify the MSIS model by accounting for IFNRs' depletion to test if it can replicate the observed saturation effect and reveal new dynamics. Additionally, we aim to improve the accuracy of cell health representation in the model by rewriting the function that calculates it to better reflect phenomena such as apoptosis and pyroptosis. The updated model should provide greater insight into the pathways relevant to developing and improving clinical therapeutic strategies for treating infected patients.

# Development of a Novel Ventriculoamniotic Shunt for Treating Fetal Hydrocephalus

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Fetal aqueductal stenosis (AS) is one of the most common causes of congenital hydrocephalus, which increases intracranial pressure due to partial or complete obstruction of cerebrospinal fluid (CSF) flow within the ventricular system. Development of a shunt for treating fetal Aqueductal Stenosis (AS) can be of great importance, since there are currently no available ventriculoamniotic shunt at this moment. This study has successfully validated the design of shunt devices and demonstrated the mechanical performance and valve functions. To demonstrate the functionality of the developed device, several in-vitro and in-vivo tests were performed to validate the device design and performance. The device is made of four primary components using different materials with different characteristics. The shunt contains a main silicone-nitinol composite tube, a superelastic 90° angled dual dumbbell anchor, and an ePTFE valve encased by a stainless-steel cage.

When a functional prototype was developed, it was positioned in the right place using ultrasound guidance. Thanks to the two-dumbbell shape of the anchor, no displacement was observed during the test. As the other main component of the device, the one-way valve successfully proved fluid flow and totally stopped amniotic fluid from flowing back into the ventricles. Even though moderate tissue growth was observed on the surface of the anchor in the fetal brain region, the device was flawless and exhibited excellent performance. Flow rates in shunts were quantified to demonstrate the valve function in low flow rates mimicking the fetal hydrocephalus condition showing "no backflow" for the valved shunt while there is up to 15 mL/h flow through the shunt with pressure difference of 20 Pa. In vivo ovine study results show that the fluid drainage performance for the harvested shunt is 92□0.32% of the flow after 30-day implantation.

# Decellularized cardiac patch for cardiovascular repair: Comparative assessment of glutaraldehyde and photo-oxidation crosslinking with fixation-free processing

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Background: cardiovascular diseases (CVD) are responsible for about 19 million deaths in 2020, with an increase of 18.7% since 2010. Biological decellularized patches are common therapeutic solutions for CVD to repair cardiac and valve defects. The Strategy in manufacturing and post processing of cardiac patches are mainly through two methods of glutaraldehyde or photo-oxidation cross-linking (fixation) as well as non-crosslinked (non-fixation).

Motivation and Approach: Despite the variety of existing on the market, cardiac patches still suffer from significant draw backs, resulting failure in mimicking the biological tissue properties. This study assesses the impact of different manufacturing methodologies on four commercially available (CorPatch, CardioCel, PhotoFix) and one newly developed decellularized cardiac patch (Adeka) on biological and mechanical performance. Four cardiac patches with different processing methodologies selected, characterized for their thickness mapping, mechanical properties by biaxial testing (BT), uniaxial tensile testing (UTT), ball burst, suture retention, and structural properties by 2D surface topography using scanning electron microscopy, 3D volume microstructure using multiphoton and histology assessments. Also, their biological response was tested in vitro on platelet and calcium deposition and their host response were tested in vivo on a rat right ventricular outflow tract (RVOT) models after 8- and 16-week implantation time.

Results: Topological analysis showed that the crosslinked cardiac patches have greater thickness when compared to the non-crosslinked patches. This was consistent with histological results demonstrating that the cross-linking process preserved collagen content, while non-crosslinked demonstrated delamination. UTT indicated an elastic modulus >50MPa for Adeka and CorPatch and >130MPa for PhotoFix and CardioCel. This was consistent with the level of collagen fiber alignment visualized through Multiphoton 3D. BT demonstrated that all cardiac patches except CorPatch recapitulate the anisotropic behavior of healthy tissue. Stronger resistance to thrombus and less calcium formation were observed in non-crosslinked patches. All patches showed biocompatibility and durability during 8- and 16-week implantation in RVOT track. Explant histology shows high cell infiltration in non-crosslinked (Adeka) than crosslinked (PhotoFix).

Conclusions: This study demonstrated that crosslinked cardiac patches have higher thickness, stiffness, and durability when compared to the non-crosslinked decellularized, although they are more prone to thrombus and calcium formation. The data obtained suggest that Adeka better resists calcium and thrombus formation while maintaining improved structural properties versus CorPatch, offering promise for cardiovascular applications.

# Comparative assessment of commercially available patches for cardiac application

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Objective: Cardiovascular diseases (CVD) are responsible for about 19 million deaths in 2020, with an increase of 18.7% since 2010. Cardiac patches are common therapeutic solutions for CVD. Available in market cardiac patches differ in their manufacturing process, some undergo cross-linking treatments to enhance their mechanical properties and durability. This process of fixation impairs their natural texture, in their interaction with biological environment. Recent generation of cardiac patches attempt to maintain the natural texture as close to their biological tissue by eliminating fixation steps as much possible. In this study, the Adeka patch, a newly developed unfixed tissue, is compared with other three clinically in use cardiac patches, two of them subjected to cross-linked processes (CardioCel, PhotoFix) and one of them unfixed (CorPatch) for their hydrodynamic performance and durability as prosthetic aortic valves.

Methods: The patches were used to manufacture four stented prosthetic aortic valves. Hydrodynamic (through a pulse duplicator, ViVitro system) and durability (through a high cycle system, BDC Laboratories) assessment were performed according ISO5840 to investigate their ability to withstand to the high mechanical load typical of the cardiac cycle. In-vitro tests were performed to investigate their ability to successful interact with the complex biological neighborhoods. Cardiac patches are treated with two calcification fluids (ISO23317) to evaluate tissue attitude to calcium deposition. Platelet deposition was evaluated through the lactate dehydrogenase (LDH) assay.

