

POSTER SESSION: 10th Annual Wisconsin Stem Cell Symposium
Engineering Limb Regeneration:
Recapitulating Normal Development and Regeneration?
April 22, 2015 – Madison, WI

NOTE: Each submitter's name is in bold and italicized.

POSTER CONTEST FINALISTS

(1) Endothelial Sprouting Arrays for Screening Vascular Disrupting Compounds

David G. Belair¹, Michael P. Schwartz¹, William L. Murphy^{1, 2, 3}

¹ Department of Biomedical Engineering, University of Wisconsin-Madison

² Material Science Program, University of Wisconsin-Madison

³ Department of Orthopedics and Rehabilitation, University of Wisconsin-Madison

belair@wisc.edu

The large quantity of known environmental and synthetic compounds calls for a robust screening assay to identify potentially toxic compounds. Many of these compounds have been characterized as putative vascular disruption compounds (pVDCs) using assays of human vasculature in a dish. However, current assay systems typically measure two-dimensional (2D) endothelial tubule formation as a measure of vascular disruption. These assays are often performed on ECM-mimicking materials such as Matrigel, which is composed of hundreds of unique proteins and exhibits lot-to-lot variability that may reduce reproducibility. Recently, we have observed endothelial tubulogenesis and sprouting in assay platforms comprising well-defined, synthetic hydrogels. Here we report the analysis of de novo tubule network formation and EC sprouting using a high throughput, multivariate approach. Human induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) were encapsulated in a synthetic hydrogel composed of poly(ethylene glycol) (PEG) and tethered cell attachment and cell-degradable crosslinking peptides. iPSC-ECs exhibited invasion that was correlated with the concentration of cell adhesion peptides and degradable peptide crosslinks. Immunocytochemistry of encapsulated cells demonstrated that iPSC-ECs expressed CD31 and CD144 and exhibited VEGF-dependent sprouting behavior consistent with endothelial cell sprouting. iPSC-EC invasion was potently inhibited by Sunitinib, SU5416, and Vatalanib (VEGFR2 inhibitors), Withaferin A, Thalidomide, SB-3CT (MMP inhibitor), Nilotinib (kinase inhibitor), and Combretastatin A4 (β -tubulin inhibitor). We further identified inhibitors that were toxic to ECs, which highlights the importance of a multivariate screening approach for measuring cell response to pharmacologic agents. The screening approaches described here are capable of quantitating single-cell invasion, sprout length, survival, and tubule network formation simultaneously and thus can efficiently distinguish the particular influence of pVDCs on multiple EC functions.

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(2) Deterministic *HOX* Patterning in Human Pluripotent Stem Cell-derived Neuroectoderm

Ethan S. Lippmann^{1,2}, Clay E. Williams^{3,4}, Maria C. Estevez-Silva^{1,2}, Joshua J. Coon³⁻⁵, and Randolph S. Ashton^{1,2}

¹Department of Biomedical Engineering, ²Wisconsin Institute for Discovery, ³Genome Center of Wisconsin,

⁴Department of Biomolecular Chemistry, and ⁵Department of Chemistry, University of Wisconsin-Madison, Madison, WI.

elippmann@wisc.edu

Colinear activation of *HOX* genes is a fundamental process that conveys positional information within many developing tissue structures, including the posterior central nervous system (CNS) consisting of the hindbrain and spinal cord. *HOX* expression patterns direct many significant events in posterior CNS development, including neural circuit organization and motor neuron innervation choreography. As such, the ability to precisely regulate *HOX* expression in human pluripotent stem cell (hPSC)-derived neural cells could be a significant contribution to disease modeling applications and a potential future

avenue for regenerative medicine. However, previous attempts at *HOX* patterning in PSC-derived neural cells have only resulted in coarse, heterogeneous *HOX* expression spanning multiple domains that would not overlap *in vivo*. To address this issue, we utilized a completely defined neural differentiation scheme to interrogate the explicit roles of soluble factor combinations in regulating *HOX* expression. Temporal activation of Wnt/ β -catenin and FGF signaling was sufficient to establish a highly pure Sox2⁺/Brachyury⁺ neuromesodermal state that mimics the axial progenitors which differentiate to posterior neuroectoderm *in vivo*, and activation of these two signaling pathways was required for colinear activation of *HOX1-9* paralogs, which constitute the hindbrain, cervical, and thoracic spinal cord. Activation of GDF signaling was then required to induce expression of *HOX10-12* lumbosacral paralogs. At any point during neuromesodermal propagation, the addition of RA transitioned the cultures to highly pure Sox2⁺/Pax6⁺ neuroectoderm and halted *HOX* progression, thus producing neuroectoderm with highly pure Hox profiles characteristic of distinct regions in the hindbrain and cervical, thoracic, and lumbosacral spinal cord. Region-specific phenotypes were observed when the neuroectoderm was differentiated to motor neurons, suggesting possible utility for understanding why different populations of motor neurons exhibit differential susceptibility to diseases such as amyotrophic lateral sclerosis (ALS). Future studies will examine how different *HOX* profiles regulate molecular diversity in neuroectoderm and its progeny.

