**Comparative Evaluation of Hydrogels As a 3D Angiogenic Matrix for Adipose-Derived Stem Cells**

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**Statement of Purpose:** The behaviors of stem cells can be controlled depending on the 3D environment. Hydrogels such as collagen and fibrin have been investigated for as culture matrices for stem cells in various tissue engineering fields [1, 2]. However, there is a limited understanding about variable regenerative capacities of those matrices depending on specific tissue targets and stem cells. Our group has been focused on developing the angiogenic matrix based on PEGylated fibrin (PFG), having demonstrated interconnected cellular networks and endothelial gene induction of MSCs in PFG culture [1]. The aims of the current study is to compare how the phenotype of adipose-derived stem cells (ASCs), a promising adult stem cell source, can be differently controlled depending on the hydrogel matrix. We examined culture of ASCs on collagen, fibrin, or PEGylated fibrin and compared cell morphology, proliferation, and phenotypic expression.

**Methods:** ASCs (PT-5006, Lonza), which are positive for CD29, CD44, CD105, CD166 and negative for CD14, CD24, and CD45 using flow cytometry, were cultured in DMEM-low glucose with Glutamax I (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) and 1% penicillin-streptomycin (Invitrogen) for expansion. Matrix 1: Collagen gel (CG) using rat tail collagen (type I) Matrix 2: fibrin gel (FG); Matrix 3: PEGylated fibrin (PG) gel. The concentrations of the gels were matched at 4 mg/ml among groups. 125 µl fibrinogen (32 mg/ml) solution in Dulbecco's phosphate buffered saline (DPBS) (pH 7.8) was combined with 125 µl SG-PEG-SG solution (3.2 mg/ml) or DPBS (pH 7.8), for 1 ml FG and PG, respectively. Cell suspension (250 µl of 200,000 cells/ml) were added into the fibrinogen-PEG (or DPBS) solutions followed by addition with thrombin solution (25 U, 500 µl) diluted with calcium chloride at the volume ratio of 1:3. After 10 minutes incubation in a humidified CO2 incubator at 37°C, the culture media was added into cell-seeded gels for further cultivation. Collagen gels were fabricated from acidic collagen stock (5 mg/ml, Trevigen) by neutralization with NaOH and incubation at 37°C for 30 minutes. In the current study, the total volume of gels were varied from 500 µl to 3 ml and cells were seeded identically at the density of 50,000 cells/ml. Biochemical and molecular analysis (*e.g.* growth factor array, western blot, MTS assay and morphology analysis) were applied to compare proliferation and differentiation as well as paracrine effects of ADSCs in different 3D gel matrices.

**Results:** Rheological analysis showed greater levels for both the storage and loss modules in CG (data not shown). However, cells showed more interconnected and elongated networks in PG, which was demonstrated through Calcein AM stained fluorescent and phase contrast imaging (Fig.1A-D). Western blot analysis showed smooth muscle alpha actin (SMA), a pericyte marker, and von willebrand marker (vWF), an endothelial marker, in CG and PG, respectively (Fig.1E and F). Moreover, ASCs proliferated in all types of gels up to one week and the metabolic activities showed greatest levels in FG, presuming enhanced cell growth in FG (data not shown).

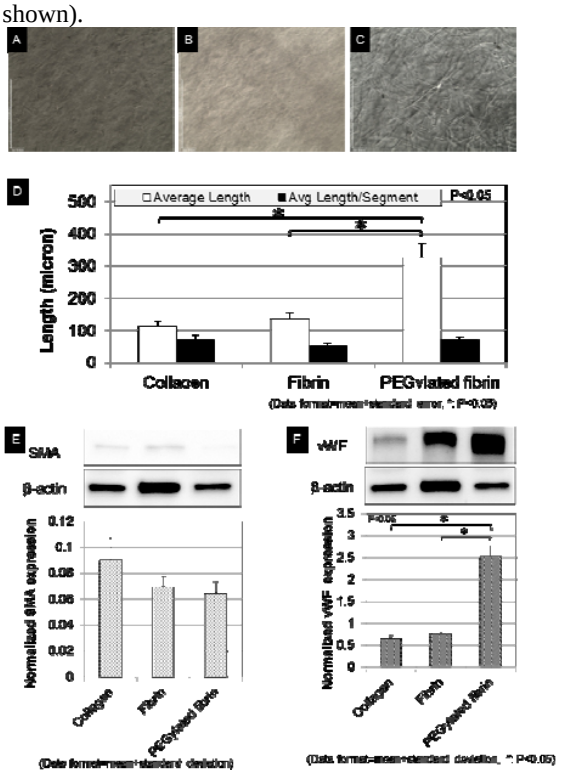


Figure 1. ASC-seeded in collagen, fibrin, and P-fibrin. ASC morphology in CG (A), FG (B), and PG. (D) Quantified cellular network length of Calcein AM fluorescence-stained ASCs. Western blot of vascular proteins (SMA and vWF) (E and F).

**Conclusions:** The current studies demonstrated how the cellular activities and morphology of ASCs were controlled depending on hydrogel culture platforms. Cells proliferated more in fibrin than the other gels examined. Moreover, PEGylated fibrin induced greater interconnected networks and expression of endothelial cell markers than fibrin and collagen. Therefore, PEGyated fibrin may have superior potential for endothelization and paracrine effects on angiogenesis. To confirm this more fully, further investigation will be focused on the paracrine effects of ASCs on endothelial cells and macrophages. In addition, ASC population studies using a fluorescent cell sorting technique and gene analysis using a real time RT-PCR will be performed to clarify ASC differentiation.

**References:** [1] S. Natesan, G. Zhang, D.G. Baer, T.J. Walters, R.J. Christy, L.J. Suggs, Tissue Eng Part A, 17 (2011) 941-953.[2] V. Falanga, S. Iwamoto, M. Chartier, T. Yufit, J. Butmarc, N. Kouttab, D. Shrayer, P. Carson, Tissue Eng, 13 (2007) 1299-1312.