Effect of Piezoelectric Scaffolds on the Osteogenic Differentiation of Mesenchymal Stem Cells

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Introduction: Smart biomaterials such as piezoelectric scaffolds are of great interest for bone tissue engineering applications due to electrical activity being generated in response to minute mechanical deformation of cells. Specifically, scaffolds made of polyvinylidene fluoride (PVDF) and its copolymers are currently being investigated for potential tissue engineering applications [1]. Mesenchymal stem cells (MSCs) are sought as a cell source due to their proliferative capacity and ability to differentiate into bone and cartilage. In addition, these stem cells are extremely responsive to the microenvironment within a scaffold. In this study, the piezoelectric synthetic material, poly(vinylidene fluoride - trifluoroethylene) (PVDF-TrFE) was processed using the electrospinning technique to form a fibrous, three-dimensional smart scaffold. Recent work has shown that annealing PVDF-TrFE at temperatures above its curie temperature enhances the presence of the piezoelectric phase within the scaffold as compared to the unannealed electrospun PVDF-TrFE scaffolds [2]. This study evaluated the osteogenic differentiation of human MSCs on annealed and unannealed PVDF-TrFE scaffolds in static cultures. We hypothesize that MSCs on annealed PVDF-TrFE scaffolds will have an enhanced osteogenic differentiation as compared to cells on unannealed scaffolds.

Methods: 25% w/v PVDF-TrFE solution was dissolved in methyl ethyl ketone. The solution was electrospun at 25 kV onto a stainless steel plate. The electrospun PVDF-TrFE was later annealed at 135°C for 96 hours. The fiber morphology and the average fiber diameter were examined using scanning electron microscopy (SEM) and image J analysis. Human MSCs obtained from the whole bone marrow were seeded on electrospun scaffolds (6mm in diameter) at 3x104 cells/cm2 and cultured in standard growth media or osteoinduction media (osteo) until day 14. At days 7, 11 and 14, samples were harvested and analyzed for cell proliferation (Pico Green) and osteogenic differentiation (alkaline phosphatase activity by quantifying the conversion of para-nitrophenyl phosphate to para-nitrophenol (p-NP)) and mineralization (Quantichrom calcium assay kit). Samples were also fixed using 4% paraformaldehyde, stained for actin using rhodamine phalloidin, nucleus using 4',6-diamidino-2-phenylindole (DAPI) and collagen Type I using alexa fluor 488 conjugated to antihuman collagen Type I antibody and viewed using confocal microscopy. Results are expressed as mean ± standard deviation. The results were initially tested for normality (Shapiro Wilk test) and Levene’s equal variance test. One-way Analysis of Variance (ANOVA) and the post hoc multiple comparison using Tukey’s tests were applied using SPSS 20.0 software. Probability (p) values < 0.05 were considered statistically significant.

Results: The PVDF-TrFE fibrous scaffolds had uniform fiber diameter and inter-fiber spacing in SEM images. The annealed scaffolds had a mean fiber diameter of 4.4 ± 1.3 μm and 5.9 ± 2.0 μm for unannealed scaffolds. The osteogenic differentiation of human MSCs on annealed PVDF-TrFE scaffolds was enhanced as determined by alkaline phosphatase activity and mineralization (Figures 1 and 2, respectively) in comparison to cells on unannealed scaffolds. Mineralization at days 7 and 14 was significantly higher for the annealed scaffolds than the unannealed scaffolds. In addition, alkaline phosphatase activity was significantly higher at day 14 for annealed scaffolds than the unannealed scaffolds. The cell proliferation results indicated an increase in the mean cell number at all time points in osteo media as compared to standard growth media. The confocal images in Figure 3 showed the synthesis of Collagen Type I on both scaffolds by day 14.

Discussion: Electrospun PVDF-TrFE supports the differentiation of human MSCs towards the osteogenic lineage. On annealed materials, which have higher levels of the piezoelectric crystal phase, MSCs expressed enhanced levels of osteogenic markers. Future studies and analyses will be measuring the electrical output from the electrospun scaffolds at various physiological loading conditions and correlating those results to dynamic and static cultures.

Significance: Collagen and other extracellular matrix molecules have been shown to exhibit piezoelectric properties under mechanical loading, which can influence cell behavior. The use of synthetic piezoelectric materials as scaffolds may have great potential for bone tissue repair applications.

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Figure 1: Alkaline phosphatase activity on annealed and unannealed PVDF-TrFE scaffolds normalized to cell number. *<0.05 between annealed and unannealed at day 14.

Figure 2: Mineralization on annealed and unannealed PVDF-TrFE scaffolds. * <0.05 between annealed and unannealed at day 7 and 14.

Figure 3: Confocal images at day 14 for cells seeded on annealed (a-b) and unannealed scaffolds (c-d). Nucleus = blue, Actin = red, and collagen Type I = green, scale = 100 μm.