(3) The Lizard Spinal Cord Induces Tail Regeneration and Enhances Healing in Otherwise Non-Regenerative Tissues via Hedgehog Signaling

Thomas P. Lozito, Rocky S. Tuan

Center for Cellular and Molecular Engineering, University of Pittsburgh, Pittsburgh, PA, USA

tomlozito@gmail.com

Lizards exhibit the remarkable ability to regenerate amputated tails, making them the closest relatives of mammals to display enhanced healing abilities as adults. Unlike salamanders, lizards are unable to regrow lost limbs, distinguishing lizards as the only vertebrates to combine regenerative tissues (i.e. tail) and non-regenerative tissues (i.e. limbs) in the same animal. These attributes specify lizards as extremely relevant model organisms for studying and manipulating adult regeneration. We have previously identified the lizard spinal cord as the critical source of signals responsible for patterning regenerated tails of Anolis lizards. In this study, we hypothesize that (1) lizard tail regeneration is induced by signals originating from the spinal cord, and (2) transplanting spinal cord pieces into amputated lizard limbs will improve healing. Towards investigating the regeneration-inductive activities of the lizard spinal cord, spinal cord pieces were removed and/or added to the stumps of amputated lizard tails. Spinal cord removal resulted in the complete loss of regeneration in amputated lizard tails, and transplantation of exogenous spinal cord pieces to tail stumps lacking endogenous spinal cords restored tail regeneration. Similarly, exogenous spinal cord pieces implanted as autografts within dorsal muscles of original tail portions induced normally structured ectopic regenerates at implantation sites, resulting in multi-pronged “forked” tails. Finally, exogenous spinal cord pieces implanted into the normally non-regenerative amputated hind limbs of lizards induced enhanced, yet tail-like, regenerates. Formation of ectopic tails was significantly diminished by co-treatment with the hedgehog inhibitor cyclopamine. In conclusion, this study has identified the lizard spinal cord as necessary and sufficient for inducing regeneration in a process involving hedgehog signaling. Furthermore, the signals produced by the lizard spinal cord are remarkably robust, capable of inducing regrowth in otherwise non-regenerative tissues. Future studies will test the abilities of lizard spinal cord-derived signals to enhance healing in mammals.

(4) A Chaperone, α B-crystallin, in Maintenance and Regeneration of Skeletal Muscle using Patient-Derived iPSCs

Katie Mitzelfelt^{1,2}, Patraranee Limphong², Qiang Dai¹, Melinda Choi¹, Michael Riedel¹, Shuping Lai¹, Elisabeth Christians², Elizabeth Wraige³, Michael Grzybowski¹, Aron Geurts¹, Ivor Benjamin^{1,2}

1 Department of Medicine, Medical College of Wisconsin, Milwaukee, WI

2 Department of Biochemistry, University of Utah, Salt Lake City, UT

3 Evelina Children's Hospital, London, UK

kmitzelfelt@mcw.edu

Introductions: The CRYAB gene encodes α B-Crystallin, a bona fide heat shock protein and molecular chaperone, with expression in skeletal muscle beginning in the cycling myoblasts and increasing with maturation. α B-Crystallin is linked to proper regeneration of skeletal muscle through effects on temporal MyoD expression, cell cycle exit, and resistance to differentiation-induced apoptosis. Several known human mutations in CRYAB cause multisystem disorders including cataracts, cardiomyopathy, and/or myofibrillar myopathy. This study focuses on one of these mutations, homozygous recessive 343delT, which has been reported to cause severe myofibrillar myopathy without a cardiomyopathy in a child, with the goals to model disease and potentially draw larger conclusions about the requirements for α B-crystallin in maintenance and regeneration of skeletal muscle.

Methods: Overexpression studies were used to analyze mutant expression and stability. Patient-derived dermal fibroblasts have been reprogrammed into fully characterized induced pluripotent stem cells (iPSCs). Heterozygous 343delT control cells were generated through genetic correction in patient iPSCs using zinc-finger nuclease-stimulated homologous recombination. iPSCs are differentiated to myogenic progenitors and myotubes using the published EZ-sphere protocol.

Results: Overexpression of 343delT results in co-aggregation of insoluble 343delT α B-crystallin with desmin and inducible HSP70, indicating potential gain of toxic function effects of the mutant protein. 343delT is degraded by both the proteasome and autophagy. 343delT protein is undetectable in patient iPSC-derived myotubes when WT α B-crystallin is present in control cells, though mRNA levels and half-life appear normal. The recessive nature of the mutation and the lack of mutant protein expression are suggestive that a loss of α B-crystallin function may be the trigger for disease initiation, with manifestation potentially occurring in the skeletal progenitor cell, as suggested by the apparent tissue specificity of this mutant and literature evidence for the requirement of α B-crystallin in proper skeletal muscle regeneration. We are beginning this investigation in iPSC-derived myogenic progenitor cells.

Conclusions: We think studies such as 343delT α B-crystallin-induced myofibrillar myopathy will expand our understanding of α B-crystallin in normal physiology including tissue maintenance and regeneration.

(5) Delivery and Endogenous Recruitment of Circulating Angiogenic Cells

Matthew Parlato^a, William L. Murphy^{a,b,c}

^aDept. of Biomedical Engineering, ^bMaterials Science Program, ^cDept. of Orthopedics and Rehabilitation, University of Wisconsin-Madison

mattparlato@gmail.com

A significant challenge facing tissue engineering and regenerative medicine is the formation of functional vasculature in vivo, and many emerging approaches harness patient-derived cell types to stimulate angiogenesis. Circulating angiogenic cells (CACs) are a particularly promising cell type to stimulate angiogenesis because of their well-characterized roles in vascular biology ranging from blood vessel maintenance, formation of new blood vessels, and repair of damaged blood vessels. Therapeutic strategies using CACs focus on either CAC delivery or endogenous CAC recruitment. CAC delivery involves CAC isolation, ex vivo culture, and re-delivery to a patient while CAC recruitment focuses on the delivery of soluble factors that encourage endogenous CAC homing and chemotaxis in vivo. Both recruitment and delivery strategies demonstrate efficacy in animal models, which suggests that they are

both promising strategies for clinical translation. We developed an adaptable, enhanced-throughput hydrogel array platform to investigate the effects of poly(ethylene glycol)-based hydrogel parameters on CAC behavior. With this approach, we aim to establish fundamental design rules for hydrogel biomaterials utilized in both CAC delivery and CAC recruitment approaches. Our initial results suggest that hydrogel stiffness strongly influences CAC behavior. Soft hydrogels (shear modulus of 2 kPa) promote CAC invasion, and soft hydrogels also promote CAC adhesion, proliferation, and viability at levels above that of tissue culture polystyrene. In our future studies, we plan to investigate other hydrogel parameters including integrin-binding peptide identity and concentration, as well as hydrogel degradability.

