SELECTIVE DEPOSITION OF ELECTROSPUN ALGINATE-BASED NANO-FIBERS ON CELL-REPELLING HYDROGEL SURFACES FOR CELL-BASED MICROARRAY

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ABSTRACT
This paper proposes selective deposition of electrospun alginate-based nanofibers through a charged shadow mask to form an array of nonwoven microspots on cell-repelling hydrogel surfaces; subsequently, the target cells are seeded onto these nanofiber-based extracellular matrix (ECM) scaffolds to create cell-based microarrays. The needle tip contains a drop of a blended solution of alginate and polyethylene oxide (PEO); an Al-coated glass shadow mask with micron-sized holes was used for selective deposition to form the patterned microspots of nonwoven mats. The shadow mask was applied with a suitable voltage to repel the nanofibers from its surface, while simultaneously forcing them into the micron-sized holes and onto the cell-repelling hydrogel surface. The experimental results show that when the shadow mask is applied with a higher voltage, a larger dense central spot will be produced within the hole. An array of 3×3 nonwoven microspots was successfully demonstrated to selectively deposit alginate-based nanofibers for cell patterning. BHK-21 fibroblast cells were shown to selectively adhere onto the nonwoven microspot surfaces due to the existence of the cell-repelling hydrogel around the microspots.

KEYWORDS: Electrospinning, Electrostatic focusing, Nanofiber, Cell patterning

INTRODUCTION
Recently, the electrospun nanofibers has attracted an increasing research interest, and shown great potential for diverse applications in areas such as tissue engineering, sensors, and advanced composite materials. Over the past few years, most studies on electropinning techniques mainly focused on producing nanofibers with unique geometrical features (e.g., uniform diameter, tubular structure, etc) and some degree of control over their selective deposition onto the collecting substrate. To maximally mimic natural ECM scaffolds, it is crucial to create structurally complex, high-definition shapes of nanofibers. To this end, some efforts have recently been proposed using the modified electrospinning to deposit the nanofiber [1–3]. However, it still remains a challenge to selectively seed the target cells onto theses arbitrarily shaped, microsized, nanofiber-based ECM scaffolds for cell biology and tissue engineering.

EXPERIMENTAL
Figure 1 shows the schematic diagram of the experimental setup used in electrodynamic focusing deposition of the electrospun nanofibers through a charged shadow mask to form the patterned micron-sized spots array on cell-repelling hydrogel surfaces. To make the alginate solution spinnable, polyethylene oxide (PEO) was used as the supporting polymer. The loaded polymer solution flows along the needle to form a pendant droplet on the sharp tip of the syringe capillary needle. The needle tip was located at a distance of 3 cm above the collecting substrate. An aluminum-coated glass coverslip with micron-sized holes, which served as a charged shadow mask, was attached to the collecting substrate by using a double adhesive as a spacer (75 μm in thickness). A high voltage (3–6 kV) source was connected between the needle tip and the collecting ITO substrate, and a positive grid voltage was simultaneously applied to the conductive Al-coated shadow mask surface. The Al-coated shadow mask was then carefully detached from the substrate, and calcium chloride ($CaCl_2$) solution was introduced to produce gelation of the deposited alginate-based nanofiber microspots on the collecting substrate. Subsequently, we seeded the BHK-21 fibroblast cells to selectively adhere onto the surfaces of the nonwoven microspot by using cell-repelling hydrogel surfaces around the microspots as shown in Fig. 2.

