MICROFLUDICS SPINNING OF FLAT FIBER WITH MICRO GROOVES FOR CELL-ALIGNING SCAFFOLDS

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ABSTRACT

In this study, we propose a method for continuously fabricating alginate flat microfibers with size tunable grooved microstructures using a microfluidic system. By introducing a sheath fluid with a high-speed flow rate, an alginate solution could be both flattened and polymerized simultaneously. Raised patterns could be engraved on the surfaces of the flat fibers in the longitudinal direction, with successful control over the number and dimensions of microscale-grooves via regulation of the slit-shaped channel.

KEYWORDS

Microfluidic spinning, Grooved flat fiber, Cell alignment on patterned hydrogel

INTRODUCTION

Diverse micro-fabrication methods have been developed in an effort to produce substrates that promote tissue-specific cell culture organization.[1] Most recently, we reported the simultaneous production of multiple fibers, each of differing composition, using coaxial PDMS microfluidic channels.[2] Although these methods permit the simple and cost-effective production of uniformly sized micro fibers, mass production and alignment for 3D tissue organization presents several challenges.

In this paper, we propose a method for continuously fabricating alginate flat microfibers with size-tunable grooved microstructures using a microfluidic system. To our knowledge, no previous studies have addressed the continuous mass fabrication of such thin flat microfibers with grooves a few microns in scale.

EXPERIMENTAL

A thin flat fiber (thickness < 10 μm) engraved with grooved patterns a few microns in scale was continuously fabricated using a PDMS microfluidic platform consisting of a slit-shaped sample channel (20 μm slit height), a thick sheath, and a reaction channel (200 μm in height), as illustrated in Fig 1(a). A sodium alginate solution was introduced through the sample channel, and calcium chloride ($CaCl_2$) solution was provided through two sheath channels. As the alginate solution traveled through the sample channel, the flow was squeezed and deformed by the engraved patterns on the surface of the slit-shaped channel ('A' region in Fig 1(b)). Extrusion of this deformed flow through the sample channel caused the sample flow to be focused by the sheath flow, at which point the alginate solution abruptly crosslinked, forming calcium alginate as the calcium ions replaced the sodium ions to crosslink the polymer ('B' region in Fig 1(c)).

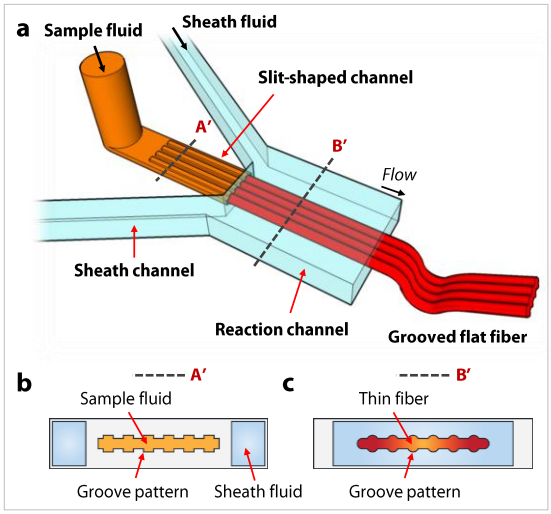

Figure 1 Production of flat fibers with microgrooves (a) Schematic diagram showing the process of generating flat fibers with microgrooves; A cross-sectional schematic diagram of (b) the slit-shaped channel (A' in a) and (c) the reaction channel (B' in d);

As shown in Fig 2(a), Slower sample flow rates or faster sheath flow rates enabled the generation of thinner flat fibers, and the sheath flow rate proved to be more critical to reducing the thickness of the flat fiber than the sample flow rate. Fig 2(b) shows the throughput of the flat fiber. In contrast with the aspect ratio, the sample flow rate critically affected the production quantity. Fig 2(c) shows SEM (FE-SEM JEOL 4701F, JAPAN) images of flat fibers produced from alginate solutions with different concentrations. A relatively low concentration of alginate (1%) yielded entwined and wavy flat fibers Fig 2(d).