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(6) Drug-loaded Nanoparticles Induce Gene Expression in Human Pluripotent Stem Cell Derivatives

Virendra Gajbhiye^{†a}, Leah Escalante^{†a}, Guojun Chen^b, Alex Laperle^a, Qifeng Zheng^b, **Benjamin Steyer^a**, Shaoqin Gong^{*a,b}, Krishanu Saha^{*a}

^aDepartment of Biomedical Engineering and Wisconsin Institutes for Discovery, University of Wisconsin-Madison, Madison, WI

^bMaterial Science Program and Wisconsin Institutes for Discovery, University of Wisconsin-Madison, Madison, WI
bsteyer@wisc.edu

Tissue engineering and advanced manufacturing of human stem cells requires a suite of tools to control gene expression spatiotemporally in culture. Inducible gene expression systems offer cell-extrinsic control, typically through addition of small molecules, but small molecule inducers typically contain few functional groups for further chemical modification. Doxycycline (DXC), a potent small molecule inducer of tetracycline (Tet) transgene systems, was conjugated to a hyperbranched dendritic polymer (Boltorn H40) and subsequently reacted with polyethylene glycol (PEG). The resulting PEG-H40-DXC nanoparticle exhibited pH-sensitive drug release behavior and successfully controlled gene expression in stem-cell-derived fibroblasts with a Tet-On system. While free DXC inhibited fibroblast proliferation and matrix metalloproteinase (MMP) activity, PEG-H40-DXC nanoparticles maintained higher fibroblast proliferation levels and more MMP activity. The results demonstrate that the PEG-H40-DXC nanoparticle system provides an effective tool to controlling gene expression in human stem cell derivatives.

(7) Engineered Substrates for Controllable Generation of Self-assembled Stem Cell Aggregates

Angela W. Xie (1); Samantha K. Schmitt (2); William L. Murphy (1,2,3,4)

University of Wisconsin-Madison, Madison, WI

Department of Biomedical Engineering (1); Materials Science Program (2);

Department of Orthopedics and Rehabilitation (3); Stem Cell and Regenerative Medicine Center (4)

awxie@wisc.edu

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. Modulating cycRGD density and Rho kinase signaling changed self-assembly kinetics, suggesting that cellular adhesion and contractility are critical to this process. 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.

(8) Characterization of Mesenchymal Stem Cells Isolated from Bone Epiphysis and Diaphysis

Yaxian Zhou^{1,2}, Tsung-Lin Tsai^{1,2}, Matthew Squire¹, and Wan-Ju Li^{1,2}

¹Department of Orthopedics and Rehabilitation

²Department of Biomedical Engineering, University of Wisconsin-Madison, Madison, Wisconsin, USA

zhou263@wisc.edu

Stem cell fate is largely determined by their microenvironment. It has been reported that human mesenchymal stem cells (hMSCs) in red and yellow bone marrow at different anatomic sites of long bone behave differently. In this study, hMSCs isolated from bone epiphysis (red marrow) or diaphysis (yellow marrow) of the same patients were cultured and analyzed for cell proliferation, multilineage differentiation and expression of MSC surface markers. Our results showed that hMSCs from bone diaphysis proliferated more rapidly than those from bone epiphysis while no significant difference in cell morphology was observed between hMSCs from different bone locations. Bone diaphysis-isolated hMSCs showed greater ALP activity and produced more calcium deposit than bone epiphysis-isolated hMSCs. Larger amounts of lipid droplets and GAG production were also shown in hMSCs from bone diaphysis than those from bone epiphysis. In addition, the results of flow cytometry analysis showed that bone diaphysis contained more CD73⁺/90⁺/105⁺/45⁻ cells than bone epiphysis. This cell population was then sorted out from hMSCs of bone epiphysis and diaphysis, cultured, and analyzed for cell proliferation as well as multilineage differentiation. Our results showed that the CD73⁺/90⁺/105⁺/45⁻ cell population from bone diaphysis was pro-adipogenic compared to that from bone epiphysis whereas there was no significant difference in cell proliferation between these two cell populations. Together, our results suggest that there exist quantitative and qualitative differences in properties of bone marrow-derived hMSCs isolated from different anatomic sites of long bone. These findings are important and useful to developing stem cell therapies for disease treatment.

GENERAL POSTER SESSION

(9) Immune Modulation with Primed Mesenchymal Stem Cells Delivered via Biodegradable Scaffold to Repair an Achilles Tendon Segmental Defect

Anna Clements¹, Erdem Aktas², Connie Chamberlain¹, Erin Saether¹, Sarah Duenwald-Kuehl¹, Jaclyn Kondratko-Mittnacht¹, Michael Stitgen¹, Jae Sung Lee¹, William Murphy¹, and Ray Vanderby¹

1. University of Wisconsin-Madison, Madison, WI

2. Ankara Oncology Research and Training Hospital, Ankara, Turkey

abriener@wisc.edu

Achilles tendon ruptures are common injuries. Surgical repair remains the gold standard in treatment, but despite advances in surgical techniques the repaired tendon does not fully recover its original mechanical properties. This is especially true with large gap defects which often require allograft reconstruction. Mesenchymal stem/stromal cell (MSC) therapy for musculoskeletal regeneration has emerged as a new treatment strategy to enhance soft tissue regeneration because of their immunomodulator capabilities, specifically in respect to macrophages and T-lymphocytes. Significant barriers remain in improving MSC therapies, including the ability to consistently control the inflammatory response, which may be improved by priming MSCs with cytokines. In this study, we sought to incorporate primed MSCs into a biodegradable three dimensional poly(lactide-co-glycolide) (PLG) scaffold for segmental repair of an Achilles tendon gap defect in a rat model. We primed rat MSCs with TNF- α , which increased PGE2 and IL-6 production 24 hours after stimulation. We then found that TNF- α primed MSC loaded PLG scaffolds improved Achilles tendon strength, reduced inflammation, and increased collagen 1 deposition.

