Alterations in metabolic activity of human umbilical vein endothelial cells cultured on gas-plasma treated poly(d,l-lactic) acid scaffolds

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Statement of Purpose: Polylactic acid (PLA) is a biocompatible and biodegradable polymer, making it an appropriate material for tissue engineering applications. Problems with this polymer as an implant biomaterial stem mostly from its hydrophobic nature. Radiofrequency glow discharge gas plasma treatment (GP) of PLA scaffolds functionalize the polymer surface with hydroxyl and carboxyl groups and etches the surface which increase its hydrophilicity, leading to increased cell attachment. Previous work with endothelial cells on GDGP treated scaffolds show increased cell proliferation at 4-6 days. It is hypothesized that an increase in vascular endothelial growth factor (VEGF) is responsible for the increase in cell proliferation. There is *in vivo* evidence of an increase of VEGF at 12, 24, and 72 days from endothelial cells on GP treated scaffolds. The development of a scaffold that would be able to stimulate growth factor secretion would improve vasculogenesis of regenerated tissues.

Methods: Gas-plasma treatment of the scaffolds was performed in a pure oxygen environment in a glow discharge system (PDC-32G, Plasma Cleaner/Sterilizer; Harrick Scientific Inc., New York) for 3minutes at 100W. After wetting scaffolds in media, HUVECs (PDL 4) were trypsinized and 1.6 X 10^5 cells seeded on 90% porous 10mm x 2mm PLA scaffolds fabricated by the vibrating particle, salt-leaching method (n=6 per group). Seeding efficiency was evaluated by determining the number of cells that did not attach to the construct and subtracting that from the seeding concentration. At 12, 24, 36, 48, 72, 168, and 288 hours, metabolic activity of the cells in the construct was evaluated with Alamar Blue (AB, Biosource International). Cell activity in basic endothelial cell medium (modified) MCDB 131 with 2% serum with and without 10 ng/ml VEGF was compared. ANOVA with repeated measures was used for statistical analysis.

Results: Seeding efficiency was 80% resulting in approximately 128,000±5000 cells seeded on each construct with no significant difference between control and treated scaffolds. The metabolic activity between the control and treated scaffolds in media without VEGF at each time point was statistically significant at all time points except 288hours ($p<0.05$). There was no statistically significant difference in metabolic activity between treatment groups in the cells cultured in media containing VEGF.

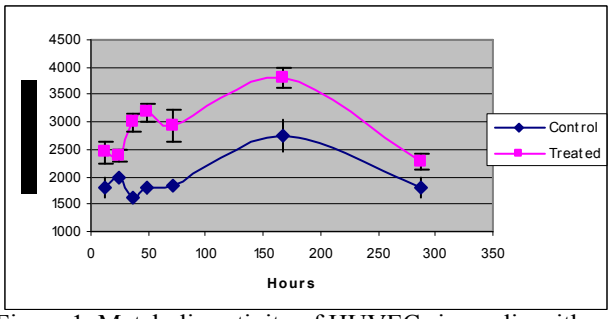

Figure 1. Metabolic activity of HUVECs in media without VEGF (n=6)

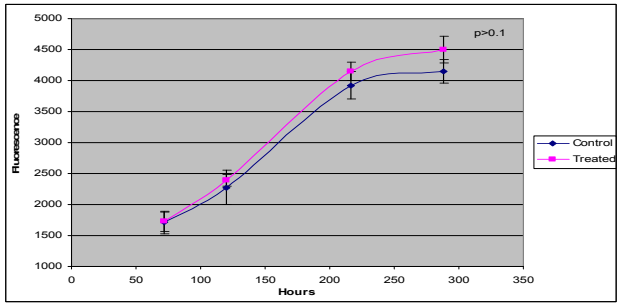

Figure 2. Metabolic activity of HUVECs in VEGF containing media (n=3)

Conclusions: The cells cultured without VEGF showed a significant difference in metabolic activity. However, the cells cultured in media containing VEGF, similar metabolic activity levels were seen in the control and treated scaffolds. These results suggest that the gas-plasma treated scaffolds may have an effect on the expression of growth factors, possibly VEGF. This expression may not be detectable in a media that contains VEGF.

The factors involved in the differential between cell growth in control and treated scaffolds needs to be determined. Gene expression analysis using microarray analysis of the cells in media without VEGF and confirmatory rtPCR will reveal up and down regulations of genes if they are present. ELISAs of the cell supernatant would ascertain differences in the amount of growth factors secreted. Gas-plasma treatment of polymer scaffolds may be a valuable surface treatment method for polymer scaffolds by not only increasing cell attachment, but also stimulating growth factor production.

References:

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