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Poster 1: Role of stiffness of aligned fibers in regulation of human mesenchymal stem cell differentiation

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Presented by: Huihua Yuan

Stiffness of biomaterial substrates plays a critical role in regulating cell behavior. While electrospun fibers have been extensively used for tissue engineering, the variable of stiffness of an electrospun fibrous scaffold on regulation of mesenchymal stem cell (MSC) activities has not been fully investigated. In this study, we simply used an annealing approach to alter stiffness of an aligned fibrous substrate without changing the material chemistry. With this unique system, we found that annealing treatment did not change the diameter of electrospun fibers but increased stiffness of aligned fibrous substrates due to increased polymer crystallinity. Larger stress fibers were formed in hMSCs cultured on stiffer substrates than those in the cells on control substrates. Proliferation of hMSCs was increased with substrate stiffness. The mRNA expression of CBFA1 was upregulated while SCX and SOX9 was downregulated with an increase in substrate stiffness, suggesting that increased stiffness favorably drives hMSCs into the osteogenic lineage. Our results also suggest that osteogenic induction of hMSCs directed by stiffness of a substrate is through activation of the AKT/ YAP/RUNX2 pathway.

Poster 2: Hydrogels loaded concurrently with mesenchymal stromal/stem cells and antimicrobials promote cutaneous wound healing and decrease Staphylococcus aureus bioburden

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Presented by Alberto Guerra

Mesenchymal stromal/stem cells (MSCs) have demonstrated favorable wound healing properties in addition to their differentiation potential including the secretion of an anti-inflammatory cytokine profile and the promotion of angiogenesis via expression of growth factors in pre-clinical models. MSCs encapsulated in poly(ethylene glycol)-diacrylate (PEGdA) and thiolated gelatin poly(ethylene glycol) (Gel-PEG-Cys) crosslinked composite hydrogels demonstrate a favorable wound healing phenotype and have led to controlled cellular presentation at wound sites. However, the therapeutic potential of MSC-loaded hydrogels may be limited by non-specific protein adsorption on the delivery matrix that could facilitate the initial adhesion of micoorganisms, leading to gene expression changes that result in toxin production, biofilm formation, and eventually device failure and patient morbidity. In this study, we developed hydrogels loaded with MSCs and two different drug combinations to include minocycline, hamamelitannin, vancomycin, and linezolid to prevent Staphylococcus aureus (SA) biofilm formation and to preserve the wound healing potential of MSC-loaded hydrogels in vivo. Hydrogels loaded with MSCs and antimicrobial combinations significantly reduced SA colony forming abilities in vitro and in wound beds in vivo, while significantly increasing wound re-epithelialization; and hydrogels concurrently loaded with MSCs, minocycline, vancomycin, and linezolid significantly increased wound closure in vivo compared to hydrogels loaded without antimicrobials. MSC and antimicrobial-loaded hydrogels hold the potential as an accelerated wound healing and wound infection prevention therapeutic.

Poster 3: High-throughput evaluation of combinatorial ECM effects on the differentiation of BMEL cells

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Presented by Andreas Kourouklis

ECM demonstrates a vast variety of biochemical and biophysical cues to control stem cell differentiation. Previous approaches have examined cell-ECM interactions with in vitro ECM models that mainly control one type of biophysical or biochemical signal. Our method presents a powerful high-throughput platform to fabricate in vitro ECM models that can evaluate the combinatorial effects of multiple biochemical and biophysical cues of the ECM on cell differentiation. Using this method, we found a special role of collagen-4 on the differentiation of bipotential mouse liver embryonic cells, which it was shown to be associated with the force that cells transmit on the extracellular substrate.

Poster 4: Synthesis and physiochemical characterization of novel fluorinated polymeric amphiphiles

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Presented by Michelle Fleetwood

With the reported environmental ubiquity and toxicity of fluorous surfactants, such as perfluorooctanoic acid, fluorocarbons as a whole have seen increased scrutiny. Alternative fluorocarbons, containing short pendant fluoroethers, have been explored to possibly minimize the potential for toxicity and environmental accumulation. This reported research focuses on the development of branched block copolymers, containing short, pendant perfluoroethers, for the encapsulation of hydrophobic pharmaceuticals and emulsification of volatile anesthetics.