(10) Influence of Embryonic stem Cell Spatial Patterning on Brown Adipogenic Differentiation

Andrew D. Dias (1), Andrea M. Unser (2), Uwe Kruger (1), Yubing Xie (2), **David T. Corr** (1)

(1) Department of Biomedical Engineering, Rensselaer Polytechnic Institute

(2) Colleges of Nanoscale Science and Engineering, SUNY Polytechnic Institute

coord@rpi.edu

Stem cell differentiation is highly controlled in vivo, i.e., in the developing embryo, facilitated in part by self-patterning and localized signals. However, typical in vitro niches do not attempt to control patterning, but employ soluble factors and/or engineered substrates with desired properties to direct differentiation. It is important to understand the effect of spatial patterning and cellular signaling on cell fate. This research investigates the influence of spatial patterning in vitro, using laser direct-write (LDW) to deposit mouse embryonic stem cells (mESCs) in precise patterns on planar substrates. To study the effect of patterning on brown adipogenic differentiation, mESCs were induced to brown adipocytes using a cocktail of specific soluble factors. Groups of mESCs were patterned in grid configurations, with two different spacings between grid nodes. These patterns have the same initial local cell density within each node, but different global cell densities across the pattern. Paracrine signaling and cell-cell contact may be different between these cases, potentially influencing differentiation. Comparisons were made to mESCs without spatial control (randomly seeded mESCs) and to mESCs that formed 3D embryoid bodies in hanging drops before seeding. Using pluripotent mESCs as a baseline, expression of twelve genes, encompassing the three primitive germ layers and adipogenic markers was studied over a time course of differentiation using qPCR. After 25 days of differentiation, ANOVA showed significant differences in adipogenic genes PPAR γ and UCP1 for different initial patterns. A multivariate linear discriminant analysis (LDA) classified some of the patterning conditions, and suggested that the mesoderm marker Myf5, endoderm marker Gata6, neural marker Nestin, and adipogenic marker PGC1 α might also be affected by spatial patterning. While the differentiated stem cells in this study did not match the brown adipocyte positive control, LDA generated a “statistical fingerprint” for each niche that will inform evaluation of future engineered niches.

(11) A High-Throughput System for Rational Control of Biophysical Cues during Cell-Fate Decisions

Ty Harkness^{1,2}, Jason D. McNulty^{1,3}, Ryan Prestil^{1,2}, Stephanie K. Seymour^{1,2}, Tyler Klann^{1,2}, Michael Murrell^{1,2}, Randolph S. Ashton^{1,2}, and Krishanu Saha¹

¹ Wisconsin Institute for Discovery, University of Wisconsin-Madison, Madison, WI, USA

² Department of Biomedical Engineering, University of Wisconsin-Madison, Madison, WI, USA

³ Department of Mechanical Engineering, University of Wisconsin-Madison, Madison, WI, USA

tharkness@wisc.edu

There is a growing appreciation that microenvironmental factors play a major role during cell-fate decisions, but the cellular microenvironment is notoriously multivariate, with complex signaling occurring among cells, soluble factors, and the surrounding proteinaceous matrix. Progress in directing cell fate in regenerative medicine has been hindered by an inability to uncouple and rationally control distinct signaling and biophysical cues.

We have developed a biomaterials based platform for high-content, single-cell analysis of cellular biophysics and decision-making by adapting microcontact printing (μ CP) technology into the format of standard 6- to 96-well plates (μ CP Well Plates). As a chemically defined platform, μ CP Well Plates allow for rational and independent control of cell geometry, substrate composition, and soluble factors and enable straightforward analysis of thousands of uniform single-cells per well-plate. Combining this technology with live-cell imaging, we probe the biophysical properties of cells under a variety of shape constraints to discover a novel relationship between cell geometry, F-actin organization, and chromatin dynamics.

Understanding how cells integrate numerous biophysical, biochemical, and genetic cues during cell-fate decisions is critically important to the field of regenerative medicine. Our data offer insights into the interplay of these cues during decision-making processes and present biomaterial-based systems for their rational control.

(12) Mbd1 Governs the Differentiation of Adult Neural Stem Cells

Emily Jobe, Yu Gao, Maggie Calkins, Janessa Mladucky, Xinyu Zhao

ejobe@wisc.edu

MBD1 is a nuclear protein that binds to both methylated and unmethylated DNA and is thought to repress gene expression by recruiting chromatin remodeling factors. Previous work from my lab revealed that knocking out Mbd1 in mice causes learning and memory problems that are linked to hippocampal neurogenesis as well as behavioral phenotypes that are reminiscent of Autism. These mice generate fewer mature neurons in the hippocampus, a deficit which is likely a major contributor to their behavioral phenotypes. In the model I propose, the cause of reduced neuronal maturation can be traced back to changes in adult neuronal stem cells (aNSCs), a unique population of cells that continuously gives rise to new neurons in the adult brain. *In vitro*, neuronal genes are upregulated in proliferating Mbd1-KO aNSCs, while neuronal differentiation is reduced. To study aNSCs *in vivo*, I crossed Mbd1-KO animals with mice that express GFP under the control of the nestin promoter, a protein expressed in neural stem cells. In these animals the progression of differentiation is disrupted: there are fewer DCX+ immature neurons, and a greater proportion of these co-express nestin-GFP. Contrary to expectations, KO mice have more nestin-GFP positive cells, revealing that precocious activation of neuronal genes may actually delay the transition to new neurons. I hypothesize that Mbd1 acts as a repressor of neuronal differentiation genes in stem cells, and in its absence activation of these genes interferes with the regular process of differentiation. Understanding the role of Mbd1 in the fate specification of aNSCs will inform our understanding how subtle alterations in gene networks during adult neurogenesis leads to lasting changes in the brain.