Results: Considering the mechanical properties, although all tissues are able to provide ISO4850 compliant valve aortic prosthesis, durability tests suggest that cross-linked patches exhibit better performances. However, among the unfixed patches, Adeka exhibits improved mechanical properties reaching superior performances than CorPatch. On the other side, Adeka showed the lowest platelet deposition ($p<0.05$) and lowest calcium deposition ($p<0.05$) compared to the other three patches.

Conclusions: The Adeka patch seems to be a good candidate for cardiac repair applications showing less calcium deposition and platelet deposition than others. The absence of crosslinked treatments, although leads to a weaker tissue texture, helps in maintaining a biological response more physiological.

# Advancements in Cardiovascular Stent Technology: Introducing a Nanostructured Electronic Stent for Continuous Monitoring of Restenosis

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Coronary artery disease (CAD) and its main complication are leading cause of cardiovascular mortality in the US. Approximately, one out of four deaths in the US are due to cardiovascular disease. While stent placement procedures have greatly improved outcomes for patients with this disease, there is still a significant risk of the life-threatening complication of restenosis, or re-narrowing of the coronary artery. To address this issue, a novel imperceptible electronic stent is presented that includes a wireless sensor capable of continuous surveillance of restenosis, neointimal proliferation, and plaque deposition. This stent incorporates a low-profile nanomembrane capacitive strain sensor, which is constructed through the printing of conductive nanoparticles and polymers on a soft elastomeric membrane. The sensor is sensitive enough to detect strains as low as 0.15%, with a sensitivity of 3% per linear strain, making it suitable for detecting small alterations produced in the coronary artery during the progression of restenosis under typical pulsatile flow. An in vitro testing platform has been developed to evaluate the sensor's performance, using both numerical analysis and computational fluid dynamics to design artery models with various levels of restenosis. Strain plots from both types of analyses successfully show the relationship between strain and restenosis levels, as well as the effects of varied pressures, artery lumens, and artery thicknesses. These findings represent a promising solution for improving the diagnosis and treatment of heart disease and other vascular diseases requiring stents.

# Dual-lumen microinjector for the organized co-delivery of viscous fluids to target tissue

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Delicate tissues such as the retina, thyroid, and skin must be treated with care to minimize trauma and preserve specialized functionality. Common procedures performed on these tissues use needles to deliver therapeutics in a safe and efficient manner. Unfortunately, this approach is limited to large gauge needles when working with viscous fluids and often requires multiple injections.

Here, we introduce a dual-lumen microinjector for the organized, simultaneous delivery of two viscous fluids. Our injector is a 3D-printed handpiece with two 32G needles embedded within a single 23G needle. This design allows for the co-delivery of therapeutics during a single injection, leveraging the mechanical strength of a larger needle along with the precise delivery of two smaller needles. The handpiece has two standard luer connections and can be used with a variety of pressure sources including a syringe pump, vacuum pump, and vitrectomy machine used for eye surgery. We demonstrate that the microinjector can reliably deliver two viscous fluids at specific volumes and locations to delicate tissues like the retina. The device fits within a standard 23G trocar cannula to perform surgery in the posterior segment of the eye. Overall, our injector shows promise as a new alternative device for vitreoretinal surgery, cataract surgery, fine needle aspiration, and the development of novel biomaterials for drug delivery.

# Therapeutic Use of an Interleukin-4 Eye Drop in a Rabbit Model of Dry Eye Disease: A Pilot Study

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Introduction: Dry eye disease (DED) is estimated to affect up to one third of the population, with a spectrum of severity. However, severe, chronic DED can lead to vision-threatening complications, negative psychological consequences, and reduced quality of life. Current treatment options have limited efficacy and either provide temporary symptomatic relief or target limited aspects of the immune system. While the exact etiology of DED may vary patient to patient, two key elements, tear film instability (of which reduction in mucin-producing ocular goblet cells contribute) and ocular inflammation, are well-recognized drivers of DED pathogenesis. The failure of current therapies to target both of these elements concurrently may be a reason for their limited efficacy. Interleukin-4 (IL-4) has been shown to cause the differentiation of epithelium into goblet cells and to induce mucin expression – both key factors in tear film stability. IL-4 is also a potent anti-inflammatory cytokine causing transition of immune cells from a pro-inflammatory to anti-inflammatory phenotype. Thus, we hypothesize that IL-4 may represent an ideal cytokine for the simultaneous treatment of both tear film instability and inflammation in DED.

Methods: After surgical lacrimal gland removal, a rabbit model of DED was allowed to develop for 4 weeks. Two drops of PBS or IL-4 in PBS were administered daily for 14 days. Clinically relevant ocular assessments (e.g., fluorescein staining) were performed at 7 and 14 days post-treatment-initiation. After sacrifice, eyes were prepared for histological assessment, which included a periodic acid-Schiff (PAS) stain for goblet cells, pan-macrophage RAM11 immunolabeling, and H&E stain for general tissue morphology and cellularity.

Results: Fluorescein staining demonstrated significant reductions in corneal damage in IL-4 treated animals, and these improvements were associated with restoration of goblet cell numbers to native values in IL-4 treated animals. H&E staining and RAM11 immunolabeling demonstrated qualitative differences between PBS and IL-4 treated animals, with PBS treated animals showing areas of dense cellular infiltrate and epithelial thinning indicative of ulceration. Interestingly, increased RAM11 labeling was also seen in the epithelial basal layer of PBS treated animals. While RAM11 is primarily considered a macrophage specific antigen, others have shown that it is expressed at low levels in the basal epithelium of multiple tissues in rabbits, with transient increases in expression following epithelial wounding, suggesting increased epithelial damage in PBS treated animals.

Conclusions: These preliminary studies suggest that IL-4 treatment results in improved ocular surface integrity as well as increased number of goblet cells when used in an eye drop in a rabbit DED model. Thus, we hypothesize that IL-4 can provide improvements in DED that are superior to those associated with currently available treatments.