Poster 5: Generation of cardiac cell lineages from human pluripotent stem cells by modulation of WNT signaling via small molecules

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Presented by Xiaoping Bao

Human pluripotent stem cell (hPSC)-derived cardiomyocytes, endothelial cells and their progenitors are important tools for cardiovascular research and have significant therapeutic potentials. During the past 10 years, rapid methodological developments for creating de novo cardiac lineages have been made. However, most of these approaches require animal cells, fetal bovine serum, or various cytokines, limiting their application for large-scale cardiac lineage production for therapeutic applications. Here, we show that temporal Wnt signaling modulation is both necessary and sufficient for cardiomyocyte and endothelial cell specification from hPSCs under chemically-defined, albumin-free conditions. Firstly, we systematically optimized our previous GiWi protocol for cardiac differentiation, and found that RPMI basal medium alone is sufficient to support hPSC differentiation to cardiomyocytes with a purity of more than 90%. In addition, we developed a robust endothelial progenitor differentiation platform that uses a minimalistic medium, consisting of only DMEM and ascorbic acid, via modulation of canonical Wnt signaling pathway. These two fully defined platforms should facilitate large-scale production of xeno-free cardiomyocytes and endothelial cells respectively for both research and clinical applications.

Poster 6: Chemically defined substrates for controlled self-assembly of stem cell aggregates

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Presented by: Angela Xie

Recent studies have demonstrated the self-assembly of specified tissue stem/progenitor cells into organoids resembling native organs. However, the low reproducibility of organoid formation in the presence of ill-defined natural materials (e.g., Matrigel) limits our understanding of fundamental mechanisms in organoid assembly, and hinders progress toward drug screening and disease modeling applications. Here we used chemically defined substrates composed of self-assembled monolayers (SAMs) to exert spatiotemporal control over a human embryonic stem cell (hESC) aggregate self-assembly process. hESCs were cultured on mixed SAMs of COOH- and OH-terminated alkanethiols adsorbed in various patterns onto gold surfaces. Cyclic RGDfK and RGDfC (cycRGD) peptides were coupled to COOH groups using carbodiimide chemistry to form non-labile amide linkages and labile thioester linkages, respectively, between peptide side chains and SAMs. We found that substrates presenting cycRGD via a non-labile amide bond allowed for two-dimensional culture of human embryonic stem cells (hESCs) over extended timeframes, similar to standard hESC culture on Matrigel. In contrast, on substrates presenting cycRGD via a labile thioester bond, patterned hESC populations underwent a reproducible self-assembly process to form three-dimensional cell aggregates. Varying the geometry of the spatial pattern led to control over the three-dimensional characteristics of cell aggregation. We observed self-assembly with hESCs, human induced pluripotent stem cells, and their differentiated derivatives, supporting applicability to a variety of cell types. Unlike traditional methods for forming stem cell aggregates, this self-assembly process occurs in the absence of exogenous cues or physical manipulation, while still enabling the high-throughput generation of aggregates of tunable size and shape. We anticipate that the tunability and reproducibility of this method will allow for critical improvements upon current methods for organoid formation.

Poster 7: Multi-transgenic human stem cells permit live visualization of cytomechanical & intranuclear dynamics

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Presented by Ryan Prestil

During differentiation and reprogramming, gene expression changes dramatically along with physical changes to the shape, size, and organization of both the nucleus and the cell body. Nuclear lamina-associated domains (LADs) have been implicated as an important repressor of gene expression via epigenetic interactions with chromatin, but the mechanisms remain poorly understood. In order to facilitate research in this area, we have created a new multi-transgenic line of human induced pluripotent stem cells (hiPSCs) known as "iHLB" combining doxycycline-inducible reprogramming factors (Oct4/Sox2/Klf4/c-Myc) with constitutively expressed Histone H2B-mCherry, LifeAct-GFP, and dCas9-BFP.

