Adipose-Derived Stem Cell Proliferation & Migration on Two-Dimensional and Three-Dimensional Substrates

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Statement of Purpose: Biocompatible scaffolds that promote the viability and proliferation of adipose-derived stem cells (ADSC) are pivotal for the advancement of the fields of regenerative medicine and tissue engineering. ADSC are readily harvested in large quantities and can be differentiated into a variety of cells types such as adipocytes, chondrocytes, osteoblasts, and neurons. Therefore, it is vital to design a construct that supports the viability, proliferation, and migration of these ADSC. In this study, we fabricate gelatin electrospun nanofibers and gelatin films and evaluate the ability of these two dimensional and three dimensional surfaces to promote cellular proliferation and migration. We hypothesize that the scaffold structure will enhance ADSC proliferation and migration compared to the film.

Methods: *Electrospinning:* A 15% w/v solution of Gelatin Type A (Sigma Aldrich) was prepared using ethanol and 10X-phosphate buffered saline (1:1 v/v) at 40°C for 4 hours. After 4 hours, the solution remained at room temperature for further dissolution. Electrospun gelatin fibers were fabricated using a custom-built electrospinning set-up utilizing the following parameters: 12 cm needle tip to collector distance, 12 kV applied voltage, and 30 μL/min fixed flow rate. The electrospun fibers were crosslinked in 0.5% glutaraldehyde vapor for 20 hours at 42°C. After crosslinking, the fibers were placed in a vacuum oven at 42°C for 24 hours to remove residual glutaraldehyde. *Cell Proliferation:* Cells (7,500 cells/well) were seeded onto the surface and incubated for 3, 5, or 7 days. After incubation, the MTT (thiazolyl blue tetrazolium bromide) assay was performed to assess cell quantity. In short, MTT solution (5 mg/mL) was added to each well and incubated for 3.5 hours. The media/MTT was aspirated, dimethyl sulfoxide was added to each well to dissolve the precipitate, and the absorbance of the resulting solution was read at 590 nm. *Cell Migration:* Outward cell migration was assessed using a non-injury cylinder assay in which cells were seeded into the center of a hollow Pyrex ® cylinder and allowed to migrate outward for 4, 24, or 48 hours. After the designated migration time, cells were fixed using 5% paraformaldehyde, rinsed 2x with PBS-T (1x PBS with 1% Tween20), and stained using 0.1% toluidine blue.

Results: *Cellular Proliferation:* Adipose-derived stem cells (ADSC) have shown high viability on the gelatin electrospun scaffolds, tissue culture plate (TCP), and gelatin film after 7 days in culture. The proliferation assay assessed cell growth on each surface over 7 days (Figure 1). The TCP and the gelatin film had similar proliferation with the film and TCP reaching confluence at day 5 and maintaining confluence at day 7. The gelatin scaffold had higher proliferation than the gelatin film or TCP at day 3, 5, and 7. This suggests that the scaffold surface supports higher proliferation than the flat, two-dimensional surfaces. *Cell Migration:* Using the non-injury cylinder assay, the gelatin film, TCP, and gelatin scaffold showed continued outward cellular migration over 48 hours (Figure 2). The gelatin film and TCP had similar outward migration after 48 hours ($p = 0.07$). The gelatin scaffold had the lowest cellular migration when compared to the TCP ($p = 0.01$) and gelatin film ($p = 0.0005$). This suggests that the two-dimensional surfaces of the gelatin film and TCP promote higher migration than the nanofibrous, three-dimensional scaffold.

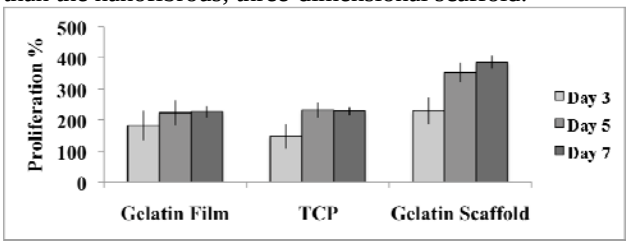

Figure 1. Proliferation of ADSC on Gelatin Film, TCP, & Gelatin Scaffold after 3, 5, & 7 days

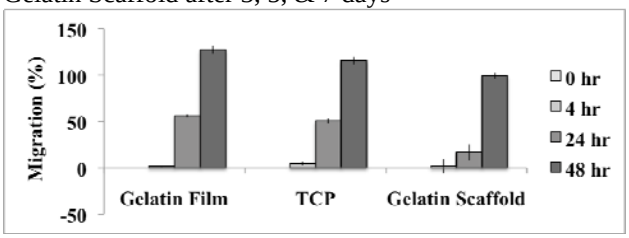

Figure 2. Outward Migration of ADSC on Gelatin Film, TCP, & Gelatin Scaffold after 0, 4, 24, & 48 hours

Conclusions: The gelatin scaffold, gelatin film, and tissue culture plate support the high viability of adipose-derived stem cells over 7 days in culture. The gelatin scaffold promotes higher cellular proliferation at all time points over 7 days than the two-dimensional surfaces (gelatin film and tissue-culture plate). In contrast, the gelatin scaffold promotes less outward cellular migration than the two-dimensional surfaces (gelatin film and tissue culture plate). Overall, the scaffold structure promotes high cellular growth and essentially entraps the cells in the scaffold for future maturation and differentiation. Additionally, we are also developing chemisorption-reaction kinetics based mathematical models to describe contact-spreading behavior on the 3-D surfaces. Contact modulation parameters and parametric sensitivity will be reported.

References: Merkle VM. BioPolym. 2013; *In Press.*
Merkle VM. Polym. 2013; 54 (21): 6003-6007