# Epoxy-silane Functional Sulfobetaine Block Copolymers for Thromboresistant Coating on an Ambulatory Assist Lung Device

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Developing an ambulatory assist lung (AAL) for patients who need continuous extracorporeal membrane oxygenation has several hurdles to solve including the design of compact components, fluid dynamics, and thrombogenicity. In an effort to improve device thrombogenicity, we have previously developed a siloxane-functionalized zwitterionic sulfobetaine (SB) coating and demonstrated positive results in a scaled-down setting. This work focused on the feasibility of the simple coating method on a full-scale AAL device designed for animal study. This study also suggests water-soluble SB block copolymers which include epoxy or epoxy-silane groups (SB-EP & SB-EP-Si) for the simple aqueous coating to the full-scale device. All modified surfaces regardless of the functional groups of the SB block copolymers showed an 80-95% reduction in platelet deposition from whole ovine blood compared to the unmodified controls without reducing the gas exchange performance of the hollow fiber membranes (HFMs). However, the SB-EP-Si coating showed better coating feasibility for the in-situ coating on a pump lung device set. The circulation of aqueous SB-EP-Si could coat a complete set of pump lung devices. The coating stability was also superior to that using other SB block copolymers. These features of SB-EP-Si indicate that this approach is promising for the full device coating and longer-term pre-clinical testing in respiratory assist devices and may ultimately allow for the reduction of anticoagulation levels in patients being supported for extended periods.

# Tissue Engineered Heart Valve Design: Influences of Material Mechanics and Macro-Scale Geometry on Performance

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After nearly two decades of development, tissue engineered heart valves are finally nearing the goal of realized clinical translation. There is a growing body of literature of implanted scaffolds in animal models that indicate that endogenous tissue growth does partially occur within the limited 1- to 2-year time frames. Additionally, no catastrophic mechanical failure has yet been reported. Despite multiple favorable outcomes in animal models, there are a wide variety of materials and approaches that have been used with little discussion pertaining to optimizing scaffolds pre-implantation. In that regard, this study seeks to provide more context in what variables should be considered in scaffold design. In particular, the effect on leaflet mechanics from material stiffness of the polymeric scaffold, as well as overall leaflet geometry, were measured. Cardiac valve scaffolds were electrospun from a base of elastomeric poly(carbonate urethane)urea (PCUU) with incrementally increasing concentrations of poly(ε-caprolactone) (PCL) in two separate geometric configurations. Markers were arrayed on the surface of valve leaflets prior to placing all valve scaffolds in a mock circulatory loop (MCL) simulating physiologic pulmonary conditions. During MCL operation, performance metrics such as orifice area and mean transvalvular pressure gradient were measured for each group, and markers were tracked stereoscopically under dynamic conditions at peak systole and mid-diastole. Contrasting marker positions with an unloaded reference condition, strain maps over the surface of the leaflets were calculated at both time points. Strain magnitude and regional strain concentration fell significantly as PCL concentration increased, but overall performance metrics such as orifice area and mean transvalvular pressure gradient similarly decreased. The greatest differences were seen, however, in contrasting the geometric configurations. There were significant differences in performance metrics and regional strain concentrations between the two geometric configurations in all of the PCL content groups. The results show that, while each of the tested variables has a significant impact on valve performance, the geometric configuration had a larger influence on valve performance and strain magnitude. It is therefore recommended that appropriate consideration for optimization of design, mechanics, and overall performance be given in the future study and application of tissue engineered heart valve scaffolds.

# Mandrel-less fabrication of biomimetic microfiber wires for soft tissue applications

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Bioimplants have been utilized as viable tissue support and surrogate in various applications. In particular, suture wires play an essential role in wound management and also in tendons, chordae tendineae, or ligament replacement and repair [1]. Despite the assortment of materials available, sutures are still affected by significant disadvantages, including failure in mimicking the mechanical properties of the tissue, excessive fibrosis, and inflammation. Moreover, understanding the underlying cell-bioimplant interaction mechanism remains relatively unexplored, hampering the development of in silico and in vitro models [2]. This study introduces a mandrel-less electrodeposition system to fabricate axially symmetric microfiber constructs with tunable mechanics and fiber architecture, potentially used as medical textiles and bioimplants [3]. The processing method used in this work is based on the notion of mandrel-less electrodeposition [4]. Two static or rotating electrically charged electrodes, with opposite and spaced tips, were used to induce the electrodeposition of polymeric microfibers. Poly(ester urethane) urea (PEUU) was used to fabricate continuous microfiber wires with controlled microarchitecture and tunable mechanics. A finite element simulation of the electric field generated during the mandrel-less fabrication was conducted using COMSOL Multiphysics. Morphological and mechanical properties of PEUU microfiber wires were characterized by scanning electron microscopy (SEM) and uniaxial tensile test. The host response to PEUU microfiber wire implantation was comparatively evaluated in vivo on a rat surgical wound model. In the second part of this study, murine fibroblasts were microintegrated into Poly(carbonate urethane) urea (PCUU) wires with limited length and highly aligned microfiber structure. The constructs were dynamically conditioned using a uniaxial stretch bioreactor for 7 days. According to the experimental observation, the electric field model predictions demonstrate how the field distribution bifurcates proportionally to the applied electric potential in the proximity of the negatively charged electrodes. As expected, the SEM qualitative evaluation confirmed the ability to properly control microfiber arrangements by tuning the electrode rotational speed and direction. The in vivo assessment demonstrates that PEUU microfiber wires reduce pro-inflammatory macrophage response, improve collagen deposition within the wound area, and preserve their mechanical properties after 30 days of use. The histological evaluation of microintegrated wires showed proper cell adhesion and infiltration within the scaffold. This study introduces a novel mandrel-less fabrication methodology for polymeric wires with controllable microarchitecture. This fabrication allows for the production of microfiber bundles with tunable ultra-structure and mechanical properties that can be used as suture materials and as tissue surrogates for tendon and ligaments repair. The microstructured wire with tailored-to-the-needs mechanical properties improved the macrophagic response and the constructive remodeling. The in vitro preliminary results on PCUU wires provided evidence of cell proliferation and viability, demonstrating proof of concept for the potential host cell recruitment and de novo tissue development.