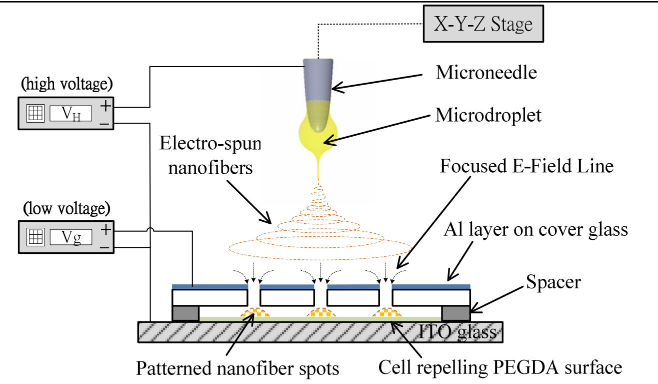

Figure 1: Selective deposition of the electrospun alginate-based nanofibers through a charged shadow mask to form the microspots on cell-repelling hydrogel surfaces.

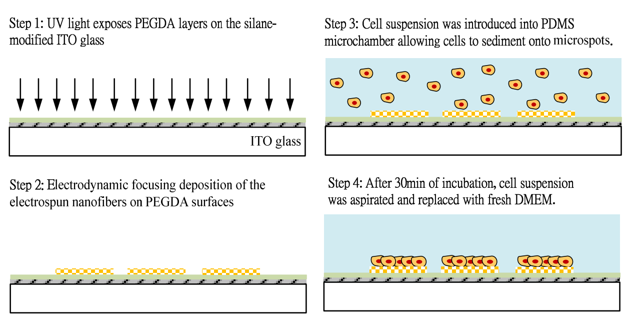

Figure 2: Schematic diagram of the target cells precisely seeded onto the nanofiber-based ECM scaffolds

RESULTS AND DISCUSSION

Figure 3 shows the optical micrographs of the deposition of the electrospun nanofibers operated without and with a shadow mask. With a shadow mask, it causes a larger dense central spot with a 600-μm diameter to be deposited onto the hydrogel surface. A dense deposition of alginate-based nanofibers with a uniform diameter was electrospun at the center of the shadow mask (Fig. 3 (d)). In addition, a large number of nanofibers deposited from the hole edges towards the center of the hole indicates electrostatic focusing; thus, the electric field on the shadow mask repelled the nanofibers from its surface while simultaneously forcing them into micron-sized holes and depositing them onto the collecting substrate (Fig. 3 (c)). Figure 4 FEM simulations of field strength for electrospinning process operated with and without a shadow mask.

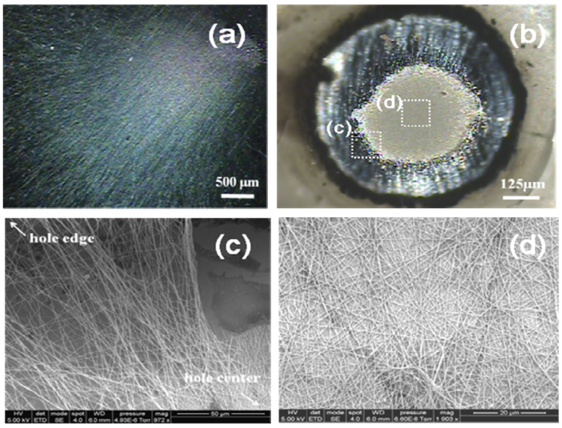

Figure 3: Optical micrographs of the electrospun nanofibers operated (a) without and (b) with a shadow mask. (c) and (d) SEM pictures of the electrospun nanofibers located in (b).

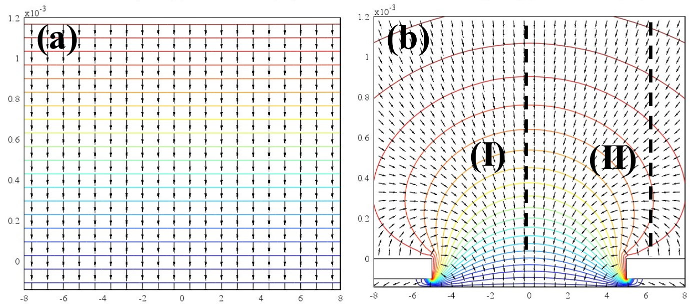

Figure 4: FEM simulations of field strength for electrospinning process operated (a) without a shadow mask, and (b) with a shadow mask applied by Vg= 100V

