Confluent and aligned growth of endothelial cells on nanoparticle arrays through focal adhesion and endocytitic mechanisms

Phat L Tran[a], Jessica R Gamboa[a], Katherine E McCracken[b], Mark R Riley, Ph.D.[ab], Marvin Slepian, M.D.[c], Jeong-Yeol Yoon, Ph.D.[ab]

[a]Biomedical Engineering Graduate Interdisciplinary Program, [b]Department of Agricultural and Biosystems Engineering, [c]Department of Medicine, The University of Arizona, Tucson, Arizona 85721-0038, USA

The ability to endothelialize and form monolayers of endothelial cells on a substrate could improve the performance of artificial small diameter vascular grafts. It has been shown that microscale technologies and bioactive ligands can promote cell adhesion, guide migration, and manipulate proliferation of endothelial cells[1,2]. Furthermore, nanopatterns can influence endothelial cell adhesion and enhance cell retention under shear stress[3]. Here, we describe methods of size-dependent self-assembly (SDSA)[4] and electron beam lithography to fabricate arrays of nanoparticles that are covalently conjugated with a cell adhesive ligand, GRGDSPK (RGD). Confluent human umbilical vascular endothelial cells (HUVECs) seeded and cultivated under static and flow conditions align and arrange in response to material properties. The nanoparticle arrays provide a very high degree of control over surface chemical and mechanical properties when utilizing bioinert substrates like polymethyl methacrylate (PMMA). HUVECs cultured under static conditions at 36 and 72 hours appear to adhere and proliferate better on arrays with RGD conjugated 100 nm nanoparticles in comparison to on other surface templates (Figure 1A). Since the bioactive ligand is spatially anchored by nanoparticles, endothelial cells are likely to habitat and saturate over the manipulated surface through the focal adhesion mechanism. After 96 hours of culture under flow conditions, focal adhesions like vinculin appear to dissipate, leaving behind a network of cells on the nanoparticle array. HUVECs are known to be capable of internalizing particles, but when the formation of actin filament are blocked by Cytochalasin B, HUVECs do not internalize particles and have decreased spreading and proliferation. Therefore, endocytic mechanisms may play a crucial role in cell adhesion, spreading and aligning when particles are anchored in the wells (Figure 1B&C). HUVECs cultured under a flow condition aligned their cell centroid in response to shear stress as well as to different surface micropatterns like serpentine and checker box. Cells aligned in the direction of the flow when grown on nanoparticle arrays, but at an angle on serpentine channels and perpendicular to flow under checker box pattern. This work has shown that endocytosis in conjunction with focal adhesions guided through nanoparticle arrays could permit a high degree of cellular locationing. Further investigation in the influence and the effect of different sizes of intracellular particles as well as different configurations of array under different flow conditions can provide greater control in endothelialized small diameter vascular grafts and may provide a better understanding of endothelial cell sensing of chemical and mechanical surface queues.

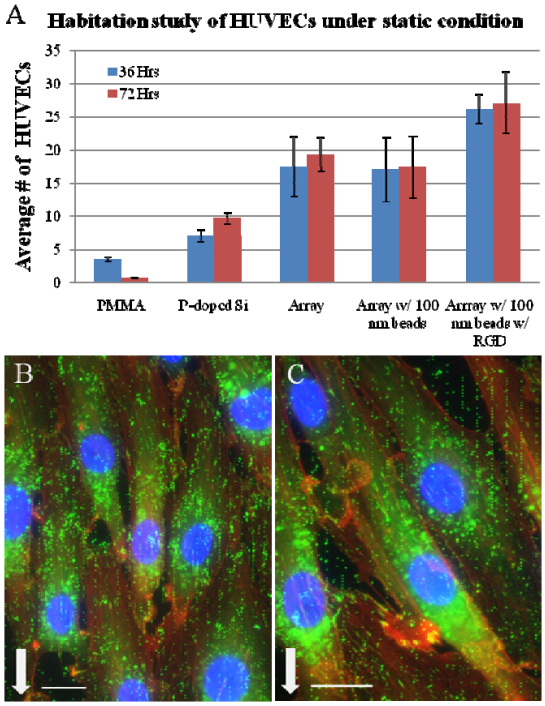

Figure 1. Habitation and alignment of HUVECs. A) Adhesion study of HUVECs under static condition over various surface templates. Results suggest that HUVECs grow better with RGD conjugated particles array. B) Under flow condition, HUVECs grow in a mixture of linear and C) mostly slanted alignment. They are also capable of internalize RGD conjugated nanoparticles array within 24 hours. The endocytosis of particles could have assisted in the habitation and endothelialization of HUVECs on PMMA surface since particles are anchored in the well. The arrow indicates the flow at about 150 ml per minute or 0.2 dyne per cm^2. The scale bar is 20 µm.

References

1. Khademhosseini A, Langer R, Borenstein J, and Vacanti JP. PNAS. 2006; 103(8): 2480-2487.
2. Moon JJ, Hahn MS, Kim I, Nsiah BA, West JL. Tissue Eng. A. 2009; 15(3): 579-585.
3. Zorlutuna P, Rong Z, Vadgama P, and Hasirci V. Acta Biomaterialia. 2009; 5:2451-2459.
4. Tran PL, Gamboa JR, You DJ, and Yoon JY. Anal. Bioanal. Chem. 2010; 398(2): 759-768.