# Endodontic Therapy Of Immature Permanent Teeth With An Acellular Drug-free Hydrogel

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Purpose/Objectives: Treatment of necrotic and traumatized immature permanent teeth is a challenge in pediatric dentistry. Regenerative endodontic therapies (RETs) work towards continued development and long-term maintenance of teeth compared to conventional non-vital therapies. Hydrogels are promising for RET of the dentin-pulp complex because they can flow into complex tooth anatomy and deliver regenerative drugs, materials, and cells. Gelatin methacrylate (GelMA) is a popular biomaterial to mimic natural extracellular matrix. However, syneresis over time is a limitation. Therefore, we incorporated methacrylated heparin (HepMA) into GelMA to create an anionic hydrogel, which provides better contact between the dentinal wall via swelling. This work evaluated the hydrogel for RET of immature teeth with open apices compared to conventional treatments.

Methodology: Photopolymerizable GelMA/HepMA hydrogel was fabricated in house. For safety assessment of the hydrogels, infection was induced in the mandibular premolars and first molars of 8-month-old beagles to simulate iatrogenic contamination. The treatment groups include hydrogel, revascularization (clinical RET procedure), apexification (routine non-vital procedure), and pulpectomy. After 2 weeks, canines were sacrificed, and the tissue response to infection was evaluated. For the regenerative application of this material, the same treatment groups were considered in canines, but the treatment outcomes were analyzed 3monthes after the surgery.

Results: The histological evaluation of the 2-week dogs showed that the periapical infection was similar between hydrogel and revascularization methods. Regarding regenerative data, continued root development was significantly higher with the GelMA/HepMA hydrogel than all other treatments. There were no significant differences in the depth of pulp-like tissue ingrowth and angiogenesis between treatments.

Conclusion/Significance: The GelMA/HepMA hydrogel is promising for RET of diseased immature permanent teeth, and it is as safe as revascularization. However, the formulated hydrogel enhanced treatment results compared to revascularization therapy.

# Histologic and Immunohistochemical Characterization of a Large Animal Model of Volumetric Muscle Loss

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Volumetric muscle loss (VML) is defined as the traumatic or surgical loss of skeletal muscle tissue which exceeds the body's repair capabilities leading to sustained functional deficits over time. There are no current approved therapies for regeneration following extensive musculoskeletal soft-tissue injuries. In an effort to contextualise repair and facilitate deposition of functional tissue, the present study seeks to provide longitudinal histochemical analysis of VML in a large animal model. Preliminary results have pointed towards a trend to earlier resolution of pro-inflammatory signalling in those treated with extracellular matrix or with electrical stimulation.

# Structural characterization of fresh human heart valves for tissue engineering application

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The four human heart valves, two atrioventricular (mitral and tricuspid), and two semi-lunar (aortic and pulmonary) exhibit significant differences in the physiology that are reflected in their different structural and mechanical properties. Heart valve disease is one of the major clinical challenges in cardiovascular care. Commercially available prosthetic devices largely neglect native tissue properties such as tensile moduli, anisotropy, bending rigidity or even simpler factors as the tissue thickness.

This, is increasingly recognized as a potential source for suboptimal performance for a number of clinical applications including healthy prosthetists, vascular graft and cardiac restrain devices. These structural parameters are not only neglected but they often remain poorly characterized, with data being extracted from animal tissue or cadaveric samples. A deep understanding of native cardiovascular tissue has become the priority in tissue engineering, primarily aiming at investigating human tissues mechanical and structural properties. In this work, we plan to fill this gap in knowledge performing a structural analysis of fresh human cardiac valves.

First, leaflet thickness of fresh human heart valves was estimated via dial indicator gage measurements according to previously established protocols. The analyses showed high heterogeneity of valve thickness distribution. Additionally, the mitral valve leaflets exhibited statistically greater thickness compared to the other valves, the pulmonary valve instead was statistically thinner compared to the others. This relationship is reflected by the different physiological transvalvular pressure values.

Then, four decellularization protocols were developed and applied on ovine heart valves to identify the best protocol to be further applied on human heart valves. All four protocols caused a significant reduction in leaflet thickness compared to the native leaflet. In addition, DNA content analysis showed that the sample treated with Triton X-100 and SDS exhibited a DNA content below the established threshold reported in the previous study, in contrast to the DNA content measured on the sample decellularized with trypsin (DNA content < 50 ngDNA/mgECM). Finally, a scanning electron microscopy (SEM) analysis was performed on native VS decellularized ovine mitral valve posterior leaflet. Using an algorithm modified by D'Amore et al. based on the algorithm developed by Chaudhuri et al. and Karlon et al. for structural parameters quantification, fibers angle distribution was quantified.

The results obtained in this study will be used to create a human heart valves structure-function properties database.

# Modeling Endometriosis Angiogenesis in Endometriotic Conditions

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Introduction: Endometriosis is gynecological disease associated with chronic pelvic pain and infertility affecting 10% of women worldwide. The main pathogenesis hypothesis, retrograde menstruation, proposes endometrial tissue sheds into the peritoneal cavity via the fallopian tubes. The presence of upregulated estrogen (E2) and a dysregulated immune environment could contribute to disease growth following retrograde menstruation. Current in vitro models of endometriosis use 2D cell culture, 3D cell (co)-culture, and microfluidic devices, but these models are limited in that they only consider the upregulated E2, not immune dysregulation. In this combined 2D and 3D cell culture study, we define endometriotic conditions as the presence of both upregulated E2 and immune dysregulation and test both E2 alone and endometriotic conditions for effects on proliferation and cord formation by endometriosis-relevant cells (endothelial and endometriotic 12Z cells).

Methods: Endometriotic conditions were modeled using THP-1 monocytes exposed to varying elevated quantities of 17-β Estradiol. 12Z cells, immortalized endometriotic cells, were used to understand how an endometriosis lesion might respond to endometriotic conditions. Human coronary artery endothelial cells (HCAECs) were used to study how endometriotic conditions might impact endothelial cell early angiogenic behavior, or cord formation. 2D cell proliferation was measured for E2 exposures of 1.0 ng/mL, 1.5 ng/mL, 2.0 ng/mL, and 4.0 ng/mL in phenol-red free Dulbecco's Modified Eagle Medium (DMEM) using alamarBlue. 3D cell culture in collagen gels were observed via an inverted light microscope.

