small vascular aggregates amount the three groups. iPSC-VSMC:HUVEC combination scaffolds that were function-ized fibronectin scaffolds demonstrated increased number of vascular aggregates when compared with control and Echistatin scaffolds ($P$ value = 0.003 and 0.002, respectively). In addition, the number of large vascular aggregates was increased in the fibronectin scaffolds containing iPSC-VSMC and HUVEC combination when compared with control and Echistatin scaffolds ($P$ value = 0.0001, both). The number of large vascular aggregates was significantly decreased in the scaffolds treated with Echistatin ($P$ value = 0.0001). However, the number of small vascular aggregate remained constant among the 3 groups.

**CONCLUSIONS:** Fibronectin plays a key role in maintaining the HUVEC’s viability, since the addition of Echistatin, a fibronectin inhibitor, dramatically decreased the HUVEC’s viability. This suggested fibronectin to have an agonistic effect on HUVEC via interaction of Alpha-v Beta-3 integrin expressed on the cells. The higher number of large vascular aggregates in iPSC-VSMC and HUVEC combination in fibronectin scaffolds suggested that fibronectin was promoting iPSC-VSMC interaction with HUVEC to promote formation and maintenance of larger and complex vascular aggregates. Echistatin scaffolds as the inhibitor caused the number large aggregates to diminish down close to none. These findings resulted in a better understanding of potentially an underlying mechanism of how to optimize iPSC-VSMC characteristics.

**METHODS:** The PEG capacity to crosslink with type I collagen was first evaluated with TNBAS assay. The physical setting of PEG scaffolds was optimized at a molar ratio of 1:1 PEG to collagen and an overall density of 4mg per ml. iPSC-VSMC were embedded into 4s-StarPEG functionalized collagen scaffolds for 3 days. At the end of 72 hours, the cultured media were collected and evaluated for enzymatic secretions: matrix metalloproteinase-9 (MMP9) and Tissue Inhibitor Of Metalloproteinase 1 (TIMP1) via ELISA. The resultant cell-scaffolds were evaluated for overall cellular viability using AlamarBlue assay. The scaffolds were also immunofluorescence stained for Calponin (Green) and NG2 (Red), which are markers for VSMC and pericytes, respectively. The morphology of immune-stained iPSC-VSMC were subsequently characterized under confocal microscope at 10× and 40×.

**RESULTS:** iPSC-VSMC embedded in PEG-crosslinked collagen scaffolds increased cellular viability ($P$ value = 0.003). iPSC-VSMC in PEG scaffolds secreted significantly more MMP9 ($P$ value = 0.014), while there was no difference in TIMP1 between the control and PEG group. Via confocal microscope, the number of elongated iPSC-VSMC, as defined by greater than 50um in length, in PEG scaffolds was much higher than the control ($P$ value = 0.0418). Furthermore, cells in the PEG scaffolds were more positively stained for NG2 ($P$ value = 0.043).

**CONCLUSIONS:** 4S-Star PEG functionalized hydrogel scaffolds promoted iPSC-VSMCs’ viability suggested that a PEG-Collagen environment is not only a safe but also a potentially preferable vehicle for iPSC-VSMC-based cell therapy. Despite MMP9 being linked with cardiovascular pathophysiology, MMP9 is also associated in neovascularization due to its ability for extracellular matrix degradation and proangiogenic paracrine factors activation. In the setting of increased MMP9 without changes in its corresponding inhibitor, TIMP1, iPSC-VSMC in PEG scaffolds may have increased migration capability via extracellular matrix degradation, potentiate neovascularization, and possibly

**4S-Starpeg Crosslinked Collagen Hydrogels Promotes iPSC-Vascular Smooth Muscle Cell Extracellular Matrix Remodel Capability Via Matrix Metalloproteinase-9**

**Presenter:** Kaiti Duan, BS  
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**PURPOSE:** Induced-pluripotent-stem-cell-derived-vascular smooth muscle cells (iPSC-VSMC) are known for their capabilities to promote angiogenesis and potentially chronic wound healing. 4S-Star polyethylene glycol (PEG) has been advocated as a potential injectable vehicle for cell-based therapy in the field of bioengineering due to its ability to form cross-links with free amine groups on collagen, which fundamentally changes the properties of collagen scaffolds and thus may affect the functionalities of cells within the collagen scaffold. In this study, our objectives were to optimize PEG collagen delivery condition and to evaluate how PEG-functionalized hydrogel scaffolds may affect iPSC-VSMC characteristics.
wound healing. The migration capability may also be supported by images of significantly more elongated cells. These findings resulted in making 4S-Star PEG functionalized hydrogel a potentially attractive platform to transfer therapeutic iPSC-VSMC onto wound sites and to promote embedment of these cells into targeted tissues. Future studies would include PEG-encapsulated iPSC-VSMC vivo studies to evaluate its efficacy in wound healing.

**Disrupting Mechanotransduction Decreases Fibrosis and Contracture in Split Thickness Skin Grafting**

**Presenter:** Kellen Chen, PhD

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**PURPOSE:** Burns and other traumatic injuries represent a significant biomedical burden for humans. Despite our best care in specialized centers, a burn patient either dies from infection or the injury itself, or lives with the devastating consequences of pain and scarring. A cornerstone of burn therapy is to excise the dead tissue and close the wound with a split thickness skin graft (STSG). While this reestablishes the skin barrier function, it is associated with severe fibrosis and scar, a process that we have recently linked to mechanical signaling in murine models.1,2 Unfortunately, these small animal models do not truly replicate human scar formation because humans are over several orders of magnitude larger, and these fundamental differences have significantly limited the ability to translate discoveries from mice to humans.

**METHODS:** We developed a clinically relevant porcine STSG model using standardized surgical techniques commonly applied for the clinical treatment of burn wounds and other soft-tissue defects. Full-thickness excisional wounds were created on the back of red Duroc pigs. STSG were harvested and secured on the wound bed with skin staples, bolster dressings and either treated with focal adhesion kinase inhibitor hydrogels or standard dressings as controls. We comprehensively characterized the tissue appearance and related porcine cell populations involved in healing at the single-cell level using scRNA-seq.

**RESULTS:** We identify an upregulation of pro-inflammatory and mechanotransduction signaling pathways in standard split thickness skin grafts. Blocking mechanotransduction using a small molecule focal adhesion kinase inhibitor, we substantially promoted engraftment, reduced contracture, mitigated scar formation, restored collagen architecture, and ultimately improved graft biomechanical properties. We demonstrate that mechanotransduction blockade results in early upregulation of anti-inflammatory pathways in myeloid cells. At later time points, mechanical signaling shifts fibroblasts toward pro-fibrotic differentiation fates, whereas disruption of mechanotransduction blocked those responses and instead drove fibroblasts toward pro-regenerative states similar to unwounded skin. We then confirmed these two diverging fibroblast transcriptional trajectories in a 3D organotypic in vitro model of skin.

**CONCLUSIONS:** Taken together, pharmacological blockade of mechanotransduction significantly improves large animal healing after STSG by promoting both acute, anti-inflammatory and chronic, regenerative transcriptional programs, resulting in healed tissue similar to unwounded skin. Our therapy could have significant translational implications and could be easily incorporated with the current standard of care to help those who experience traumatic and burn injuries.

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