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(13) Synthetic Hydrogel Substrates for Xeno-Free, ROCK Inhibitor-Independent Human Pluripotent Stem Cell Culture

Ngoc Nhi T. Le¹, William L. Murphy^{1,2,3}

¹Materials Science Program, University of Wisconsin-Madison, Madison, WI, USA; ²Department of Biomedical Engineering, University of Wisconsin-Madison, Madison, WI, USA; ³Department of Orthopedics and Rehabilitation, University of Wisconsin-Madison, Madison, WI, USA

ntle@wisc.edu

Recent studies suggest that substrates that facilitate increased intracellular cytoskeletal tension promote human pluripotent stem cell (hPSC) self-renewal [1]. 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 [1]. Reliance on animal proteins limits clinical translatability and preliminary studies suggest that routine dependence on ROCK inhibitor can limit hPSC differentiation potential [2]. Here, we developed synthetic hydrogel 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 in short-term culture. With the enhanced throughput capabilities afforded by our 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 fully-defined, 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.

References:

1. Fan Y, Wu J, Ashok P, Hsiung M, Tzanakakis ES. Stem Cell Reviews. 11, 96, 2015.
2. Gharechahi J, Pakzad M, Mirshavaladi S, Sharifitabar M, Baharvand H, Salekdeh GH. Molecular bioSystems. 10, 640, 2014.

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(14) Stimulating Mouse Digit Regeneration by Embryonic Limb Progenitor Cell Transplantation

Gufa Lin and Ying Chen

Stem Cell Institute, University of Minnesota, 2001 6th Street SE, Minneapolis, MN 55455, USA

linx519@umn.edu

We have recently shown that transplantation of embryonic limb progenitor cells can stimulate limb regeneration in the non-regenerating forelimb of adult frogs (*Xenopus laevis*). Here we report that this approach can also be used to induce regeneration in the mouse digits. We have optimized method for transplanting limb progenitor cells onto the mouse middle phalanx (P2) stumps and identified a panel of growth factors that can promote proliferation of embryonic limb progenitor cells and maintain the differentiation potential of limb progenitor cells in vitro. Transplantation of embryonic limb progenitor cells, together with combinations of growth factors, can significantly enhance digit regeneration after P2 amputations in the adult mice. The transplanted cells have mainly contributed to the bone lineage. Our results indicate that limb progenitor cell transplantation can be utilized to treat limb and digit injuries.

(15) Investigating Axolotl Limb Regeneration via Single Cell Transcriptomics

Jeffrey Nelson

nelson@morgridge.org

The Mexican axolotl salamander (*Ambystoma mexicanum*) possesses regenerative capabilities that are extraordinary among vertebrates. Following injury, these animals can regenerate body parts ranging from regions of the brain and heart to entire limbs and tails. While this phenomenon was first observed about 250 years ago, the biological mechanism by which axolotls accomplish such incredible feats is still relatively poorly understood. Following limb amputation, a cluster of cells known as a blastema forms at the wound site. This blastema then goes on to proliferate and fully regrow the missing limb. Previous work in our lab has identified coordinated gene expression programs that likely govern various stages of limb regeneration via blastema outgrowth. However, these experiments were performed on bulk blastema tissue samples, leaving open the critical question of just how individual adult cells coordinate themselves to complete such elaborately patterned tasks. Are blastemas made up of de-differentiated multipotent progenitor cells? Or, are they populated by fate-restricted tissue-specific adult cells that migrate from stump muscle, cartilage, nerve, and blood? Luckily, recent technological advances have allowed us to address these questions. Using single cell microfluidic capture platforms we have isolated and extracted total RNA from dissociated individual blastema cells in regenerating axolotl limbs. We then performed next generation sequencing on these samples, yielding axolotl transcriptomes at a single cell resolution. With bioinformaticist collaborators, we are currently developing methods by which we may identify gene expression patterns that represent sub-populations of cells participating in the regeneration process over time. Ultimately, we hope that the knowledge gained through this work will someday be applied in a biomedical setting to encourage tissue regeneration after injury or disease. *JDN was supported by an NHGRI training grant to the Genomic Sciences Training Program 5T32HG002760.*

(16) High Throughput Screening Format Identifies Synthetic Mimics of Matrigel for Tubulogenesis Screening