Results: It was found that in 2D culture of HCAECs and 12Zs, ECs and 12Zs respond differently to the E2-supplemented media compared to endometriotic conditions. Furthermore, 12Z proliferation decreased in endometriotic conditions compared to only estrogenic conditions. In 3D culture, it was found that HCAECs and 12Zs on collagen gel demonstrated preliminary cord formation in endometriotic conditions. These findings will be helpful in developing a more advanced model of endometriosis angiogenesis by demonstrating desirable cell seeding densities and expected cellular performance in different conditions.

Conclusion: This work shows cord formation due to endometriotic conditions and promise in maintaining cell viability in 3D cultures under endometriotic conditions. Future work will include transmigration and invasion studies to understand the interaction between ECs and 12Zs in endometriotic conditions. In the future, a microfluidic model using endometriotic conditions will be a novel way to study endometriosis angiogenesis and pathogenesis. This research contributes a novel and standardized method for creating in vitro endometriotic conditions and will provide a platform for studying disease pathogenesis in vitro.

# Inhibition of Vasa Vasorum Angiogenesis Induces Thoracic Aortic Aneurysm

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Background: Thoracic aortic aneurysm is a life-threatening condition resulting in dilatation and potential rupture of the thoracic aorta. The underlying molecular and biomechanical causes of aortic wall weakening remain incompletely defined. The adventitial (outer) layer of the aorta contains numerous small blood vessels known as vasa vasorum. Previous work by our team revealed deficient and dysfunctional vasa vasorum associated with medial hypoxia in human aortic aneurysm. Based on this knowledge, we posit that reduced perfusion due to loss of vasa vasorum leads to hypoxia and aortic wall weakening. We hypothesize that application of ABT-510 to the ascending thoracic aorta induces aneurysmal degeneration through its antiangiogenic effects on the vasa vasorum and consequent reduction in aortic wall perfusion. We tested this hypothesis in a rabbit model via peri-adventitial delivery of ABT-510, an antiangiogenic thrombospondin analogue, to inhibit angiogenic signaling in the adventitia.

Methods: The ascending aorta was exposed through a median sternotomy in New Zealand White rabbits (n=6). The proximal ascending aorta was treated with ABT-510-soaked sponge while the distal portion was treated with a PBS-dextran-soaked sponge as vehicle control. Transthoracic echocardiography (TTE) was undertaken immediately prior to the procedure and 14 days post-operatively to measure the aortic diameter prior to euthanasia. The aortic tissue was harvested and adventitial vasa vasorum number, size, and density were quantified from paraffin-embedded H&E stained sections (n=2 rabbits) while elastin content was assessed with Verhoeff-Van Gieson (VVG) staining. Immunodetection of Hypoxyprobe™ (100 nM, administered 2 hours prior to euthanasia) and αSMA were also undertaken to identify hypoxia and smooth muscle cell content, respectively.

Results: TTE revealed aorta treated with ABT-510 had exhibited an increase in aortic diameter over 14 days when compared with aorta exposed to vehicle control (36.08% ± 7.35 vs 13.84% ± 3.63, p=.0313, Fig 1). On histologic analysis, aorta treated with ABT-510 had decreased vasa vasorum count (80.75 ± 4.37 vs 100 ± 4.30, p=.041), decreased vasa vasorum cumulative area (56,637 ± 1004 vs 128,470 ± 2890 μm2, p<.0001), and decreased vasa vasorum indexed cumulative area (0.017 ± 0.0001 vs 0.029 ± 0.0008 μm2/ μm2 adventitial area, p<.0001) relative to control. Immunodetection of Hypoxyprobe™ showed evidence of increased hypoxia in the medial and adventitial layers of aorta treated with ABT-510 (Fig 2). VVG staining and immunodetection of αSMA appeared to reflect decreased elastin content and areas of smooth muscle cell loss reminiscent of cystic medial degeneration in ABT-510 treated aorta (Fig 3-4).

Conclusions: Our findings suggest that inhibition of adventitial vasa vasorum via anti-angiogenic signaling and a reduction in aortic wall perfusion cause aneurysmal degeneration. Clarifying adventitial-based biomolecular pathways of aneurysm formation could enable development of targeted, pro-regenerative and less-invasive therapies that maintain and/or restore perfusion to the aortic wall and prevent the degenerative sequelae of elastic fragmentation and smooth muscle cell loss.

# Placement of an elastic biohybrid patch on the right ventricle following pulmonary artery banding

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Right ventricular function plays an important role in the prognosis for both congenital heart disease and pulmonary hypertension, as well as in dilated cardiomyopathy and left heart failure. Failure of the right ventricle (RV) has arguably been understudied, in contrast to left ventricular failure. RV dysfunction or failure has been focused on as a strong negative contributor to morbidity and mortality, in fact, the in-hospital mortality of patients with acute heart failure with RV failure ranges from 8 to 17%. There are still minimal direct therapies that remedy the failing right heart. We have previously shown the therapeutic effects of a biodegradable patch incorporating cardiac extracellular matrix (ECM) in a rat model of ischemic cardiomyopathy following myocardial infarction. This cardiac patch includes two important features; ventricle mechanical support and the capacity to deliver a bioactive factor that could influence the cardiac wall remodeling pathways. The biohybrid patch with ECM has shown favorable inhibition of left ventricular (LV) remodeling in terms of macrophage polarization and angiogenesis as well as LV global function and geometry. With these results in LV failure, we hypothesized that epicardial placement of the ECM patch might have therapeutic effects in an RV failure model caused by pressure overload achieved with pulmonary artery banding. The results demonstrate an improvement of RV global function and geometry in the patch group measured with echocardiography and cardiac catheterization. We also demonstrated reduced wall thickness and fibrosis, higher vascular density, and reduced myocyte size in the patch group. In conclusion, introducing the biohybrid ECM patch into an RV failure model with fixed afterload improved hemodynamics and myocardial perfusion and reduced right ventricular fibrosis.

