Adipose-derived Stem Cell Attachment to Biomaterials

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Statement of Purpose: Adipose tissue contains a stromal population that consists largely of microvascular endothelial cells, smooth muscle cells, fibroblasts, and stem cells[1]. These adipose-derived stem cells (ASCs) share many characteristics with bone marrow mesenchymal stem cells and may prove useful in a variety of applications. ASCs are particularly suitable for tissue engineering due to their abundance, ease of isolation, and high proliferative potential. In addition, ASCs could prove useful in altering the foreign body response to implants, such as glucose sensors. The foreign body reaction often results in a fibrous and avascular capsule that impedes mass transport and leads to device failure. Since the foreign body reaction in adipose tissue is less aggressive than in lean tissues[2,3], the attachment of ASCs to a biosensor could mitigate the response and increase vascularity around the implant. For application in either tissue engineering or sensor wound healing, ASCs will need to be attached to a suitable biomaterial. This study was designed to isolate ASCs and adhere them onto medical grade polyimide and polyurethane, two commonly used glucose sensor materials. In addition, cell retention following shear was quantified.

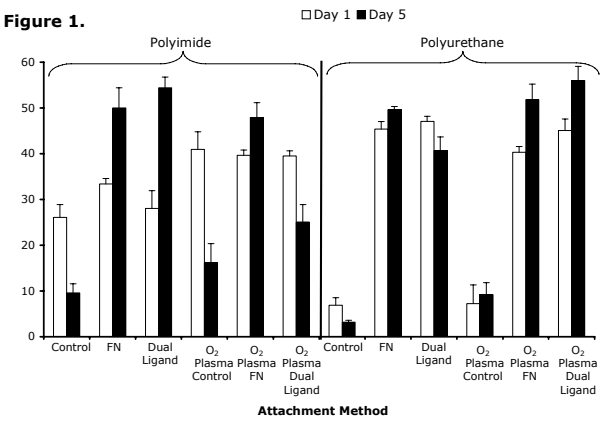
Methods: Cells: Fat was harvested from the inguinal fat pads of Lewis rats and processed by collagenase digestion and centrifugation to obtain ASCs. The cells were frozen at passage 1 and thawed as needed. All cells used for attachment were at passage 2.

Attachment: Three methods of cell attachment were analyzed: (1) control, (2) fibronectin, and (3) dual ligand, avidin/biotin plus fibronectin. In addition, these three cell attachment methods were also performed after the materials had been exposed to oxygen plasma etching at 100 watts for 40 minutes. For all attachment methods, cells were seeded at 800 cells per mm^2 of material, allowed to attach and grow, and assessed on either day 1 or day 6. The control attachment protocol consisted of washing the material with PBS prior to the addition of cells. The fibronectin attachment protocol was composed of treating the materials with 25 µg/ml fibronectin for 2 hours prior to cell seeding. The dual ligand protocol consisted of treating the material with a heterogenous mixture of 25 µg/ml fibronectin and 0.4 mg/ml biotinylated bovine albumin (BSA) for 2 hours followed by 0.5 mg/ml avidin for 40 minutes, and the cells were biotinylated by treating with 1 mM sulfosuccinimidyl-6-(biotinamido) hexanoate (NHS-LC-biotin) for 30 minutes. For imaging, the cells were stained with a live/dead stain, pictures were taken, and the percent of surface coverage by cells was analyzed.

Strength of Attachment: ASCs were attached to materials for 12 hours using the treatments described above with a cell density of 125 cells/mm^2. Flow experiments were performed for 5 minutes at room temperature with increasing sheer stresses of 5 dyne/cm^2, 20 dyne/cm^2, and 50 dyne/cm^2. Additionally, a final flow of water for 10 minutes at 40 dyne/cm^2 was performed to cause detachment. The flow chamber was opened between each change in shear rate and 15-20 microscopic fields were photographed. The percent surface coverage by cells was measured.

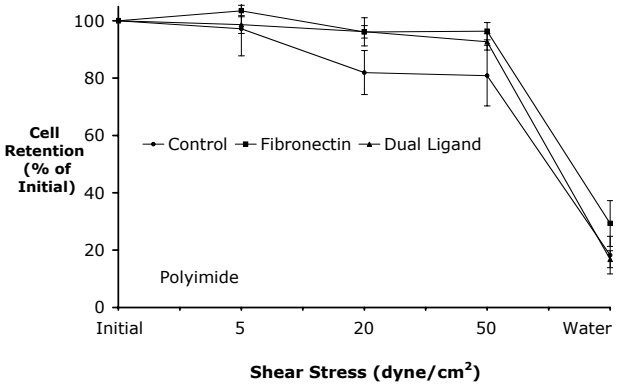
Results / Discussion: Figure 1 shows the percent cell coverage (±SEM) for the six attachment methods.

Figure 1.



Fibronectin and dual ligand treatments both resulted in increased cell coverage. For polyurethane, the oxygen plasma etching alone did not increase cell coverage; however, for polyimide the oxygen plasma etching did result in slightly higher cell coverage.

Figure 2.



The cells were adhered strongly to polyimide even after exposure to flow at 20 dynes/cm^2. The fibronectin and dual ligand treated materials had increased cell retention at the higher shear rates of 20 and 50 dynes/cm^2 when compared to the control.

Conclusions: These results demonstrate that ASCs can be isolated, cultured, and firmly attached to both polyimide and polyurethane. The success of attachment depended on the material and the attachment method used. Several attachment protocols yielded similarly low cell detachment after high shear exposure.

References:

1. Zuk, PA et al, Tissue Eng (2001), 7, 211-28.
2. Wisniewski, NA et al, Am J Phys Endo & Met (2002), 282, E1316-23.
3. Williams, SK et al, J Biomed Mat Res (1997), 35, 473-81.

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