The iHLB line permits simultaneous live imaging of nuclear shape and fluidity, cytoskeletal structure and remodeling, and the location and movement of targeted genomic loci in order to provide cheaper, faster, and more detailed profiling of differentiation and reprogramming. Preliminary experiments have demonstrated that actin is necessary to physically transduce extracellular stresses to deform the nucleus, and both the soluble fraction of histones and the overall movement of DNA decrease as the nucleus is elongated and constricted.

iHLB cells present new opportunities to study the real-time mechanics of differentiation and reprogramming as well as the complex ways cells interact with their environment. Further studies will correlate gene location with gene expression and provide insight into the role of LADs on human cell fate determination. A rich portrait of cell states and transitions is beginning to take shape, and the physical signaling mechanisms involved in human development—and how they may be manipulated and engineered in vitro—is being educed in unprecedented detail.

Poster 8: Synthetic hydrogel substrates for xeno-free, ROCK inhibitor-independent human pluripotent stem cell culture

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Presented by Nhi Le

Recent studies suggest substrates that facilitate increased intracellular cytoskeletal tension also promote human pluripotent stem cell (hPSC) self-renewal. These insights have been used to develop chemically-defined substrates for cell culture; however, current chemically-defined substrates are only suitable for hPSC culture in media containing animal proteins and Rho- associated protein kinase (ROCK) inhibitor. Reliance on animal proteins limits clinical translatability and studies suggest that routine dependence on ROCK inhibitor can limit differentiation potential. Here, we developed synthetic substrates capable of promoting hPSC attachment, proliferation, and pluripotency maintenance in fully-defined, xeno-free, ROCK inhibitor-independent media. We utilized a hydrogel array-based platform to systematically and independently control substrate stiffness, peptide identity, and peptide density to examine the combinatorial and synergistic effects of these properties on hPSC behavior. With the enhanced throughput capabilities of the array platform, we also examined the influence of cell seeding parameters (seeding density and colony vs. single-cell seeding) and seeding and maintenance media (with or without ROCK inhibitor) to identify the first chemically-defined synthetic hydrogel substrates that promote hPSC expansion in xeno-free, ROCK inhibitor-independent media. Results suggest that substrates presenting peptides with higher integrin-binding affinity increased total number of cells attached and substrates with increased peptide density facilitated faster initial cell attachment and enhanced survival. Additionally, substrate stiffness and peptide density synergistically promoted increased intracellular cytoskeletal tension necessary for stable cell adhesion and pluripotency maintenance over the course of 10 days.

Poster 9: Intravitreal drug delivery using targeted nanoparticles protects retinal ganglion neurons

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Presented by: Lian-Wang Guo

Retinal ganglion cell death is a characteristic of glaucoma. Effective pharmacological treatment of glaucoma has yet to be established. Unimolecular nanoparticles (NPs) afford an ideal drug delivery platform because of high drug loading capacity, prolonged release, and versatility of bioconjugations. We conjugated the cholera toxin B domain (CTB) onto NPs for retinal ganglion cell (RGC) targeting, and tested the effect of DHEA-loaded CTB-NPs on protection of RGCs treated with intravitreally injected NMDA. In control eyes injected with non-targeted DHEA-loaded NPs, NMDA induced ~50% cell loss in the RGC layer 14 days after injection; whereas in the eyes injected with targeted DHEA-loaded CTB-NPs only ~10% cell loss was observed. Moreover, more cells in the RGC layer were preserved in the eyes injected with DHEA-loaded CTB-NPs compared to control eyes injected with free DHEA. Thus, targeted CTB-NPs provide a promising drug-delivery platform for prevention of retinal ganglion cell degeneration.