# Injectable ECM-based embolic-releasing therapeutic agents for treating cerebral saccular aneurysms

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Aneurysms are vascular lesions that result from the biomechanical failure of the vessel wall because of hemodynamic stress and inflammation. Aneurysms are at a high risk of rupture resulting in subarachnoid hemorrhage often leading to death or disability. Embolization using a metallic coil (e.g. Platinum) is the current clinical gold standard for saccular aneurysm treatment. But it has limitations including coil compaction, coil migration, and recanalization causing retreatment. On the other hand, injectable embolic agents and hydrogel (e.g. Onyx™, TRUfill™ n-butyl cyanoacrylate, and EmboGel™) are commercialized and showed positive results in the treatment, however, these injectable embolic also showed recanalization after treatment. In this regard, an alternative material induces rapid embolization of the sac of an aneurysm and stimulates tissue ingrowth while the material is absorbed into the body may be necessary for ideal permanent treatment. We suggest that injectable hydrogel which delivers therapeutic agents has a high potential for aneurysm embolization and progressing to in vivo tissue engineering. An extracellular matrix (ECM) based stimuli-responsive injectable gel system which consists of thiolated gelatin (Gel-SH) and vinyl sulfonated hyaluronic acid (HA-VS) was prepared. Their chemical structures were confirmed by proton nuclear magnetic resonance. Contrast agent iohexol and therapeutic agents (Monocyte Chemoattractant Protein-1 (MCP-1) or substance p (SP)) were incorporated in the injectable embolic and their morphology, hemocompatibility, thrombogenicity, cell penetration, and proliferation, von Willebrand factor (VWF) or α-smooth muscle actin (αSMA) expression were evaluated in vitro. After three weeks following the injection into the murine carotid aneurysm model, pro-inflammatory cytokine production assay and immunohistochemistry including H&E staining were performed to evaluate the potential use in cerebral aneurysm treatment. The Gel-SH and HA-VS became a gel after injection through 30-gauge needles at pH 7.4 and 36°C within 30 sec and the gel showed a highly porous structure at scanning electron microscope images. The iohexol-incorporated gel showed thrombogenicity, and rat smooth muscle cell penetration and proliferation. Also, the gel showed better VWF or αSMA expression when SP was incorporated. In the animal study, the injectables demonstrated their potential use in the treatment of aneurysms in terms of successful embolization of the sac with tissue ingrowth.

# Creating and Transferring an Innervated Vascularized Muscle Flap Made from an Elastic, Cellularized Tissue Construct Developed in situ

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Reanimating facial structures following paralysis and muscle loss is a surgical objective that would benefit from improved options for harvesting appropriately sized muscle flaps. The objective of this study was to apply electrohydrodynamic processing to generate a cellularized, elastic, biocomposite scaffold that could develop and mature as muscle in a prepared donor site in vivo and then be transferred as a thin muscle flap with a vascular and neural pedicle. First an effective extracellular matrix (ECM) gel type was selected for the biocomposite scaffold from three types of ECM combined with poly(ester urethane)urea microfibers and evaluated in rat abdominal wall defects. Next, two types of precursor cells (muscle-derived and adipose-derived) were compared in constructs placed in rat hind limb defects for muscle regeneration capacity. Finally, with a construct made from dermal ECM and muscle-derived stem cells, proto-flaps were implanted in one hindlimb for development and then microsurgically transferred as a free flap to the contralateral limb. Stimulated muscle function was confirmed. The implanted, cellularized constructs generated with dermal ECM and skeletal muscle derived stem cells showed strong evidence of muscle regeneration and could be transferred with pedicles to provide functional recovery for defects made in the contralateral limb. This construct generation and in vivo incubation procedure might allow the generation of small-scale muscle flaps appropriate for transfer to the face, offering a new strategy for facial reanimation. Furthermore, this research could serve as a broader basis for tissue engineering of flaps allowing better management of soft tissue reconstruction.

# An Innervated Synovium-Cartilage Chip for Modeling Joint Inflammation and Associated Pain

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Osteoarthritis (OA) is a painful and debilitating disease, as well as the 11th global contributor to disability. However, the correlation between OA and pain is not well understood. To date there are not any disease modifying osteoarthritis drugs that have reached FDA approval leaving pain management to non-steroidal anti-inflammatory drugs or invasive surgeries such as total joint arthroplasty (TJA). Due to the lack of appropriate models to recapitulate the whole-joint disease nature of OA in humans, there is a clinical need to establish safe and effective methods for the treatment OA-associated pain. Here we report the creation of an innervated organ-on-chip model to enable the dynamic interplay between the nervous system and the synovial joint. The objective of this study is to utilize the incorporated neurons to model the relationship between pain and OA in the established Neu-microJoint.

Cartilage tissue constructs treated with IL-1 β showed robust inflammation compared to the negative control follow qRT-PCR. Fluorescence imaging of rodent and human dorsal root ganglion (DRG) neurites showed neurite extension through the microchannels towards a growth factor gradient and fibrous tissue in the absence of a growth factor gradient. Live imaging was used to track neuronal activity and electrical stimulation of the neurites evoked calcium transients in the corresponding soma. Importantly, neurons continue to respond to algogenic stimuli including "synovium fluid" from the “osteoarthritic” microJoint. Furthermore, the macrophage-fibrous tissue constructs showed and increase in tumor necrosis factor $\alpha$ (TNF- $\alpha$) after two days of treatment with INF- $\gamma$ and LPS, indicating the successful polarization of the macrophages into M1 macrophages.