Eric Nguyen

ehnguyen@wisc.edu

Introduction: Matrigel™ is a commonly used material for testing the effects of chemical compounds on endothelial cell tubulogenesis but is poorly characterized and composed of >1800 individual proteins. This leads to a number of limitations including high batch-to-batch variation and presence of confounding growth factors. We used a high-throughput screening assay to determine chemically defined synthetic hydrogel formulations that permit tubule formation by Human Umbilical Vein Endothelial Cells (HUVEC) and human induced pluripotent derived endothelial cells similar to tubule formation observed on Matrigel™. Methods: Synthetic hydrogel formulations contained varied concentrations of cell adhesion peptides, crosslinking peptides and a matrix-linked Vascular Endothelial Growth Factor (VEGF)-binding peptide (VBP). Endothelial cells were seeded on an array of hydrogels and cultured in EGM2-supplemented endothelial growth medium (Lonza) for 48 hours before fixation. Samples were imaged at 24 and 48 hours and tubulogenesis was quantified using Nikon Elements image analysis software as mean tubule area, total tubule area, mean tubule length, total tubule length and mean fluorescence intensity of objects. Results: The hydrogel screening format identified three synthetic formulations that mimicked Matrigel's™ ability to induce endothelial cell tubulogenesis. Endothelial cells cultured on Matrigel™ and synthetic hydrogels demonstrated similar sensitivity to multiple vascular inhibitors including Sunitinib, Valatinib, Semaxanib, soluble Flt-1 receptor and VEGF antibody. Additionally, the presence of matrix-linked VBP minimized the effects of inhibitors on disrupting vascular morphogenesis. Conclusions: Our screening format identified synthetic hydrogels that are amenable to screening bioactive chemical compounds via tubulogenesis quantification. Initial screening of known vascular inhibitors showed similar sensitivity in optimized synthetic hydrogel formulations when compared to Matrigel™.

(17) Polymer Coatings on Plastic Substrates for Chemically Defined Culture of Human Mesenchymal Stem Cells

Samantha K. Schmitt¹, Angela W. Xie², William L. Murphy^{1,2,3}, and Padma Gopalan^{1,4}

¹Department of Materials Science and Engineering, ²Department of Biomedical Engineering, ³Department of Orthopedics and Rehabilitation, ⁴Department of Chemistry, University of Wisconsin-Madison, Madison, WI 53706. samanthakschmitt@gmail.com

Human mesenchymal stem cells (hMSC) are a widely available and clinically relevant cell type with a host of applications in regenerative medicine. Current clinical expansion methods can lead to changes in hMSC phenotype potentially resulting from relatively undefined cell culture surfaces. Chemically defined synthetic surfaces can aid in understanding the influence of cell-material interactions on stem cell behavior. Here we develop a copolymer coating that is 30 nanometers thick for hMSC culture on standard tissue culture plastic substrates. The random copolymer is synthesized by living free radical polymerization and characterized in solution before application to the substrate, ensuring a homogeneous coating and limiting the sample-to-sample variations. The polyethylene glycol based copolymer, specifically polyethylene glycol methyl ether methacrylate-*ran*-glycidyl methacrylate-*ran*-vinyl dimethyl azlactone, or P(PEGMEMA-*r*-GMA-*r*-VDM) is ethanol and water soluble which allows coating onto multiple substrate types and to cover large surface areas. Arg-Gly-Asp (RGD)-containing peptides are incorporated into the coating under aqueous conditions via their lysine or cysteine side chains, resulting in amide and thioester linkages, respectively. Polarization modulation-infrared reflection-adsorption spectroscopy (PMIRRAS) and X-ray photoelectron spectroscopy (XPS) verified the azlactone ring opening and percent incorporation of peptides. Depth profiling by XPS using an argon sputter gun confirmed that peptides are incorporated through the entire thickness of the coatings. Stability studies show amide linkages to be stable and thioester linkages to be labile under standard serum-containing culture conditions. Lastly, we show chemically defined passaging of hMSCs using only ethylenediaminetetraacetic acid (EDTA) on large area (150 cm²) polystyrene dishes coated with the copolymer. After passage, the hMSCs can be seeded back onto the same plate, indicating potential reusability of the coating. This coating platform can potentially elucidate cell-material interactions *in vitro* and have far-reaching effects on stem cell culture methods.

(18) Collagen Matrix Reorganization during Zebrafish Tail Regeneration

Jayne M. Squirrell, Danny C. LeBert, Jens Eickhoff, Yuming Lui, Kevin W. Eliceiri, Anna Huttenlocher
jsquirre@wisc.edu

The extracellular matrix (ECM) plays various roles in a diversity of biological processes, including development, wound healing, and regeneration. Collagen can have both positive and negative effects on healing and subsequent regeneration. To assess the role of the ECM during regeneration, we are using zebrafish larvae as a model, combined with second harmonic generation imaging (SHG), to examine collagen fiber regrowth during tail regeneration post-transection and how that collagen reorganization, and subsequent regeneration, is altered when ECM regulation is manipulated. Following an acute tail transection, MMP9 expression was induced and collagen fibers thickened as the tail regenerated. Additionally, depletion of mmp9 impaired this fiber thickening and disrupted tail regeneration, implicating mmp9 in proper acute wound repair. In animals that are mutant for the hepatocyte growth factor activator inhibitor gene 1 (*hai1*), which mimics chronic wound conditions including the increased mmp9 expression, the collagen tail fibers were disorganized. Interestingly, this disorganization was rescued by depletion of mmp9. Further, modification of the immune response by inhibiting macrophages impaired fiber re-growth at the wound site. Thus, collagen fiber reorganization, ECM regulation and interactions with immune system appear to be essential for proper regeneration of the larval zebrafish tail.

(19) Collaborative Rewiring of the Pluripotency Network by Chromatin and Signaling Modulating Pathways

Tran KA, Jackson SA, Olufs, ZP, Zaidan NZ, Leng N, Kendziorowski C, Roy S, and Sridharan R
katran@wisc.edu

Reprogramming of somatic cells to induced pluripotent stem cells (iPSCs) represents a profound change in cell fate. Here we show that combining ascorbic acid (AA) and 2i (MAP kinase and GSK inhibitors) increases the efficiency of reprogramming from fibroblasts and synergistically enhances conversion of partially reprogrammed intermediates to the iPSC state. AA and 2i induce differential transcriptional responses, each leading to the activation of specific pluripotency loci. A unique cohort of pluripotency genes including *Esrrb* require both stimuli for activation. Temporally, AA-dependent histone demethylase effects are important early, whereas Tet enzyme effects are required throughout the conversion. 2i function could partially be replaced by depletion of components of the epidermal growth factor (EGF) and insulin growth factor pathways, indicating that they act as barriers to reprogramming. Accordingly, reduction in the levels of the EGF receptor gene contributes to the activation of *Esrrb*. These results provide insight into the rewiring of the pluripotency network at the late stage of reprogramming.