Poster 10: Enhancing 14 helical -peptides antifungal activity by targeting C. albicans biofilm formation using small-molecules

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Presented by Angelica Rodriguez

Background: *Candida albicans* is an opportunistic pathogen that causes superficial and invasive infections in humans. The principal source of these infections is often associated with structured microbial communities, known as biofilms, which are resistant to common antimicrobial therapies. In prior work we developed a class of antifungal β-peptides, structurally modeled from natural a-helical antimicrobial peptides, that kill selectively *C. albicans* planktonic cells and prevent biofilm formation. However, these β-peptides show toxicity to host cells at the concentrations needed to kill *C. albicans* in biofilms. Therefore, it is important to develop selective anti-biofilm strategies. Disrupting biofilm formation in combination with cytotoxic β-peptides may overcome the resistance of biofilms to antimicrobial therapies. Several groups have shown the potential of using small molecule morphological effectors, quorum sensing agents (QS-agent) and dispersal enzymes to disrupt biofilm formation. Here, we investigate whether targeting biofilm formation, using small-molecules in combination with β-peptides, will disrupt *C. albicans* in biofilms *in vitro* without compromising β-peptides selectivity towards microbial cells.
Methods and Results: Extracellular addition of dodecanol, isoamyl alcohol and QS-agent farnesol block the yeast-to-hyphae transition characteristic of *C. albicans* biofilm formation without causing an effect on the cell viability. Susceptibility testing, using a checkerboard assay, confirmed that disruption agents enhance the β-peptides cytotoxic effect on the prevention of *C. albicans* biofilm formation. In addition, biocompatibility results show that disruption agents studied were non-hemolytic, at the concentrations used in this study, and all synergistic combinations have a low percentage of hemolysis (10%). However, although these disruption agents enhance β-peptides cytotoxic effect for biofilm prevention, no synergistic effect was observed for *C. albicans* in biofilms. Finally, the disruption of *C. albicans* ECM matrix with β-glucanase resulted in the dispersal of *C. albicans* biofilms without causing significant *C. albicans* cell death. These dispersed biofilms show to be susceptible to β-peptide treatment at 4-fold lower concentrations of β-peptide compared to the β-peptide treatment alone.
Conclusions and Remarks: Our experiments revealed that small molecule disruptors of biofilms enhance β- peptide effects on the prevention of biofilm formation in a selective manner. The addition of β-glucanase to disperse biofilms also enhance the β-peptide effect against pre-formed biofilms. Further studies will include a mechanistic analysis to study the effect of biofilm dispersal and β-peptide in *C. albicans* morphological regulation and virulence in vitro.

Poster 11: Multifunctional unimolecular micelles loaded with the anti-cancer drug aminoflavone for targeted triple negative breast cancer therapy

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Presented by Guojun Chen

Triple negative breast cancer (TNBC) is an aggressive subtype of breast cancer for which there is no available targeted therapy. TNBC cases contribute disproportionately to breast cancer-related mortality, thus, the need for novel and effective therapeutic methods is urgent. Previous studies identified aminoflavone (AF) as a growth inhibitory agent in TNBC in vitro models. However, in vivo studies and human clinical trials revealed that AF exhibited dose-limiting pulmonary toxicity. Because it is well established that nanoparticle (NP)-based drug delivery systems can be used to reduce systemic toxicity of therapeutics, we engineered unimolecular micelle NPs loaded with AF and conjugated with GE11, an EGFR-binding peptide, due to the high frequency of EGFR amplification in TNBC tumors. These NPs were stable, uniform, and preferentially released drug payload at endosomal pH levels rather than blood pH levels. Use of the GE11 targeting peptide resulted in enhanced cellular uptake and growth inhibitory effects in TNBC cells. Further, intravenous injection of AF-loaded, GE11-conjugated (targeted) unimolecular micelle NPs to nude mice bearing orthotopic TNBC tumors reduced tumor volume in a statistically significant manner compared to mice treated with AF-loaded, GE11-lacking (non-targeted) unimolecular micelle NPs or free AF. Processed plasma collected from mice treated with targeted NPs was found to contain the highest concentrations of AF, yet tissues from these animals were not significantly different at the time of sacrifice. Together, these results offer support for the use of AF-loaded and EGFR-targeted unimolecular micelle NPs as an effective therapeutic option for EGFR-overexpressing TNBC.