# “Artificial mesenchymal stem cells” fabricated from conditioned media enhance acute patency in silk-based vascular grafts

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Purpose: Coronary artery disease leads to 360,000 deaths annually, primarily due to myocardial infarction/dysfunction downstream of the occluded coronary artery. Restoration of blood flow typically requires a bypass vascular graft, but current clinical standards have high long-term failure rates. Tissue engineered vascular grafts (TEVGs) have been sought as alternatives to current clinical grafts, under the premise of allowing regeneration of the grafts into native tissue. Mesenchymal stem cells have been sought as a source of regenerative factors for tissue engineering applications, including TEVGs. The purpose of this study was to validate the use of “artificial mesenchymal stem cells” (artificial MSCs) in the context of a TEVG. Specifically, artificial MSCs combined poly-lactic co-glycolic acid microparticles (PLGA) with mesenchymal stem cell conditioned media (MSC-CM), to harness the regenerative nature of MSCs in a cell-free silk scaffold TEVG.

Methods: Conditioned media microparticles (CM-MPs) were fabricated by encapsulating MSC-CM in PLGA MPs. For in vivo experiments, CM-MPs and Blank microparticles (Blank-MPs) were loaded in lyogel silk scaffolds (a blend of Bombyx Mori and Antherea Assama silk) and implanted as 1 week and 8 week interpositional grafts in Lewis rats. Explanted grafts were assessed for patency and recruitment of macrophages, endothelial cells (ECs), and smooth muscle cells (SMCs). For in vitro analysis, CM-MPs and Blank-MPs were released over 7 days to assess release kinetics of total protein and vascular endothelial growth factor (VEGF). Their releasates were also assessed for cytotoxicity and ability to promote SMC proliferation.

Results: CM-MP 1 week explants had significantly higher patency rates (9/9 (100%)) compared to Blank-MP 1 week explants (7/14 (50%)) ($p=0.0189$). The Blank-MP explants also had higher numbers of macrophages in both the outside ($p=0.0355$) and inside ($p=0.0222$) regions compared to CM-MPs explants. At 8 weeks, there were no significant difference in patency between CM-MP and Blank-MP explants (56% (5/9) and 40% (2/8) respectively). The CM-MP explants showed both contractile SMCs and ECs within the neotissue layer of the graft. Characterization of the CM-MPs revealed a burst release of protein and VEGF within 6 hours of incubation. Additionally, CM-MPs had no negative effect on cell viability, but also did not have an effect on SMC proliferation. Conversely, CM alone increased proliferation in SMCs ($p<0.0001$).

Conclusions: CM-MPs had positive effects on acute remodeling of the grafts, but lost efficacy in the longer term. CM contained regenerative factors that stimulated vascular cell activity, and these factors can be encapsulated and then released from microparticles. However, further optimization of the fabrication of CM-MPs will be required for better regeneration both in vivo and in vitro.

# Development of a TGFB2 Eluting Tissue Engineered Vascular Graft with Tunable Delayed Release

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It is well known that a prominent source of failure for small diameter vascular grafts is compliance mismatch between the native artery and the graft. Clinically used synthetic and autologous grafts tend to be overly stiff compared to the native vessel. This mismatch alters hemodynamic flow in the vessel and contributes to the development of intimal hyperplasia and eventual graft failure. To combat this failure mode, we are working on continuing development of a tissue engineered vascular graft (TEVGs) that remains compliance matched throughout host integration. Our lab has previously developed a pre-implantation compliance matched vascular graft made from layers of electrospun polycaprolactone (PCL) and gelatin. These compliance matched grafts showed improved acute remodeling and immune response. However, these scaffolds degraded quickly, leading to a hypercompliant environment. Additionally there were issues with cellular infiltration. To minimize this scaffold compliance loss we aim to create a compliance matched biopolymer TEVG that remains compliance matched through the entire host remodeling process by introducing TGFB-2 - a key regulator in vascular smooth muscle cell (VSMC) fate. Precise release of TGFB-2 allows for the of increase cellular infiltration and encourages ECM remodeling to replace the TEVG as it degrades. Therefore, the goal of this specific study is to fabricate Poly(lactic-co-glycolic) acid (PLGA) microparticles (MPs) that exhibit tunable delayed burst release of TGFβ2 and incorporate them into our compliance matched TEVGs.

Through this work we have shown that PLGA microparticles can successfully delay the burst release of TGFβ2 for over 50 days as well as create sustained protein release over 90 days depending on the polymer formulation chosen. This delay is sufficiently long to begin TGFβ2 release before scaffold degradation leads to a hypercompliant environment. As expected, the formulation with the highest LA:GA ratio displayed delayed burst release. As this work continues we hope to increase the efficiency of release, evaluate the release profiles of the MP containing grafts and evaluate VSMC migration and infiltration in vitro.

# Assessing the Macrophage-Directed Remodeling Kinetics of a Peripheral Nerve Matrix Hydrogel

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Peripheral nerve injuries (PNI) are a frequent result of traumatic injury and can cause deficits in sensory or motor function including paralysis. Acute nerve gap injuries (when the nerve is severed) very rarely recover spontaneously and require surgical intervention to restore function. Despite advances in PNI treatment, recovery remains largely unsatisfactory and functional deficits become permanent.

This study investigates the regenerative nature of a decellularized nerve hydrogel (peripheral nerve matrix; PNM) as a therapeutic for gap injury. PNM has demonstrated improved recovery in a nerve gap, however the mechanism responsible for functional returns remains unclear. Macrophage polarization is believed to enhance regeneration in the presence of PNM as alterations to the microenvironment are more conducive to an anti-inflammatory, more pro-regenerative/M2-like phenotype. To understand their contribution to PNM-induced nerve healing, a Macrophage fas-induced apoptosis (MaFIA) mouse model is being used to eliminate macrophages at the time of injury. Firstly, this model was assessed to determine the extent of macrophage elimination, specifically at the systemic level and at the tissue level within injured nerves, to evaluate deplation. Flow cytometry on a peritoneal lavage and CD11b staining on nerve sections demonstrated near complete ablation of macrophages upon depletion and showed limited recovery to the macrophage population 1 week after depletion.

Small gap nerve injuries were assessed histologically, electrophysiologically, and functionally for nerve recovery with PNM and macrophage depletion as factors. This work is ongoing with the purpose to observe if PNM retains its regenerative capacity when macrophages are not present in the acute healing response at 1 and 8 weeks post-injury. Data presented includes the 1 week time point as further experimentation is underway.

