Responses of Vascular Endothelial Cells to Photo-embossed Topography on Polymer Films and Fibers

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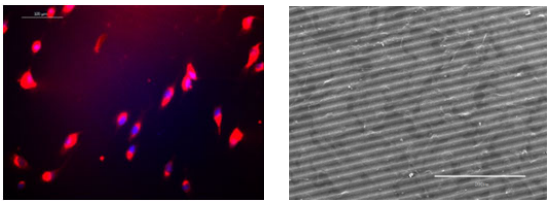
Introduction: A crucial problem of artificial vascular implants, especially those with diameters less than 4 mm, is thrombus and neointima formation. One approach to prevent thrombosis and to improve the biocompatibility of artificial implants is to create a functional, quiescent monolayer of endothelial cells on the lumenal surface of implants (Lin et al. J Biomed Mater Res. 1994; Uttayarat et al. J Biomed Mater Res. 2005). With the premise that surface topography influences cell behavior and the increased surface roughness could enhance the adhesion and growth of endothelial cells (Chung et al. Biomater, 2003), we patterned polymer films and electrospun fibres with photo-embossing and investigated responses of human umbilical vein endothelial cells (HUVECs) on these patterned substrates.

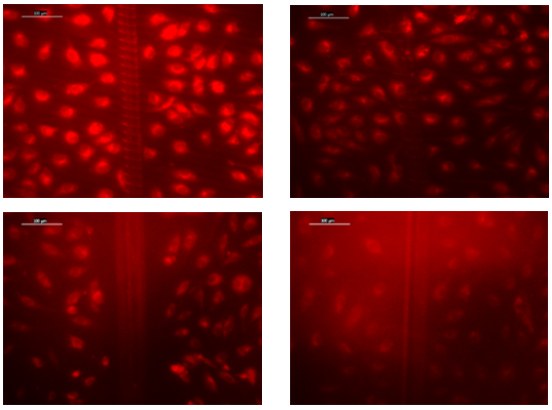
Methods: Photo-embossing is a new and simple technique to create surface textures, where a mixture of a multifunctional monomer, a polymer binder and a photo-initiator are used. The photo-embossing procedure involves creating a thin film from the mixture by ultra violet (UV) light irradiation through a patterned contact photo-mask and followed by heating and flood UV exposure (Hughes-Brittain et al. MRS Proceedings, 2012) In the study, the polymer binder Poly(methyl methacrylate) (PMMA) with triacrylate (TPETA) monomer and biocompatible photo-initiator Irgacure 369 are used. The film is prepared by wire bar coating which resulted in a thickness of 60 ± 5μm. It is then photo-embossed with the pitch and height of 20μm and 2.5μm respectively. Fibres are prepared by electrospinning with a diameter of ~1μm, and then photo-embossed with the pitch at 1μm. HUVECs are seeded on to these substrates in 24 well-plates. Cell viability and cell migration are tested on the film. Cell adhesion is studied on fibres. Comparisons are made between substrates with and without photo-embossed textures.

Results: After 7 days of culture, HUVECs on glass slides (GS), PMMA, non-embossed photopolymer films (non-em PMMA-TPETA) and embossed photopolymer films (em PMMA-TPETA) are studied. Good biocompatibility is observed for the photopolymer film. On em PMMA-TPETA film, clear cell alignment to the surface texture is observed at day 1. Cells spread as the time increases (Figure1). Wound-healing assay are used to investigate cell migration both on smooth and photo-embossed films. The size of the wound is 200±10μm. Cells are stained and observed under a fluorescence microscope. The results show that after 2 hours, there are noticeable differences between the embossed films and the smooth films. After 3 hours, cells on the textured film have almost filled the wound, whereas cells on the smooth film show a significant gap at the wound (Figure 2).



a) b)

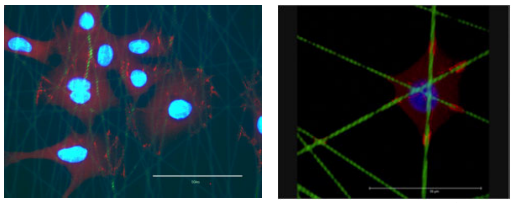
Figure 1 HUVECs on em PMMA-TPRTA film. a) day 1, b) day 7.



a) b)

Figure 2. Wound-healing assay on photo-embossed (top) and smooth (bottom) films. a) 2 hours, b) 3 hours.

Fibre diameter and orientation are seen to have strong influence on cell attachment. Cells begin to grow from the fibre crossings and align with fibres (Figure 3a). Focal adhesions show stronger signal intensities at the groves of the surface texture on fibres (Figure3b).



a) b)

Figure 3. HUVECs on photo-embossed fibres. a) Cells grow at fibre crossings and align with fibres. b) Close look of cell adhesion sites.

Conclusions: The photopolymer has good biocompatibility and can be used as a potential material in cell and tissue engineering with relative easy preparation. Photo-embossed surfaces accelerate HUVEC migration along the texture and enhance their adhesion to fibres. It could be used to promote HUVEC monolayer on the surface of artificial vascular implants