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Influence of micropattern width on differentiation of human mesenchymal stem cells to vascular smooth muscle cells

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ABSTRACT

In recent years, various approaches have been taken to generate functional muscle tissue by tissue engineering. However, *in vitro* methods to generate smooth muscle with physiologically aligned structure remains limited. In order to mimic the *in vivo* highly organized structure of smooth muscle cells, we used micropatterning technology for engineering parallel aligned cells. In this study, a gradient micropattern of different width of cell-adhesive polystyrene stripes (5, 10, 20, 40, 60, 80, 100, 200, 400, 600, 800 and 1000 μm) was prepared and the effects of micropattern width on human mesenchymal stem cells (hMSCs) orientation, morphology and smooth muscle cell differentiation were investigated. The width of micropattern stripes showed obvious effect on cell orientation, morphology and smooth muscle cell differentiation. The cells showed higher degree of orientation when the micropattern stripes became narrower. Higher expression of calponin and smooth muscle actin was observed among the narrow micropatterns ranging from 200 μm to 20 μm, compared to the non-patterned area and wide micropattern areas which showed similar levels of expression.

1. Introduction

Vascular smooth muscle cells (VSMCs) perform a crucial function in angiogenesis, mechanical support of vessels and blood pressure control [1]. To successfully construct tissue-engineered blood vessels, regeneration of functional VSMC layer is required. Since the lifespan of autologous VSMCs derived from elder donors who are the majority of potential recipients of vascular grafts for the treatment of cardiovascular diseases [2,3] is limited, increasing the lifespan of autologous VSMCs from elder donors is still a big challenge. Human mesenchymal stem cells (hMSCs) can differentiate into a variety of cell types including myocytes [4] and they are considered to be nonimmunogenic [5]. Their potential as a source of smooth muscle progenitor cells for vascular engineering approach has received widespread attention.

To direct hMSCs differentiation to a VSMCs lineage, an appropriate microenvironment that mimics the *in vivo* physicochemical and biological cues is desirable. One approach using micropatterning technique to regulate cell alignment is considered effective on mimicking the structure, composition and function of muscular tissue. Huang et al. [6] have reported that myoblasts on micropatterned polymer surfaces had well organized F-actin assembly to the direction of microgrooves, together with enhanced level of myotube formation at early time point. In another report, Tay et al. [7] have developed a biomimetic PLGA micropattern to modulate hMSCs response. The cells were well aligned along the micropattern and the myogenic activity of hMSCs was promoted. It has also been reported to use micropatterning network structure to spatially control MSCs fate within 3-dimensional hydrogels [8]. These researches highlight the importance of physical cues in creating aligned muscle for tissue engineering and muscle regeneration applications. However, systemic comparison of cell alignment influence on stem cells behavior and differentiation

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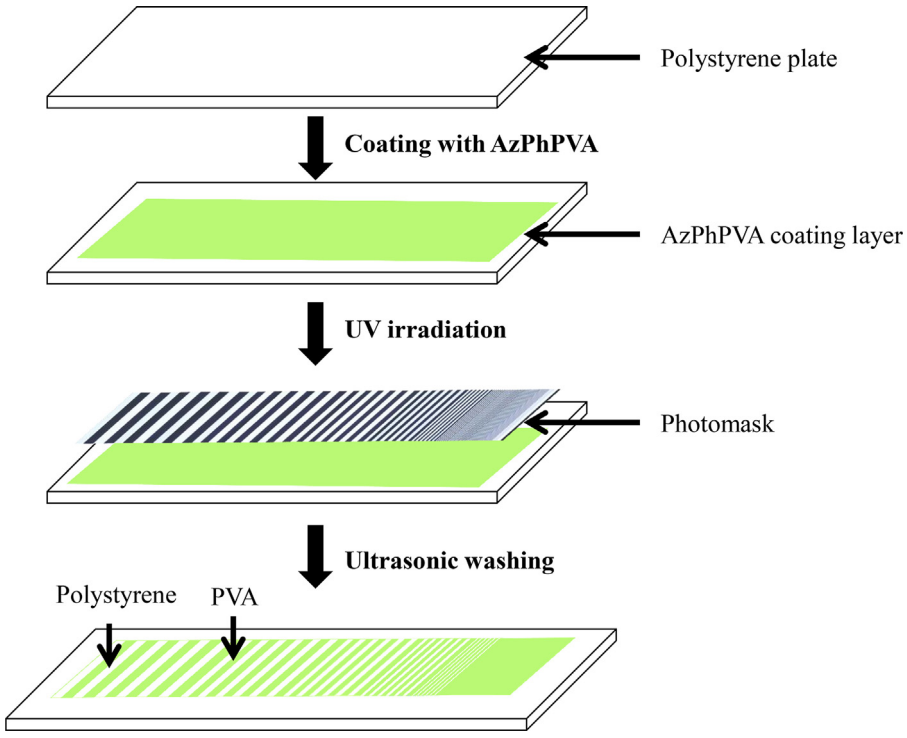