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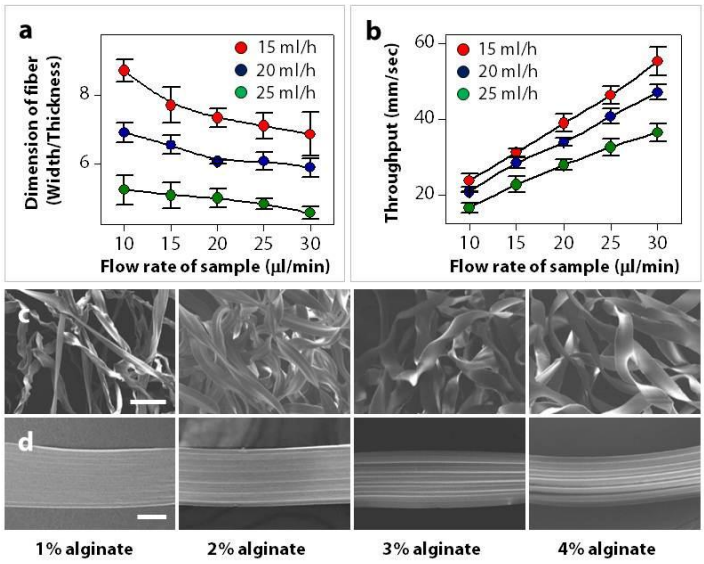

Figure 2 Size and shape of the flat fibers. (a) fiber dimensions (width/thickness) as a function of the sample flow rate (10–30 mL/h) and sheath flow rate (15–25 mL/h); (b) fiber throughput as a function of the sample flow rate and sheath flow rate; (c) SEM images and (d) magnitude images of the grooved flat fibers made from different concentrations of alginate solutions (1–4%). Scale bar indicates 20 μm in (c) and 100 μm in (d)

RESULTS AND DISCUSSION

Fig 3(a, b) show SEM images of smooth and grooved flat fibers. The flat fibers were continuously and stably produced without breakage, and the size and shape of the flat fibers and groove patterns were uniform along the longitudinal dimensions. Fig 3(c) shows a cross-sectional SEM image of a flat fiber with 5 grooves, indicating that the shape of the groove was rounded. The widths of the walls separating the grooves were approximately 11 μm, whereas the grooves themselves were approximately 6 μm in width. The thickness of a flat fiber was approximately 6 μm; in contrast, the height of a groove was approximately 3 μm, as shown in the magnified image in Fig 3(d).

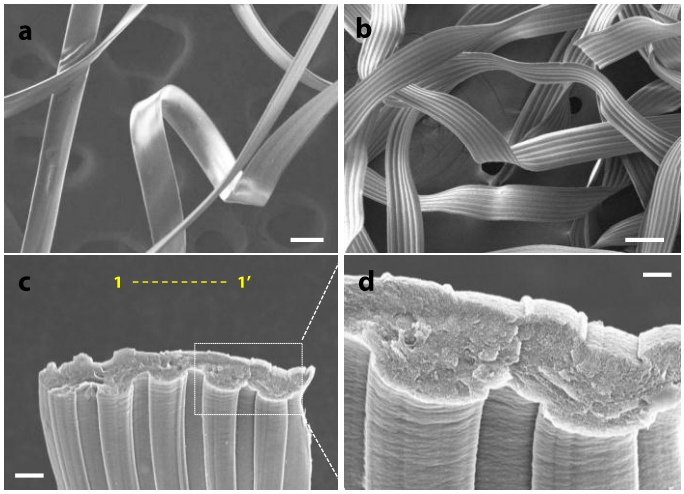

*Figure 3 SEM images of the microgrooved flat fiber. SEM images of a (a) smooth flat fiber and (b) grooved flat fiber: (c) cross-sectional image (line 1-1' in d) and (d) magnified image (white box in c). Scale bar indicates 100 μm in (a, b) 10 μm (**c**), and 3 μm (**d**).*

Biomedical applications of the grooved flat fibers were demonstrated by isolating neuron cells from the embryos of pregnant Sprague Dawley rats on the 16th day of gestation. Fig 4(a) shows a fluorescence image of neuron cells on the smooth fibers. Most neuron cells on the smooth fibers migrated toward the edges and aggregated to form networks across the fibers. In contrast, on the grooved fiber, many neuron cells adhered to the ridges of the grooves and the neurites were projected along the ridges of the grooved fiber (Fig 4(b, d)). Grooved flat fibers can be used to align other types of cells in culture, including fibrous and epithelial cells. We cultured myoblast cells (Rat skeletal muscle cell, L6) on smooth and grooved flat fibers. Fig 4(c) shows fluorescence micrographs of the muscle cells on the fibers. Muscle cells grown on the grooved flat fibers aggregated within the microgrooves indicating that the deep microgrooves prevented the myoblasts from spreading over the ridges. This shows that cells were patterned along the microgrooves with topographic guidance.

