Evaluation of the Effect of Donor Variability on Stem Cell Response to Biomaterials

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Statement of Purpose: Previous research from our group indicated that nanofiber scaffolds promoted osteogenic differentiation of human bone marrow stromal cells (hBMSC) and similar gene expression to osteogenic supplement (OS) induced osteogenic differentiation [1]. Additionally, biological variation between hBMSC donors can result in differences in cell response to materials. To advance tissue engineering treatments into the clinic and to develop robust characterization of cell-material interactions, the response to materials for several donors needs to be characterized. In this study, we examined the cell response to five material groups (three material substrates and two substrates plus OS) for six hBMSC donors evaluated by differences in gene expression.

Methods: Poly(ε-caprolactone) (PCL) nanofibers (NF) were fabricated via electrospinning and PCL films via spin-coating (SC). Scaffold structure was visualized using scanning electron microscopy (SEM). hBMSCs were obtained from Tulane University and cultured in α-minimum essential medium with 16.5 % by vol. fetal bovine serum, 4 mmol/L L-glutamine and penicillin/streptomycin. Cells were expanded until passage four and then 130,000 cells were seeded onto 6-well plate sized substrates (13,000 cells/cm^2). There were five treatment groups for the study, including: tissue culture polystyrene (TCPS); TCPS plus OS (TCPS+OS); PCL spun coat film (PCL SC); PCL SC plus OS (PCL SC+OS); and PCL nanofibers (PCL NF), with six donors per treatment for a total of 30 samples. The samples were cultured for 14 d in a humidified incubator at 37 °C with 5 % by vol. CO_2 with regular media changes. mRNA was collected using a RNEasy Kit (Qiagen) and the collected mRNA was analyzed with Illumina microarrays (Human HT-12 v4, 47231 probes for 28688 transcripts). The data was analyzed using BRB Array Tools and DAVID Bioinformatics Resources 6.7 to identify differentially expressed genes and related gene ontologies. Microarray data was normalized to average of all TCPS samples and a 1.5 fold filter was applied.

Results: Out of 391 significantly expressed genes hierarchical cluster analysis was performed for the thirty samples evaluated. The clustering showed that OS treated samples predominantly grouped together. This indicated the strong effect of OS on hBMSC response. Removing the OS samples from the analysis to highlight the differences in donor response to materials indicated 402 significantly expressed genes (Figure 1). In this analysis, three donors sorted together (black boxes) while the other three donors sorted according to nanofiber treatment (dashed box). This suggested that there are two groups of donors present in the study: donors insensitive to nanofibers (sort by donor) and donors sensitive to nanofibers (sort by nanofiber). Further analysis of the differential gene expression highlighted differences in the gene ontologies and pathway analysis for these two groups.

Conclusions: Comparison of hBMSCs from 6 donors cultured in five treatment groups indicated that OS had the largest effect on gene expression. The second most influential parameter was donor and material effects were third (NF vs SC). Taken together, these data suggest that the regenerative response to biomaterials may vary by person, where implantation of nanofiber scaffold may enhance osteogenic differentiation of hBMSCs in some patients but not in others.

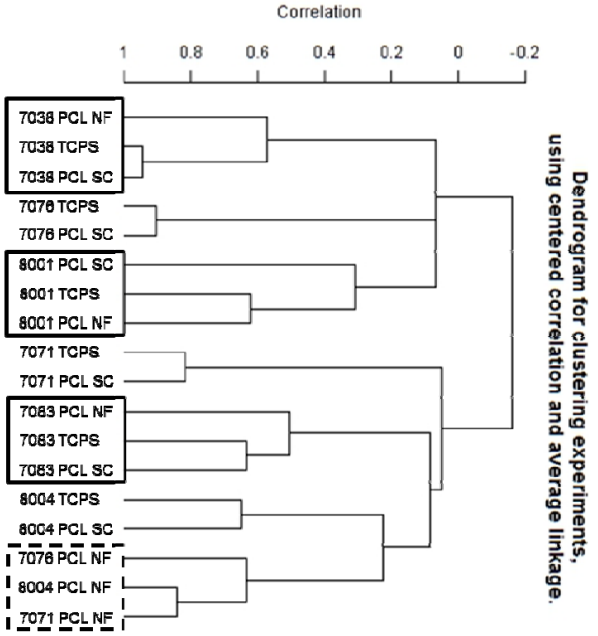

Figure 1. Hierarchical clustering of 402 significantly expressed genes related to hBMSC response to different materials. Three of six donors clustered by donor (black boxes) while the remaining three donors clustered by response to NF scaffolds (dashed box).

Reference: [1] Kumar G, et al. Biomaterials 2011; 32: 9188.

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