Comparing Endothelial Cell Function on Self-Assembled Type IV Collagen and Its Components

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Introduction:

It was suggested by Dixit, et al. that covalent binding of extracellular matrix proteins to the material surface could greatly increase the patency of a small diameter vascular graft (SDVG). Previous work in our laboratory has focused on the covalent binding of Type IV Collagen (CNIV) to the surface of hydroxylated polytetrafluoroethylene (PTFE) and its self-assembly. CNIV is the primary scaffold component in the vascular extracellular matrix and is important in biochemical regulation of gene expression, differentiation, migration and adhesion of endothelial cells (Xu, et al.).

On-going research in our laboratory has shown that endothelial cells grown on CNIV self-assembled on PTFE does not show increased superoxide levels when triggered with hydrogen peroxide (unpublished results). Due to the high cost of CNIV, we are comparing the functionality of CNIV versus its components.

Currently, our lab has primary data suggesting that endothelial cells on RGD modified hydroxylated PTFE show a decrease in oxidative stress compared to endothelial cells tissue culture polystyrene when exposed to hydrogen peroxide. We are now working on proving that the pathways in Figure 1 are relating to the cell biomaterial contact.

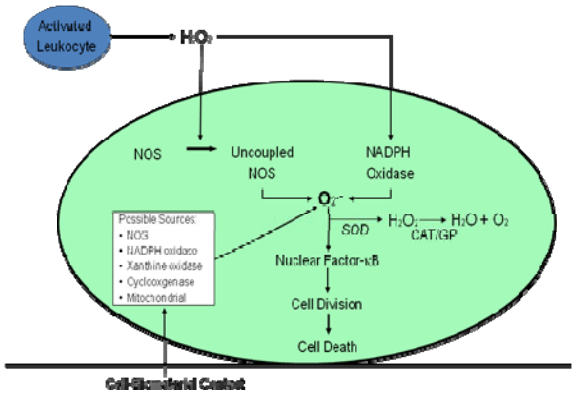
Materials and Methods:

A self assembling collagen IV layer on PTFE was made according to Yoder et al. Briefly, PTFE was washed with acetone and then removed. Then 42 ml of acetone and 30.9 µL of EDC were allowed to react with the PTFE for 1 hour. After an hour, the solution was removed and the PTFE was washed with PBS. Following the washing, 42 mL of PBS and 0.4µM of collagen IV were added for 24 hour. For the coupling of GRGDS, 30µM of the peptide in PBS was substituted for the CNIV in the previous reaction.

To test endothelial cell function on these surfaces, 200,000 porcine aortic endothelial cells (PAEC) were cultured in medium 199 with 10% fetal bovine serum on 5cm x 5cm pieces of the CNIV- and GRGDS- coated PTFE. Once the cells reached confluence they were analyzed for increases in intracellular superoxide levels before after exposing the PAEC to hydrogen peroxide.

Briefly, PAEC were exposed to vehicle or 60 µmol/L H_2O_2 in serum-free M199 for 1 hour. Incubation was continued for an additional 30 minutes in the presence of 2 µM dihydroethidium. After incubation, PAEC were washed, placed in phenol-red-free and serum-free M199. At the end of the testing period, samples were washed and placed into a Perkin-Elmer LS-55 fluorometer. Fluorescence intensity of the total sample was used as an indicator of intracellular $O_2^{\cdot-}$.

Results and Discussion:



Initial results show an eight time increase in intracellular SO induced by hydrogen peroxide on tissue culture polystyrene compared to the self assembled collagen IV and GRGDS on PTFE. Primary data has also showed an increase in intracellular superoxide in the PAECs on TCP versus the CNIV only. These results were before SO was induced with hydrogen peroxide.

Conclusions:

The attachment of biological molecules including adhesion proteins is a critical need for the development of biomaterials. We have shown a method by which that can be affected using a novel, proprietary hydroxylation process.

Our initial results have shown an importance in optimizing the biomaterial with adhesion peptides and CNIV. The initial results show that the PAECs have a greater ability to handle an oxidative stress when the surface is optimized with CNIV or the adhesion peptide RGD. We are currently working on other factors in the PAECs to compare against tissue culture polystyrene and CNIV and GRGDS on PTFE.

We hypothesize, that interfacing the PAECs with the biomaterial via the RGD adhesion peptide, we can create a cost effective, suitable interface for the PAECs and their ability to handle certain oxidative stress factors.

References:

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