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# Circumferential alignment of vascular smooth muscle cells in a circular microfluidic channel

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ABSTRACT

The circumferential alignment of human aortic smooth muscle cells (HASMCs) in an orthogonally micropatterned circular microfluidic channel is reported to form an *in vivo*-like smooth muscle cell layer. To construct a biomimetic smooth muscle cell layer which is aligned perpendicular to the axis of blood vessel, a half-circular polydimethylsiloxane (PDMS) microchannel is first fabricated by soft lithography using a convex PDMS mold. Then, the orthogonally microwrinkle patterns are generated inside the half-circular microchannel by a strain responsive wrinkling method. During the UV treatment on a PDMS substrate with uniaxial 40% stretch and a subsequent strain releasing step, the microwrinkle patterns perpendicular to the axial direction of the circular microchannel are generated, which can guide the circumferential alignment of HASMCs during cultivation. The analysis of orientation angle, shape index, and contractile protein marker expression indicates that the cultured HASMCs reveal the *in vivo*-like cell phenotype. Finally, a fully circular microchannel is produced by bonding two half-circular microchannels, and the HASMCs are cultured circumferentially inside the channels with high alignment and viability for 5 days. These results demonstrated the creation of an *in vivo*-like 3D smooth muscle cell layer in the circular microfluidic channel which can provide a bioassay platforms for in-depth study of HASMC biology and vascular function.



## 1. Introduction

One of the main issues in tissue engineering is mimicking the well-defined three dimensional microvascular architecture found in native tissues in the human body [1]. To understand the functional tissue engineered vascular remodeling, it is essential to mimic the *in vivo* environments with *in vitro* model. The vascular structure consists of intima, media, and adventitia from inner to outer with distinct patterns. While the endothelial cells (ECs) are oriented longitudinally in the intima, the smooth muscle cells (SMCs) are aligned circumferentially in the media [2,3]. Thus, to mimic the vascular vessel structure, it is important to consider the alignment and orientation depending on the cell type. Recently, microfabrication techniques have been utilized for constructing biomimetic structure due to its fine controllability for cellular microenvironments, and the microfluidic based artificial blood vessel system has been extensively studied due to its ability to produce vascular architectural similarity. Initial researches explored the culture of ECs on the 2D flat surface to investigate cell adhesion and alignment. The micro/nanopattern and the direction of shear stress were applied to guide the cell migration and elongation in the *in vitro* model [4–6]. In the case of SMCs, the cells were also aligned on the micropatterned flat surface or in the rectangular microfluidic channel, and the regulating orientation and functional contractility were investigated [7–12]. However, since such a flat substrate is quite different from the real vascular structure, recent reports were dedicated to the fabrication of circular microchannels to mimic the blood vessel system. Introducing a stream of gas into PDMS oligomers [13], stamping based replication [14], and rolling membrane methods [15] were employed to generate the circular microchannel, and the *in vivo*-like network was presented. While there are a few reports regarding the EC cultivation inside the circular microchannels [13,16,17], the presentation for the circumferential SMC culture in a circular microfluidic channel has been rarely reported so far. Since the SMCs in a native blood vessel are known to be circumferentially aligned and elongated, it remains very challengeable to construct the *in vivo*-like vascular microenvironments and produce contractile and spindle-shaped morphology of SMCs. Thus, an advanced *in vitro* model is necessary to mimic the

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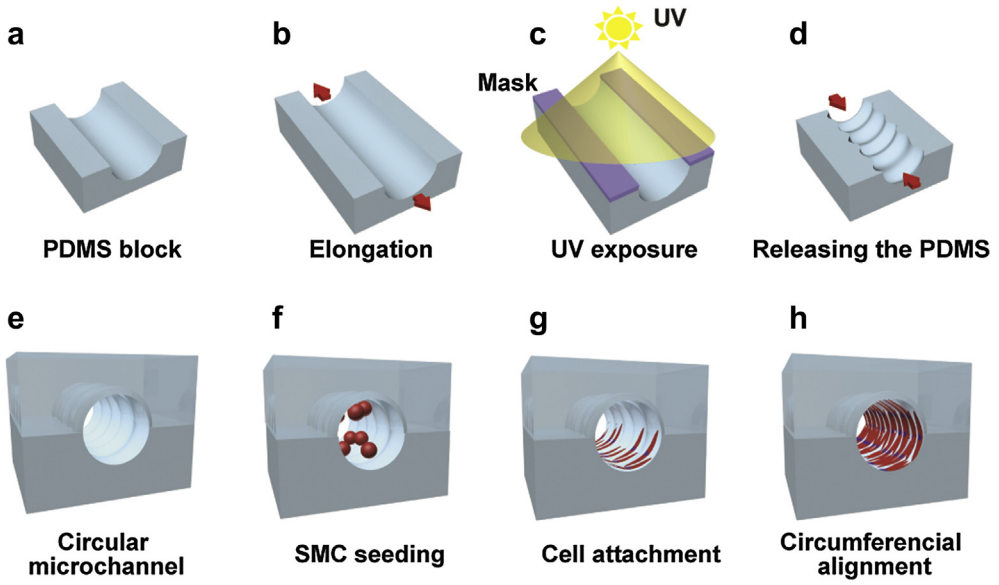


**Fig. 1.** Schematic illustrations to create an orthogonal microwrinkle structure inside the circular microchannel which guided the circumferential HASMC alignment.