Figure 5 the vertical electric field versus height above the shadow mask surface and above the center of the holes for Vg= 0 to 500 V. For a shadow mask without an applied grid voltage (Vg= 0V), the electric field has a positive value (E > 0 kV/m) for both the height above the shadow mask's surface and above the center of the holes. This indicates that the positive electric field attracts the electrospun nanofibers to deposit on the shadow mask's surface and into the hole. However, while applying the grid voltages from Vg = 100 to 500V, the electric field had a negative value (E < 0 kV/m) at a height approximately above the shadow mask's surface (dash lines) The electric field at the center of the holes (solid lines) has a positive value towards the collecting substrate. The repulsive electric field (E < 0 kV/m) on the shadow mask's surface and the attractive electric field (E > 0 kV/m) at the center of the holes provide a focusing action that forced the positively charged nanofibers to be deposited within the center of the holes. Figure 6 shows the voltage dependence on the diameters of the electrospun nanofibers and the dense central spots of nonwoven mats. Increasing the grid voltage (Vg) can cause more nanofibers to be deposited onto the collecting substrate and significantly increase the size of the dense central spot within the shadow mask's hole. Increasing the grid voltage (Vg) can also significantly decrease the diameter and the standard deviation of the electrospun nanofibers. This result is attributed to reinforcement of electrostatic focusing because increasing the grid voltage (Vg), which significantly increases the attractive electric field at the center of the hole, accelerates the deposition of positively charged nanofibers onto the collecting substrate.

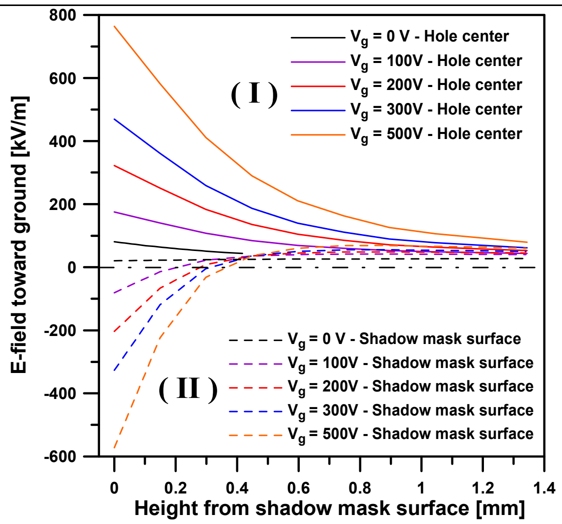

Figure 5: The vertical electric field versus height above the shadow mask surface (dash lines) and above the center of the holes (solid lines) for Vg= 0 to 500 V

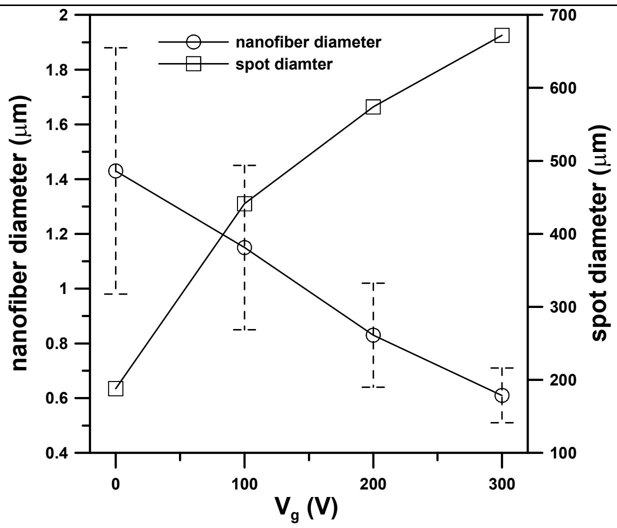

Figure 6: Voltage dependence on the diameters of the electrospun nanofibers and the dense central spots of nonwoven mats applied with focusing voltage (Vg)