# 3D Bioprinting of Functional iPSC Derived Islet Organoids and Human Islets in Hydrogel Constructs

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One of the largest medical problems today is the lack of organs available for transplantation. In the US as of February 2021, over 107,000 people were waiting for a transplant. 3D bioprinting is designed to fight this by printing cell embedded bio-layers that work to recreate a tissue construct. Primarily bioprinting has focused on constructing hard tissues, with less work going towards supplementing soft-tissue environments. This requires bioinks that can act as supports, while maintaining the correct biophysical properties for cells. Another technology used to address organ shortages is induced pluripotent stem cells (iPSCs), which can be patient-specific and differentiated to most tissues. The current aim of this project is combining the advantages of bioprinting and iPSC organoids to form a complex soft-tissue environment. In this study, iPSC islets were bioprinted with the goal of engineering functional pancreatic tissue.

The ideal bioink was determined by optimizing the ink to produce a construct that could achieve a resolution below 1000 μm at printing pressures below 30 kPa. At higher pressures cells experience more shear stress, which negatively affects viability. It was also important that the ink could facilitate transport of oxygen and nutrients. A 3% w/v alginate and 6% w/v methylcellulose bioink crosslinked with 50 mM CaCl2 met those qualifications. The bioink was tested by printing human islets, and culturing for 7 days. Printing did not affect the islet viability, and they also expressed key islet markers. Functionality was observed through Glucose Sensitive Insulin Secretion (GSIS), where islets were exposed to high (16 mM) and low glucose (3 mM), which should prompt an similar pattern of insulin secretion. The islets demonstrated appropriate insulin response, producing a stimulation index (SI) of 3. Having confirmed primary islet function, printing was adapted for iPSCs-derived islets. As seen in human islets, printing didn't affect viability, and the iPSC islets maintained their identity. GSIS demonstrated that iPSC islets retained functionality, with an SI = 5. Importantly, iPSC-derived islets printed under the same conditions as human islets were able to demonstrate comparable insulin secretion.

This work marks the first successful printing of iPSC-derived islets and human islets that maintained functionality after 7 days of culture. Work is ongoing to analyze single-cell RNAseq data of printed iPSC-derived islets and determine the effect printing has on metabolic pathways. This project acts as proof of concept for bioprinting functional islets, with work focused on bioprinting a more detailed pancreatic micro-environment.

# Coupling Electric Field Manipulation at Mesoscale Patterned Collecting Target to Enhance Engineered Heart Valve Fabrication

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Current heart valve scaffold processing technologies do not allow combined control of macro morphology and micro-fiber architecture. Near-field electro-deposition techniques result in a high control of fiber alignment, yet they suffer limitations in fiber diameter and construct porosity. Far-field electro-deposition techniques provide adequate scaffold porosity and thinner fiber diameters in the [0,1÷10]μm, yet they show some restrictions on the control. To overcome these limitations, in this study, we further advance Double Component Deposition (DCD) by manipulating the electrical field of the collecting target using different designs and combinations of materials. Surface mesoscopic grooves were investigated to induce local scaffold anisotropy and large-scale fiber undulation.

Different heart valve DCD collector designs were modeled by changing shape and material proportions. The electric field distribution of the electro-deposition process was investigated in COMSOL Multiphysics®. A micro-grooved cylindrical copper mandrel was used to control scaffold micro-structure under quasi-static deposition conditions. The grooves' width, depth, and wall thickness were set as equal to 50, 100, and 200 μm in different combinations. A poly-(ester urethane) urea solution was electro-deposited onto the mandrel. Scaffold morphological and mechanical properties were characterized by SEM microscopy and biaxial tensile test. The in-silico model simulations allowed to visualize the electrical field distribution at the macro and the micro-scale. For the grooved pattern, the width was the most influential parameter in terms of its capacity to induce statistically significant levels of circumferential fiber alignment and mechanical anisotropy.

This seminal study introduces a novel approach to heart valve collecting target design in electro-deposition techniques that aims to advance biomimetics in heart valve engineering. Both experimental observations and numerical models revealed the likely mechanism of fiber deposition associated with local anisotropy at the tissue scale and with fiber network alignment ad orientation at the organ-level scale.

# Histologic Comparison of Abdominally vs Vaginally Implanted Polypropylene Mesh in the Nonhuman Primate

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Polypropylene mesh is a common surgical material used in both hernia and Pelvic Organ Prolapse (POP) repair. Mesh complications occur in up to 20% of POP patients, most commonly mesh exposure through the vagina and pain. In contrast complications are observed in < 5% of hernia repairs. We aimed to perform a histomorphologic analysis of samples from POP reconstructive surgery and abdominal implantation in non-human primates.

Rhesus Macaques were implanted with mesh on the vagina via sacrocolpopexy (N =10) and onto the abdominal wall (N =8) during the same surgery, with explantation of the mesh tissue complex at 90 days. H&E and Masson's trichrome staining were performed on to analyze cellular density and collagen deposition at the site of implantation. CD31 labeling was performed to assess blood vessel formation. And Macrophage labelling using CD68 was used to observe macrophage response at site of implantation. Results were compared across the two implantation sites by t-test put in statistical tests. Animals were 11.73 ± 2.35 years of age, 9.18 ± 1.24 (lbs) weight, and 4 ± 3 parous. There were no significant differences in the number of cells per um between the vagina and abdominal sites. We observed increased collagen deposition in the vagina as compared to the abdomen ($p=0.0003$). Staining of CD31 showed increased area of vessels in abdomen ($p=0.0421$) surrounding the area of implantation as well as increased average perimeter of vessels ($p=0.0002$).

Our data comparing abdominal to vaginally implanted mesh showed increased angiogenesis in and decreased collagen encapsulation of mesh in the abdomen relative to the vagina despite comparable cellularity. Additional studies phenotyping the cellular response to mesh in these tissue locations are ongoing.