Poster 12: High-throughput screening format identifies synthetic mimics of Matrigel for angiogenesis assays

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Presented by Eric Nguyen

Matrigel is a commonly used material for testing the effects of chemical compounds on endothelial cell tubulogenesis (1), but it is a complex, poorly characterized and composed of >1800 individual proteins. (2). We hypothesize that synthetic materials that recapitulate ECM properties critical to the induction of angiogenesis can be a suitable substitute for Matrigel in toxicity screens. Here we used a high-throughput screening assay to determine chemically defined synthetic hydrogel formulations that permit Human Umbilical Vein Endothelial Cell (HUVEC) tubule formation to occur similar to Matrigel and tested its efficacy in evaluating the effects of a library of known angiogenesis inhibitory compounds on tubulogenesis.

The hydrogel screening format used here identified one culture environment that induced endothelial cell tubulogenesis over 24 hours and maintained structural stability over 48 hours. Comparison of the optimal PEG culture system to Matrigel showed that the PEG system demonstrated increased repeatability, increased sensitivity to vascular inhibitors and the ability to observe inhibitory mechanisms not observable on Matrigel. We performed the tubule formation assay on a PEG hydrogel containing a VEGF binding peptide and demonstrated that VEGF binding and sequestration to the hydrogel nullified the effects of VEGF inhibitors on tubulogenesis, suggesting a mechanism behind decreased sensitivity of HUVECs plated on Matrigel.

Our screening format identified synthetic hydrogels that are amenable to screening bioactive chemical compounds via tubulogenesis quantification. Further studies will demonstrate the efficacy of the synthetic hydrogels to quantify the effects of 53 chemicals of the ToxCast Library on HUVEC tubulogenesis.

References:

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Poster 13: Nanogel-deferoxamine conjugate: a biodegradable nanomedicine for iron chelation therapy

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Presented by Shaurya Chanana

Deferoxamine (DFO) is one of the most commonly used chelation therapies in current treatment for iron-overload diseases. However, its fast elimination from blood circulation and various side effects have caused considerable patient compliance issues. We are developing a formulation in which DFO molecules are conjugated to biodegradable nanogels, in the hopes of attenuating its toxicity and extending the circulation time in the body. We have successfully fabricated the nanogel scaffold by reverse - emulsion polymerization technique yielding different sized nanogels. Each formulation has been characterized by Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM) and zeta-potential measurements. The level of DFO conjugation has also been determined using UV-Vis spectroscopy and Atomic Absorption Spectroscopy (AAS). The capability of hNG - DFO to chelate iron has furthermore been demonstrated by a ferritin co-incubation assay. The in-vitro cytotoxicity of hNG - DFO was also tested on two separate cell lines and shows promise. Further plans to determine PK parameters are underway.

Poster 14: Oxidation responsive polymeric micelles for iron chelation

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Presented by Deepa Acharya

Patients with sickle cell anemia and β-thalassemia require chronic blood transfusions. Since the body has no innate mechanism to eliminate the excess iron that comes with the transfusion, it tends to accumulate in its free form, which then causes toxicity to vital organs. This disorder is known as transfusional iron overload. Currently there are very limited therapies available and these either have issues regarding compatibility and/or adverse effects.

As a more efficient alternative therapy, we have designed a stimuli responsive diblock copolymer that can form micelles to chelate the excess iron from the body. We have engineered the micelle such that it can effectively pick up free iron and also undergo oxidation, following which the micelles disassemble into constituent polymer chains and can be renally cleared. The design using poly hydroxamic acid as the chelating moiety has the disadvantage of forming precipitates after it binds to iron. However, using Deferroxamine (DFO) can alleviate this problem since each molecule of DFO binds very strongly to one molecule of iron. In addition to this, future version of this polymer would incorporate a biodegradable backbone, which would make our polymeric chelating micelle a viable marketable option for iron chelation therapy.