(20) Human Mesenchymal Stem Cell and Endothelial Cell Interaction through Endothelin-1

Tsung-Lin Tsai^{1,2}, Bowen Wang³, Matthew W. Squire¹, Lian-Wang Guo³ and Wan-Ju Li^{1,2*}

¹Department of Orthopedics and Rehabilitation, ²Department of Biomedical Engineering, ³Department of Surgery, University of Wisconsin-Madison, Madison, Wisconsin, USA

tsai@ortho.wisc.edu

Introduction: Human mesenchymal stem cells (hMSCs) reside in a perivascular niche of the body, suggesting that they interact closely with vascular endothelial cells (ECs) through cell-cell interaction or paracrine signaling to maintain cell functions. Endothelin-1 (ET1) is a paracrine factor mainly secreted by ECs. We thus hypothesize that ECs can regulate cellular activities of hMSCs and direct their stem cell fate.

Methods: We investigated whether cocultured human aortic endothelial cells (HAECs) were able to regulate expression of potency- and lineage-related markers in bone marrow-derived hMSCs. We further explored the regulatory effects of ET1 on cell proliferation, expression of surface antigens and pluripotency-related markers, and multilineage differentiation in hMSCs. Activation of the AKT signaling pathway in hMSCs was also analyzed to identify its mechanistic role in the ET1-induced regulation.

Results: Cocultured HAECs enhanced expression of mesenchymal lineage-related markers in hMSCs. Treatment of ET receptor antagonist downregulated the increased expression of CBFA1 in hMSCs cultured with HAEC-conditioned medium. Human MSCs treated with ET1 showed cell proliferation and expression of surface antigens, CD73, CD90, and CD105, comparable with those without ET1 treatment. ET1-treated hMSCs also expressed upregulated mRNA transcript levels of OCT3/4, NANOG, CBFA1 and SOX9. When induced for lineage-specific differentiation, hMSCs pre-treated with ET1 showed enhanced osteogenesis and chondrogenesis. However, adipogenic differentiation of hMSCs was not affected by ET1 pretreatment. We further showed that the ET1-induced regulation was mediated by activation of AKT signaling.

Conclusion: Our results demonstrate that ET1 secreted by HAECs can direct bone marrow-derived hMSCs for osteo- and chondro-lineage differentiation through activation of the AKT signaling pathway, suggesting that ET1 plays a crucial role in regulation of hMSC activity. Our findings may help understand how hMSCs interact with ECs in a perivascular niche.

(21) Local CXCR4 Up-regulation in Injured Arterial Wall Contributes to Intimal Hyperplasia

Xudong Shi*, Lian-Wang Guo*, Stephen Seedial*, Toshio Takayama, **Bowen Wang**, Sarah R. Franco, Yi Si, Daniel M. DiRenzo, Bo Liu, K. Craig Kent.

Department of Surgery, University of Wisconsin Hospital and Clinics, 600 Highland Avenue, Madison, WI 53792-7375

bwang98@wisc.edu

* These authors contributed equally to this work

Rationale: CXCR4 is a stem/progenitor cell surface receptor specific for the cytokine stromal cell-derived factor-1 (SDF-1 α). There is evidence that bone marrow-derived CXCR4-expressing cells contribute to intimal hyperplasia (IH) by homing to the arterial subintima which is enriched with SDF-1 α . We have previously found that the transforming growth factor- β (TGF β) and its signaling protein Smad3 are both up-regulated following arterial injury and that TGF β /Smad3 enhance the expression of CXCR4 in vascular smooth muscle cells (SMCs). It remains unknown, however, whether locally induced CXCR4 expression in medial SMCs plays a role in neointima formation.

Objective: To investigate whether elevated TGF β /Smad3 signaling leads to the induction of CXCR4 expression locally in the injured arterial wall, thereby contributing to IH.

Methods and Results: We found prominent CXCR4 up-regulation (mRNA, 60 fold; protein, 4 fold) in TGF β -treated, Smad3-expressing SMCs. Chromatin immunoprecipitation (ChIP) assay revealed a specific association of the transcription factor Smad3 with the CXCR4 promoter. TGF β /Smad3 treatment also markedly enhanced SDF-1 α -induced ERK1/2 phosphorylation as well as SMC migration in a CXCR4-dependent manner. Adenoviral expression of Smad3 in balloon-injured rat carotid arteries increased local CXCR4 levels and enhanced IH, whereas SMC-specific depletion of CXCR4 in the wire-injured mouse femoral arterial wall produced a 60% reduction in IH.

Conclusions: Our results provide the first evidence that up-regulation of TGF β /Smad3 in injured arteries induces local SMC CXCR4 expression and cell migration, and consequently IH. The Smad3/CXCR4 pathway may provide a potential target for therapeutic interventions to prevent restenosis.

