METHODS: Polydimethylsiloxane molds were created using custom-designed 3D-printed Poly lactic acid molds (a Makerbot Replicator 5th Generation). 3D-printed Poly lactic acid stamps and 22G catheters were used for the fabrication of BC hemispheroid “buttons” and putative vascular channels within the same Z coordinate, with the buttons separated by 3.5 mm from each other and 1.5 mm from either vascular channel. The biomimetic platform was fabricated using adipocytes and other patient-derived tissue components mixed within neutralized 0.6% (w/v) Type I collagen to form the main structure in Polydimethylsiloxane molds; red-fluorescent MDA-MB-231 cells mixed with 0.6% collagen at a density of 40,000 cells/1.6 µL were added into the wells that were pre-formed with Poly lactic acid stamps in the biomimetic platform bulk to create the tumor hemispheroid “buttons”. Twenty-four hours after plating, fluorescently labeled smooth muscle cells and endothelial cells were seeded sequentially within the channels at a concentration of 3 million cells/mL. Control constructs were made by generating vascular structures and BC “buttons” within a collagen-only matrix. Constructs were cultured for 7, 14, and 21 days, imaged with confocal microscopy, and analyzed with H&E and immunofluorescent staining.

RESULTS: Patent vascular channels lined with smooth muscle cells and endothelial cells were visualized within the platform at Day 1. Confocal and H&E staining showed that EC sprouts had formed from vessels oriented preferentially toward BC buttons by day 7 and 14 of culture; this was more prevalent after 21 days. Concurrently, the hemispheroid tumor “buttons” were noted to increase in size and individual cells were seen invading preferentially toward the vessels to a much greater degree over time. Compared with collagen-only control group, the biomimetic group displayed increased cell invasion on Day 7, and H&E staining revealed successful fabrication of biomimetic containing patient-derived adipocytes, stromal vascular fraction, and ductal organoids.

CONCLUSIONS: We have successfully engineered an advanced, patient-specific, biomimetic platform of the breast cancer microenvironment that not only replicates patient tissue characteristics, but also includes vascular structures and cancer hemispheres that closely resemble early tumors. Such a platform represents a highly potent tool that holds significant promise for diagnostic and therapeutic applications in the study of breast cancer.

Vascular Smooth Muscle Cells in the Presence of Fibronectin Functionalized Collagen Scaffold Increases the Size of Endothelial Cell-based Vascular Aggregates

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PURPOSE: Fibronectin-functionalized collagen scaffolds can promote induced-pluripotent-stem-cell-derived-vascular smooth muscle cell (iPSC-VSMC) to enhance their pro-angiogenic paracrine profiles. However, the influence of fibronectin collagen on human umbilical vein endothelial cells (HUVEC), a key component of angiogenesis, is still unknown. In this study, our objectives were to evaluate the behaviors of HUVEC within fibronectin-functionalized scaffolds and evaluate the integrity of iPSC-VSMC:HUVEC combination vascular formation in the setting of fibronectin-functionalized collagen scaffolds.

METHODS: Fibronectin was added to type I collagen to obtain a final scaffold density of 4mg per ml. HUVECs were incubated within the scaffold for a total of 7 days, and after the first 24 hours Echistatin, an integrin inhibitor of Alpha-v Beta-3 was added to scaffolds. The resultant scaffolds were evaluated for cellular viability via AlamarBlue assay. The scaffolds were immunofluorescence stained with CD144. Confocal microscope was used to count the total number of endothelial vascular aggregates, and then categorized based on sizes, greater or less than 50 µm. Next, we combined iPSC-VSMC and HUVEC at a ratio of 1:4 and embedded them into fibronectin-functionalized collagen for 7 days. The resultant scaffolds were then immunofluorescence stained with Sm-22alpha, NG2 and CD144. The number and sizes of the vascular aggregates were also evaluated.

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RESULT: iPSC-VSMCs embedded in fibronectin-functionalized collagen scaffold demonstrated no significant cellular viability from control collagen scaffold. However, the addition of Echistatin, an integrin inhibitor, to fibronectin scaffolds resulted in significant decrease in HUVEC viability when compared with control and fibronectin scaffold groups (P = 0.0001). The total number of vascular aggregates was significantly higher in fibronectin scaffolds than in control and Echistatin scaffolds (P value = 0.03). There was no significance between the number of large or
small vascular aggregates amount the three groups. iPSC-VSMC:HUVEC combination scaffolds that were functionalized 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 iPS-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 4 mg per ml. iPS-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 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.

4S-Starpep Crosslinked Collagen Hydrogels Promotes iPS-Vascular Smooth Muscle Cell Extracellular Matrix Remodel Capability Via Matrix Metalloproteinase-9

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PURPOSE: Induced-pluripotent-stem-cell-derived-vascular smooth muscle cells (iPS-VSMC) are known for their capabilities to promote angiogenesis and potentially chronic