In Vitro Assembly of Micropatterned Cell Sheets for Vascular Tissue Engineering

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Statement of Purpose

Vascular disease is the leading cause of death in the United States. While there have been developments in synthetic materials to create small diameter artificial vessels, the lack of vessel strength and compliance has been a deterring issue. Ideally it would be advantageous to use human cells instead of synthetic materials to prevent an immune response. The organization of cells is an important factor to consider when developing grafts to properly mimic the in vivo extracellular matrix (ECM) deposition to form properly functional vessels. In this study we describe a practical method to form organized cell sheets which can be assembled layer by layer to form the tunica media of small diameter vessels.

Methods

Micro ridges and grooves, with groove periods of 5µm, 10µm, 15µm and 20µm, were created on polydimethylsiloxane (PDMS) as previously described[1]. 3-Aminopropyltriethoxysilane (APTES) was vapor phase deposited on the oxidized micropatterned PDMS samples. Samples were then placed in 12 well dishes and sterilized by placing under UV light for 30 minutes. Mesenchymal stem cells (MSCs) were seeded at 20,000 cells per well. Cells were then stained with Calcein AM and Ethidium homodimer 1 (Invitrogen L3224) at Day 1, 2, 4, and 7 and pictures were taken under fluorescent microscopy. MSCs were seeded on another set of samples at 20,000 cells per well and stained with MTT (Invitrogen V-13154). Absorbance at 570nm was recorded at Day 1, 3, 5 and 7.

Results

Mesenchymal stem cells attached to the deposited APTES micropatterned PDMS and aligned on top of the ridges within 2 days of seeding. Cells seeded in ridges 5µm spaced apart had 5-10% of cells not aligned along the ridges due to cells being larger than this spacing. 10µm spacing was found to be most optimal for cell alignment (Figure 1). 15 and 20µm spaced ridges aligned cells well but the spacing is thought to be too far for cells to communicate with each other and deposit a proper extracellular matrix.

A MTT proliferation assay was performed in order to determine if the deposited APTES hindered proliferation. Figure 2 shows that MTT absorbance increased with the number of days cells were seeded, illustrating APTES is a cell friendly substrate.

Discussion

The ability of MSCs to attach, align, proliferate, and finally detach as cell sheets, while retaining their orientation, with intact ECM shows great promise for tissue engineering applications. Poly *N*-isopropyl-acrylamide (PNIPAAm) and its derivatives belong to a group of thermo-responsive smart polymers. They are hydrophobic above a critical temperature (e.g. 32°C) at which the cells can attach and grow, whereas below the critical temperature they become hydrophilic, thus enabling cells to detach from the surface. We have shown that sheets of highly aligned cells can be formed using surfaces containing microgrooves that are created by a simple peeling process.[1] Future research will be to fabricate micro-grooved PNIPAAm surfaces that allow the creation of aligned cell sheets to be assembled *in vitro* for vascular tissue engineering applications.

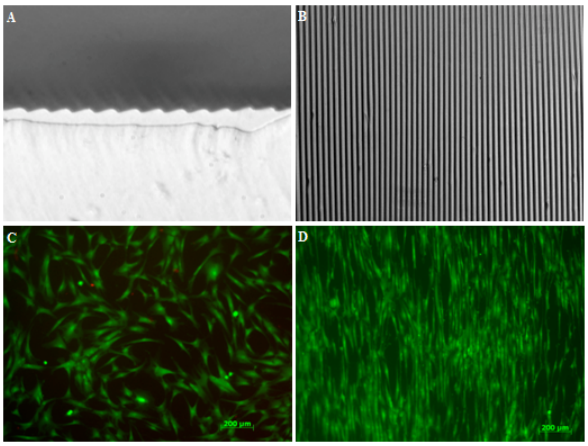

Figure 1: (A) Phase contrast of cross section of ridges at 60x. (B) Phase contrast top view of 10µm ridges at 10x. Flourescent image of MSCs at Day 4 on a smooth surface (C) and on a surface containing10 µm ridges (D).

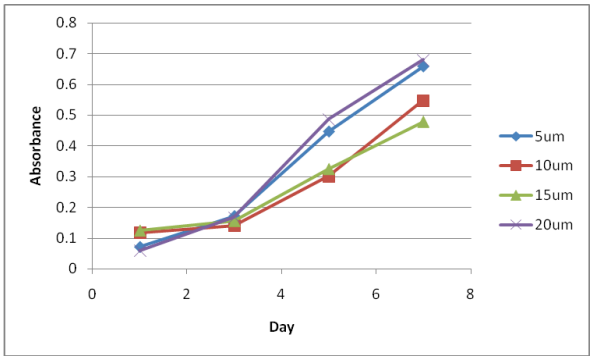

Figure 2: MTT absorbance of MSCs at day 1, 3, 5 and 7.

References

1. Cai Yj, Zhang Newby B-m, Fracture-induced formation of parallel silicone strips. *J. Mater. Res.* 2010; 25(5): 803-809.