Topography of nanofibers regulates the phenotypic expression of Adipose Stem Cells

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Statement of Purpose: An ideal scaffold for wound healing should mimic the structural and functional properties of native skin extracellular matrices (ECMs). Electrospun scaffolds have been found to closely mimic the native ECM in terms of composition and physical structure [1], however, they are still far from ideal in scar - free wound healing, because wound repair is a complex process requiring the coordination of several biological responses, including the organization of ECMs [2,3]. Thus, we hypothesize that the specific underlying nanofiber organization will guide the wound healing related behaviors of adipose stem cells (ASCs), a potential stem cell in skin tissue engineering. In our study, the effects of fiber organization (aligned *vs* random) on the responses of human ASCs, including morphology, proliferation, and migration were explored.

Methods: Aligned and random nanofiber scaffolds based on polycaprolactone (Sigma, St. Louis, MO) and collagen type I (Elastin Products CO, Owensville, Missouri) were fabricated using electrospinning as previously described [4]. hASCs were then seeded on the scaffolds at a density of 3×10^4 cells/cm^2 and cultured for 14 days. Cell morphologies were evaluated on day 3 using immunostaining for anti-α-tubulin (DSHB, Iowa City, IA). Cell proliferation was determined by CyQUANT®-Cell Proliferation Assay Kit (Molecular Probes, Eugene, Oregon). Gene expressions of proteins involved in cell attachment and migration, including integrin a2, a5, ß3 and matrix metalloproteinase (MMP1) were measured via reverse transcriptase polymerase chain reaction. Cell migration on the nanofiber scaffolds was assessed via collagen gel drop assay [5]. The paired student's *t* test was used for statistical analysis. *P* values less than 0.05 were considered statistically significant.

Results:

Effects of nanofiber organization on cell morphology The cells displayed distinct morphologies on the two types of nanofiber scaffolds. Specifically, cells grown on the aligned fibers adopted an elongated morphology with the orientation in the direction of the fiber long axis. In contrast, cells seeded on the random fibers exhibited a polygonal morphology without preferential orientation. (Fig.1)

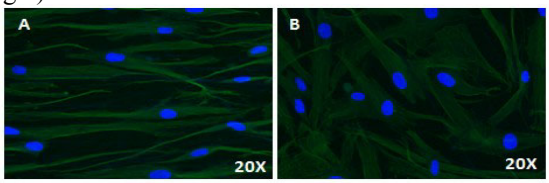

Figure 1. Cell morphology via a-tubulin immunostainning, A) Aligned; B) Random

Effects of nanofiber organization on hASC proliferation hASCs proliferated faster on the aligned fiber than those on random fibers during 1-week culturing period, as shown in figure 2.

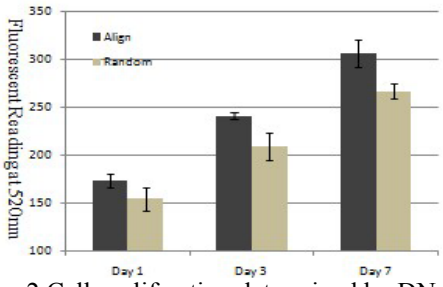

Figure 2.Cell proliferation determined by DNA assay

Gene Expression The expression of integrins and MMP-1 were compared over time on the aligned, random scaffolds and coverslips (Fig. 3). Cells on aligned nanofibers expressed less integrin- a5, which negatively regulated cell growth, compared to the other two groups. But aligned group had significant higher expression level of integrin- ß3 and MMP1, both of which could promote cell migration.

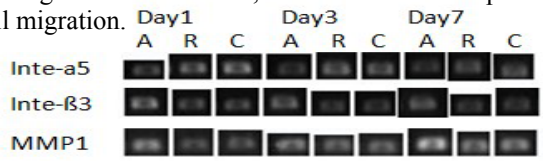

Figure 3. Gene expression on aligned (A), random (B) scaffolds and coverslips(C, control) over time

Cell Migration hASCs grown on aligned scaffold migrated much faster along the fiber alignment direction than those on random scaffold.(Fig. 4)

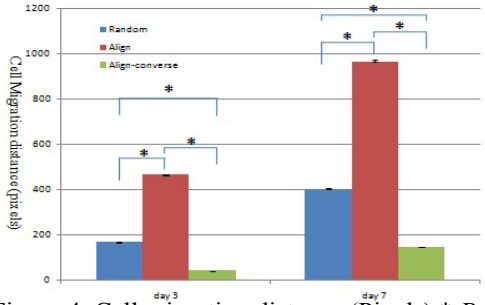

Figure 4. Cell migration distance (Pixels) * $P<0.05$

Conclusions: Our results clearly show the behavioral difference of hASCs on nanofibers with distinct organizations. hASCs grown on aligned scaffolds have elongated morphology, higher proliferation and migration rate, all of which occur during wound healing process. Moreover, the higher expression levels of MMP-1 and integrin ß3 and lower expression level of integrin a5 were consistent with the facts of upgraded cell proliferation and migration. These findings demonstrate the potential application of aligned nanofiber for wound repair.

References:

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