Nanobased Fiber Matrices for Wound Repair: Optimization for Human Skin Fibroblast Growth

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Statement of Purpose: In cases of severe and large amounts of skin loss, immediate coverage of the wound surface with a dressing is needed for rapid recovery. Wound dressings have been aimed to achieve the functions of natural skin, protecting the area from the loss of fluid and proteins, the removal of exudates, the inhibition of exogenous microorganism invasion and improved appearance. We have designed electrospun nanofiber matrices as wound repair materials. In this study we examined human cellular response to these matrices.

Methods: PLAGA 50:50 of $\overline{Mw}$ 71,000 (Lakeshore Biomaterials Inc., Birmingham, AL), THF, DMF (Fisher Scientific, Atlanta, GA), CellTiter 96® (Promega Madison, WI), Live/dead cell viability kit (Molecular Probes (L-3224), Human skin fibroblasts (hSF), EMEM (ATCC, Manassas, VA), FBS, Antibiotics and Trypsin–EDTA, were purchased from Sigma (St. Louis, MO). PLAGA solutions at concentrations of 0.2, 0.225, 0.24, 0.27 g/mL in THF: DMF (3:1) when electrospun using the optimized parameters of 20-gauge needle, voltage gradient of 1kV/cm and flow rate of 2 mL/h resulted in the fiber matrices having diameters in the nanometer range and were named as Matrix 1, Matrix 2, Matrix 3, and Matrix 4 respectively. Concentrations of 0.3, 0.35, 0.42 g/mL resulted in fiber diameter in micrometers named as Matrix 5, Matrix 6, and Matrix 7 respectively. hSF were seeded with a density of 50,000 cells/scaffold and cell study was continued for 28 days. hSF proliferation (MTS assay), viability (live/dead cell viability kit), and interaction with fiber matrices (SEM) were followed at various time points. Expression of various genes namely collagens type I, type II, type III and elastin were determined using a real time RTPCR [Applied Biosystems, ABI Prism, 7900 HT Sequence Detector System, 134 USA].

Results/Discussion: Fiber matrices, Matrix 1– Matrix 4 resulted in fiber diameters 150-225, 200-300, 250-467 and 500-900 nm whereas Matrix 5- Matrix 7 resulted in fiber diameters of 600-1200, 2500-3000 and 3250-6000 nm respectively. hSF showed fiber diameter dependent proliferation behavior at all time points studied (Figure 1A) as assessed by MTS assay. Nanofiber Matrices 3, 4 and 5 showed a statistically significant higher proliferation rate than Matrices 1, 2, 6 and 7 at all the time points studied. Figure 1B presents the proliferation trend with respect to fiber diameter. Statistically significant higher proliferation of hSF was observed on Matrix 3, Matrix 4 and Matrix 5. More uniformly distributed live cells were observed on Matrices 3-5. Progressive hSF growth was also visualized by SEM micrographs at all the time points (data not shown). Expression of collagen level followed the order of collagen II>III>I however, elastin expression remained constant (fold change ~1.5) at all the time points. Expression of collagen III peaked on day 28 whereas collagen I, on day 7 and Collagen II on day 14. Collagen III (figure 3 A) expression showed fiber diameter dependent behavior significantly higher on Matrices 3-5 on day 14 and 28.

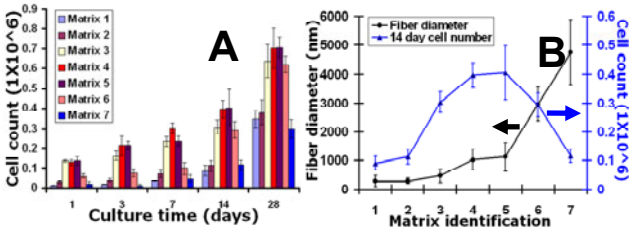

Figure 1(A) hSF proliferation (MTS) and (B) General observed hSF proliferation trend with varying fiber diameter.

Figure 2 (A) Confocal micrograph of Matrix 5 on day 28. hSF maintained normal morphology on all nanofiber matrices (B) SEM micrograph of Matrix 2 (C) Matrix 3 after 1 h hSF seeding. Rounded morphology was observed on Matrix 1, 2, 6 and 7 whereas Matrix 3, 4 and 5 showed spread morphology.

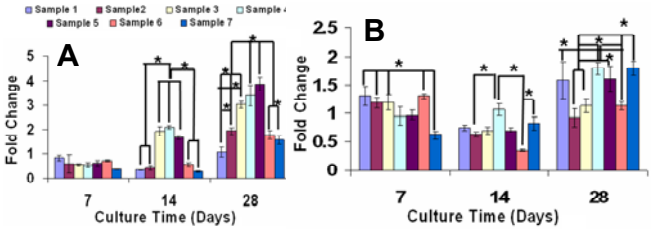

Figure 3 Gene expression (A) Collagen type III (B) Collagen type I by hSF on fiber scaffolds. Quantitative values were determined by the Delta-Delta method and normalized with the house keeping gene, β-Actin (Statistical significance at $p<0.05$, n=3).

Conclusions: This study has optimized fiber diameter range favorable for fibroblast proliferation. Nanofiber diameters ranging between 500-1200nm showed the highest proliferation and maintained fibroblast phenotype at all the time points studied. The design of fiber matrices with these optimized fiber diameters with appropriate growth factors to be used as bio-bandages are currently underway.

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