*in vivo* blood vessel for in-depth study of strength, elasticity and contractility of vascular smooth muscle cells.

To this end, we have prepared the circular microchannel with the orthogonal micro-grooves perpendicular to the axis of the microchannel by using the PDMS based replica molding process [18]. Such an orthogonal microwrinkle embedded in the curvature surface of the circular microchannel can lead to the circumferential alignment of HASMCs during cultivation. The analysis of orientation angle, shape index, contractile protein marker expression, and live/dead cell assay was performed to investigate the circumferential alignment of the cultured HASMCs, spindle elongated morphology, contractile state, cellular viability and coverage to demonstrate the biomimetic 3D HASMC layer formation.

## 2. Materials and methods

### 2.1. Materials

HASMCs and a smooth muscle cell growth medium (SmGM™) bullet kit, which contains a smooth muscle cell basal medium (SmBM) and SmGM SingleQuots™ (supplements and growth factors) for cell culture, were obtained from Lonza (MD, USA). Glutaraldehyde, 10% neutral buffered formalin, phalloidin and tetramethylrhodamine B isothiocyanate (TRITC) conjugate were purchased from Sigma Aldrich (MO, USA). A LIVE/DEAD® viability/cytotoxicity assay kit was obtained from Molecular Probes (Invitrogen, CA, USA). A positive photoresist (S1818) and MF™-CD-26 developer were ordered from Rohm and Haas Electronic Materials Limited Liability Company (MA, USA). A <100> Si wafer was purchased from iTASCO (Seoul, Korea). An isotropic wet etching solution was prepared from a mixture of hydrofluoric acid (DC chemical, Korea), nitric acid (Junsei, Japan), and glacial acetic acid (SAMCHUN, Korea). A PDMS prepolymer and a curing agent were obtained from Dow Corning Corporation (Sylgard 184 elastomer kit, USA). Hexamethyldisilazane (HMDS) was ordered from Sigma Aldrich.

### 2.2. Preparation of a half-circular PDMS microchannel

A half-circular PDMS microchannel was prepared as follows [18]. First, the half-circular microchannels on a Si wafer were generated according to the isotropic etching procedure. A 300 nm thickness of a $Si_3N_4$ layer was used as an isotropic wet etching hard mask (Fig. S1a). Microchannel patterns of a photomask were transferred to the $Si_3N_4$-deposited silicon wafer via a conventional photolithography process. After developing, the exposed $Si_3N_4$ hard mask was removed by a reactive ion etching (RIE) in $CF_4$ plasma (SAM-HAN VACUUM TECH. Co., Ltd, Korea), and the remaining positive S1818 photoresist was cleaned with acetone (Fig. S1b-d). Then, an isotropic wet etching was followed in a HF, $HNO_3$, and $CH_3COOH$ mixture solution (the used volume of HF, $HNO_3$, and $CH_3COOH$ is 20, 35, and 55 mL, respectively) for 100 min to form a half-circular microchannel with around 400 μm in diameter (Fig. S1e). After vigorous washing with distilled water and drying, the remaining $Si_3N_4$ layer was removed via RIE with $CF_4$ plasma (Fig. S1f). A PDMS prepolymer and PDMS curing agent were mixed at a volume ratio of 10:1, poured on the patterned Si wafer and cured in an oven at 80 °C for 12 h (Fig. S1g). The PDMS elastomer was then carefully peeled off from the Si wafer, and the produced PDMS mold has convex half-circular microchannels (Fig. S1h). To fabricate concave half-circular PDMS microchannels, the PDMS mold was first exposed to UV for 1 min and then treated with HMDS for 15 min to make the surface hydrophobic. A PDMS prepolymer was poured on the flat substrate and replicated by the pretreated PDMS elastomeric mold (Fig. S1i). After curing in an oven at 80 °C for 12 h, the microchannel-patterned PDMS block was manually removed from the PDMS mold (Fig. S1j).

### 2.3. Generation of orthogonal microwrinkles in the half-circular microchannels

A half-circular PDMS block was clamped and uniaxially stretched to 40% (1 cm → 1.4 cm) by a custom-made apparatus (Fig. 1a and b). The UV treatment was carried out in an UV chamber (Ahtech LTS Co., Ltd, Korea) for a designated time (10, 20, 30, 40, 50 and 60 min), and then released in the opposite direction to recover its original shape (Fig. 1c and d). The intensity of the UV light was fixed at 28 mW/cm$^2$ and the distance between the lamp and the PDMS block was 1 cm. The resultant UV-treated PDMS block contains orthogonal microwrinkle patterns inside the curvature surface, and the wavelength of microwrinkles was controlled by the UV irradiation time. To form the orthogonally microwrinkle pattern