Fig. 1. Preparation scheme of PVA-micropatterned polystyrene plate surfaces.

requires the cells to be controlled at different degree of alignment on a single culture plate. Kim et al. have used anisotropic micro- and nanotopographic pattern arrays with variable groove widths to compare the their influence on cell shape, orientation and migration [9].

In our previous studies, a photolithographic method was developed to prepare poly(vinyl alcohol) (PVA)-micropatterned surfaces to investigate the influence of cell spreading area, cell geometry and surface composition on osteogenic and adipogenic differentiation of hMSCs [10–12] at a single cell level. The method has also been used to prepare gradient micropatterns to control cell density for investigation of cell density influence on osteogenic and adipogenic differentiation of hMSCs [13,14]. This method has the advantage over other micropatterning methods for creation of

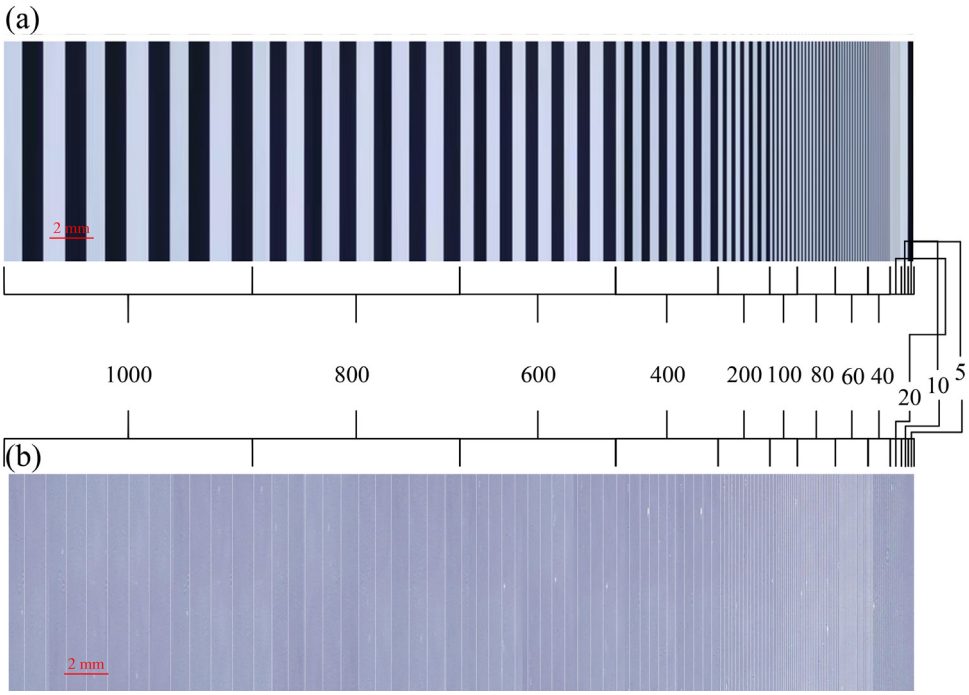

Fig. 2. Phase contrast micrographs of a photomask (a) and the PVA-micropatterned surface (b). The width of stripes was 5, 10, 20, 40, 60, 80, 100, 200, 400, 600, 800 and 1000 μm.