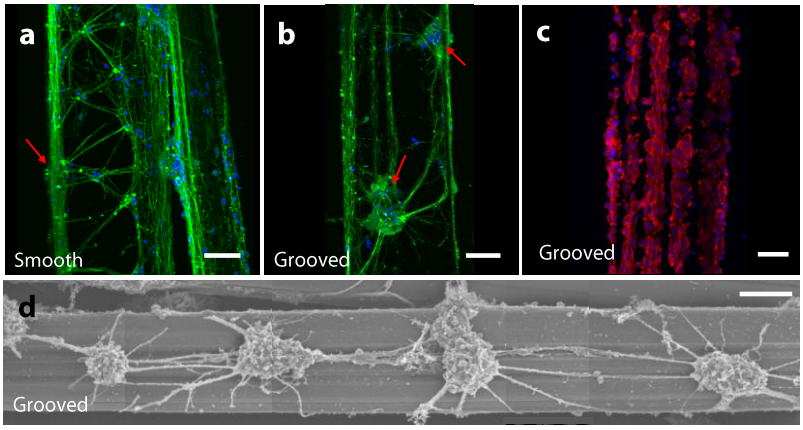

Figure 4 Cell alignments on the microgrooved flat fibers. Fluorescence micrographs of neurons (a) on a smooth flat fiber and (b) on a grooved flat fiber; (c) Fluorescence micrograph of muscle cells on the grooved flat fiber and (d) SEM image of neurons on a grooved flat fiber. Scale bar indicates 50 μm in (a, b, c and d)

As shown in Fig 5(a), the cell orientation angle was evaluated by measuring the angle between the main axis of the cell and the grooves. The alignment angle on the grooved fibers was narrowly distributed around 0°, indicating that most neurites were aligned well along the fiber, whereas the alignment angle distribution on the smooth fibers was broad, reflecting the randomly directed neurites. As with neurite alignment on the fibers, The angular alignment distributions of the muscle cells on grooved and smooth flat fibers were 12° and 41° respectively, (on the control culture dish, the angle was 45°). Our results indicated that cells may sense the microtopographic features of the flat fiber, which guide cell migration, interaction, organization, and neurite orientation.

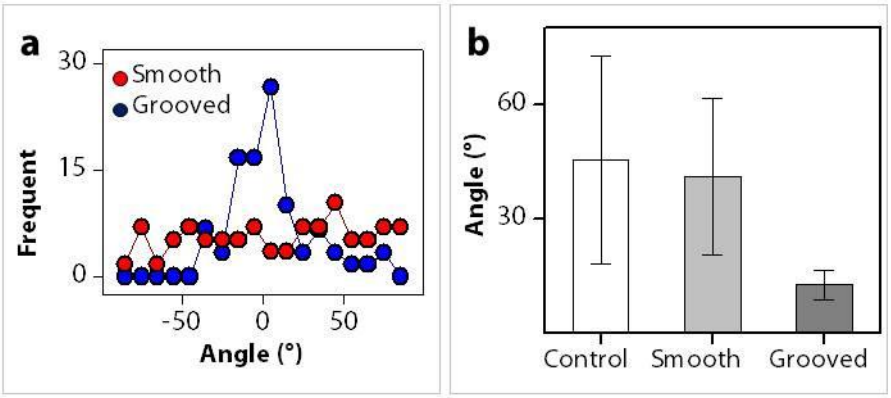

Figure 5 Quantification of (a) the neurite orientation on the grooved and smooth flat fibers and (b) Rat skeletal muscle cells (L6) orientation on a control culture dish, smooth flat fibers and grooved flat fibers.

CONCLUSION

In summary, we present a novel and simple spinning method for continuously producing thin flat fibers with groove patterns using a microfluidic channel. The microgrooved flat fibers can be used not only to guide the morphogenesis of various types of cells, but also to integrate topographic control over cell alignment with the design of scaffolds for tissue engineering purposes, such as scaffolds for regeneration in SCI or PNI, or for reconnecting severed muscle tissue.

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