(22) Engineering Three-Dimensional Anisotropic Human Mesenchymal Stem Cells Tissue Constructs

Qi Xing, Feng Zhao

Department of Biomedical Engineering, Michigan Technological University

qxing@mtu.edu

Human mesenchymal stem cells (hMSC) are highly multipotent, regenerative and capable of self-renewal. An anisotropic and hierarchical engineered hMSC tissue construct holds great promise for multiple tissue regeneration as it effectively mimics the organization characteristics of some native tissues, which are essential to the tissue mechanical strength and function. We have previously derived a natural extracellular matrix (ECM) scaffold from aligned human fibroblast cell sheets. The scaffold contains parallel organized protein nanofibers that not only direct the orientation of cells, but also potentially recapitulate the complex in vivo microenvironment for in vitro cell growth. In this study we further examined the feasibility of using aligned ECM nanofibers in engineering a completely biological, structurally and mechanically anisotropic hMSC tissue construct. The hMSCs-incorporated ECM scaffolds were layered to form a three-dimensional (3D) construct and matured in a rotating wall vessel (RWV) bioreactor. The dynamic culture environment provides efficient exchange of nutrients and waste to maintain the construct with higher cell density. In addition, the mechanical stress generated in the RWV bioreactor stimulated the formation of thick aligned ECM fiber bundles and organized groove-like structure formed in the outer surface, which resulted in a hMSC tissue construct with enhanced anisotropic property and mechanical strength in the direction of nanofibers. The multi-lineage differentiation potential of hMSCs was preserved in the construct, which indicates the possible applications of the anisotropic hMSC construct in engineering various tissues.

(23) The Role of HP1 Family of Proteins in the Acquisition of Pluripotency

Nur Zafirah Zaidan and Rupa Sridharan, University of Wisconsin-Madison

nzaidan@wisc.edu

Somatic cells can be converted to an embryonic stem cell like state by transcription factor-mediated reprogramming to induced pluripotent stem cells. Chromatin organization is notably different between pluripotent cells and non-pluripotent cells and is thought to play a role in maintaining the plasticity of pluripotency. However, whether such spatial organization is required for the acquisition and maintenance of pluripotency has not been investigated. H3K9 methylation, a repressive histone modification, is enriched in mouse embryonic fibroblasts (MEFs) but decreases as cells become reprogrammed to a pluripotent state. The HP1 family of proteins recognizes H3K9 methylation. Interestingly HP1 gamma, changes localization from a mostly gene body enrichment to a more promoter specific localization between pluripotent and non-pluripotent cells. Additionally, a decrease in HP1 expression increases reprogramming efficiency. This phenomenon is greater in HP1g compared to other members of the HP1 family. Therefore we are interested in further evaluating this protein in the acquisition of pluripotency.

(24) The Role of Substrate Elasticity in Influencing the Differentiation of Human Pluripotent Stem Cells to Neurons

Yefim Zaltsman¹, Samira Musah², Paul J. Wrighton¹, Xiaofen Zhong³, Qiang Chang^{3,4}, and Laura L. Kiessling^{1,2}

¹Department of Biochemistry, ²Department of Chemistry, ³Waisman Center, ⁴Department of Medical Genetics and Neurology, University of Wisconsin–Madison, Madison, WI, USA.

zaltsman@wisc.edu

Human pluripotent stem (hPS) cells possess the remarkable capacity to self-renew indefinitely and differentiate into virtually all cell types. hPS cells thus represent an unlimited source of cells with potentially transformative applications such as cell-based regenerative medicine and drug discovery. These applications, however, require efficient and reproducible methods for directing the differentiation of hPS cells to desired cell types. To date, focus has been on soluble factors such as growth factors and small molecules, while the role of insoluble signals, such as substrate elasticity, in influencing hPS cell fate decisions is less clear. We found that, even in the presence of soluble factors that promote pluripotency, compliant substrata, with elasticity similar to human brain tissue, override these signals to induce efficient differentiation of hPS cells to neurons. The neurons derived by substrate induction alone—in absence of neurogenic factors—express mature markers and possess action potentials. The molecular mechanism is through modulation of the subcellular localization of the transcriptional coactivator YAP. Nuclear exclusion of YAP in cells cultured on compliant substrata or the depletion of YAP by RNA interference effects neuronal differentiation. Our findings indicate that mechanical cues can override soluble signals, suggesting that their contributions to early human development and in vitro differentiation are profound. Current hPS cell differentiation protocols are almost exclusively carried out on tissue culture polystyrene, a substrate which is orders of magnitude stiffer than biological tissues. Therefore, we anticipate that utilizing substrates with more biologically relevant mechanical properties will augment differentiation protocols.

(25) Effects of Oxygen Tension on Microvessel Network Formation on Human Mesenchymal Stem Cell Sheet for Prevascularized Three-Dimensional Tissue Engineering

Feng Zhao, Department of Biomedical Engineering, Michigan Technological University
gendzhao@mtu.edu

Microvessel networks inside engineered tissues are required to support cell survival and accelerate anastomosis after implantation. Three-dimensional (3D) pre-vascularized tissues based on human mesenchymal stem cells (hMSCs), show great promise in regenerating multiple types of connective tissues. This study investigated the role of oxygen tension in microvessel formation by human umbilical vein endothelial cells (HUVECs) seeded on top of hMSC sheets, which are subsequently used for 3D tissue construction. It was found that the HN condition, in which hMSC sheets formed under physiological hypoxia (2% O₂) and HUVECs co-cultured under normoxia (20% O₂), enabled longer and more branched microvessel network formation. The observation is corroborated by the higher levels of angiogenic factors in co-culture medium. Additionally, the hypoxic hMSC sheet was more uniform and less defective, which facilitated fabrication of a 3D pre-vascularized tissue construct by layering the pre-vascularized hMSC sheets and maturing in rotating wall vessel (RWV) bioreactor. The hMSCs in the construct still maintained multi-lineage differentiation ability, which indicated the possible application of the 3D construct for various connective tissue regeneration. These results demonstrated that hypoxic hMSC sheets benefit the microvessel growth and it is feasible to assemble the 3D pre-vascularized tissue construct using the pre-vascularized hMSC sheets.