To demonstrate the ability to selectively deposit microspots of nonwoven mats on cell-repelling hydrogel surfaces for cell patterning, a shadow mask with an array of 3×3 holes with 700-μm diameters was operated at Vg= 300 V; subsequently, the target cells were seeded onto these nanofiber-based ECM scaffolds. After electrospinning was completed, the shadow mask was carefully detached from the collecting substrate, and $CaCl_2$ solution was introduced to produce gelation of the deposited alginate-based nanofiber mats. Figure 7(a) and (b) show optical micrographs of 3×3 nonwoven microspots that were deposited onto the hydrogel surface before and after the shadow mask was detached. As can be seen, 3×3 nonwoven microspots with approximately identical sizes were successfully demonstrated to selectively deposit onto the hydrogel surface after the shadow mask was detached. Figure 7 (c) shows the optical image of the BHK-21 fibroblast cells that were seeded onto the nanofiber-based ECM scaffold after 12 hours in culture. The results demonstrated that BHK-21 fibroblast cells selectively adhere to the nonwoven microspot surfaces with a high level of cell viability due to the nanofiber-based ECM scaffold and the existence of cell-repelling hydrogel surfaces around the microspots. The presented technique not only has the ability to selectively deposit high-definition nanofiber patterns, but it also enables selective seeding of the target cells onto a microsized, nanofiber-based ECM scaffold for potential applications in cell biology and tissue engineering.

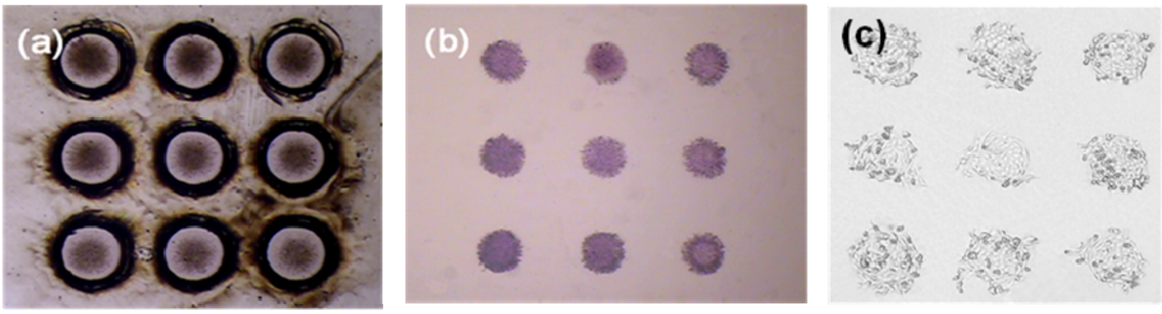

Figure 7: An array of 3×3 nonwoven electropun nanofiber microspots (a) with and (b) without a shadow mask. (c) BHK-21 fibroblast cells selectively adhere on the nonwoven microspot surfaces due to the existence of the cell-repelling hydrogel surfaces around the microspots.

CONCLUSION

In conclusion, this paper has introduced a simple method to selectively deposit electrospun alginate-based nanofibers and to precisely seed the target cells onto these nanofiber-based ECM scaffolds by using electrostatic focusing and cell-repelling hydrogel surfaces. It was shown that increasing the grid voltage (Vg) can increase both the repulsive and attractive electric fields to reinforce the electrostatic focusing of the produced electric fields, which can significantly increase the size of the dense central spot and decrease the diameter as well as the standard deviation of the electrospun nanofibers within the shadow mask hole. An array of 3×3 nonwoven microspots was successfully demonstrated to selectively deposit alginate-based nanofibers; the target cells were shown to selectively adhere onto these nonwoven microspot surfaces. The presented technique is simple and does not require lithography, a controllable moving tip or complex surface modification to selectively seed the target cells onto these microsized, nanofiber-based ECM scaffolds for potential applications in cell biology and tissue engineering.

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