Shear-Dependent endothelial cell attachment to polymeric biomaterials

Xin Wang, Daniel E. Heath, Stuart L. Cooper
William G. Lowrie Department of Chemical and Biomolecular engineering
The Ohio State University,Columbus,OH 43210,USA

Statement of Purpose: This research studies the adhesion of endothelial cells (ECs) and human blood outgrowth endothelial cells (HBOECs) to polymeric biomaterials under flow. Very few studies have been conducted on the adhesion of these cell types under shear stress; however, both cell populations are present in small quantities in blood presenting the possibility of scavenging them from circulation and using them in the endothelialization of blood-contacting medical implants.

Methods: The polymers used in this research are Tissue Culture Polymer Styrene (TCPS) and a methacrylic terpolymer (H20) polymerized from 20 mol% hexyl methacrylate (HMA), 78 mol% methyl methacrylate (MMA) and 2 mol% methacrylic acid (MAA) (Fussel GW, et al, Biomaterials. 2004; 25; 2971-2978).
Cell adhesion was carried out in a radial flow chamber allowing real time quantification of cell adhesion as a function of shear rate. The radial flow chamber was mounted on a Nikon inverted microscope, and ImagePro software was used to automatically select observation fields and count cell number (Dickinson RB, et al, Bioengineering, Food and Natural Products.1995, 41; 2160-2174).

Results and discusssion: HUVEC and HBOEC adhesion studies were carried out on TCPS and H20. At low flow rates, both cell types adhered linearly with time, but at higher shear rates, a steady state was reached at lower cell densities illustrating shear dependent cellular adhesion as observed in Figure 1.

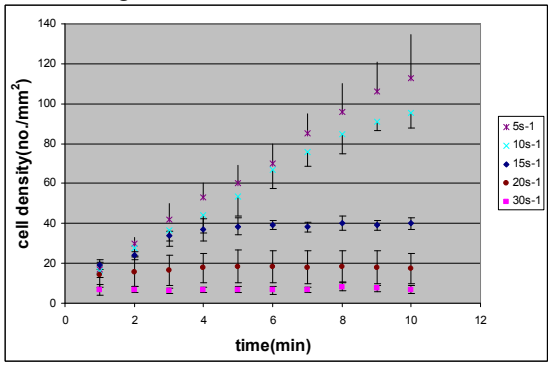

Figure 1. Real time shear dependent HBOEC adhesion to TCPS

An important and often neglected consideration in studies of cell adhesion is the cell lifting conditions. It was observed even in the absence of serum proteins that HUVEC and HBOEC adhesion was highest on EDTA lifted cells, somewhat lower for 0.025% trypsin/EDTA lifted cells and lowest on 0.25% trypsin/EDTA lifted cells (Fig 2). While these changes in cell adhesion may be attributed to damage of transmembrane proteins due to trypsin digestion, it was interesting to see an effect on adhesion even though there was minimal adsorbed protein on the polymers (Brown MA, et al, Biomaterials. 2007, 28; 3928-3935) (Charles CA, et al, Langmuir. 2009, 25; 5725).

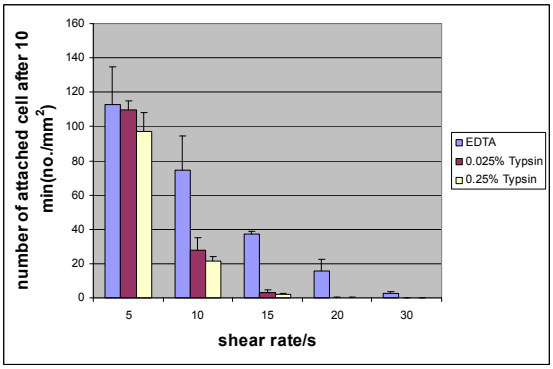

Figure 2. HBOEC attachment on TCPS with different lifting conditions after 10 minutes

A comparison of EDTA-lifted HUVEC and HBOEC cell attachment at 10 minutes on TCPS is shown in Figure 3. The number of adherent HUVECs is less than the number of HBOECs for the range of shear rates examined (5 to 30s-1). At higher shear rates, the adhesion ability of HBOECs is higher by a factor of 3 to 4 compared with that of HUVECs (Figure 3). Figure 3 also shows that HBOECs are less sensitive to shear rate when adhering to H20.

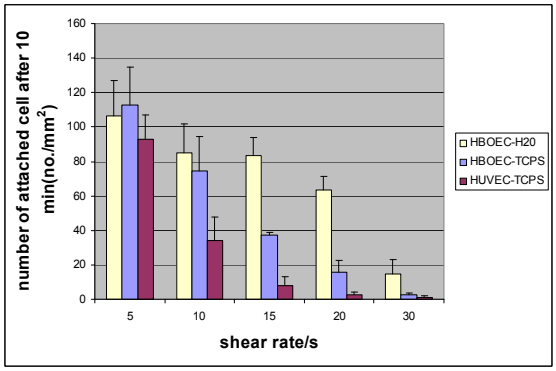

Figure 3. Dynamic adhesion of HUVECs and HBOECs to TCPS and H20 after 10 minutes

Conclusions: Our results suggest that the highly proliferative HBOECs (Lin Y, et al, J of Clin Invest. 2000, 105; 71-77) also have higher adhesion propensity than EC under flow and thus may have the potential to contribute to endothelialization of a device such as a vascular graft containing high affinity HBOEC binding sites. Further work on the H20 biomaterial involves the incorporation adhesion ligands to promote specific binding of HBOEC (Anka NV, et al, Biomaterials. 2008).