A polymer-based artificial microenvironment for enhancing cell adhesion

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Introduction

Dynamic control of cell adhesion to a surface is important for understanding cell networks, their involvement with the extracellular matrix, and the formation of controlled structures for cell-matrix interactions similar to those in native tissues [1–3]. The ability to control the cell-surface network is widely used to observe the regulation of host-biomaterial interactions, predict cell behavior, and perform solid organ tissue engineering [4,5].

Generating thin films as cell adhesion platforms is of great interest because of their applications in biosensor, drug, and delivery research and soft robotics [6]. These platforms should be able to support cell growth while maintaining stability and control cellular ability in order to provide insights into cellular interactions and dynamics, while forming scaffolds and cell-based biosensors [7,8]. In recent research, cellular micro-patternning and organization have been widely used to control cell adhesion onto substrates. Dynamic control of cells has been achieved using surface chemistry based on self-assembled monolayers [9–13], etching [14,15], microfluidic techniques [16,17], photolithography [18,19], and screen printing [20,21]. Surface modifications based on biomolecules, such as enzymes...
and other proteins, have also been used to enhance cell adhesion [22,23]. Despite their considerable advantages, these methods still suffer from limitations in being applied for flexible and soft substrates because of their rigidity. The Haraguchi group described a flexible substrate with a micro- or nano-pattern that had the ability to enhance cell growth and adhesion, generating ordered and functional cell sheets [22].

Many studies on cell adhesion platforms on flexible, micro- and nano-patterned substrates have reported the use of polystyrene film, which is deposited using spin coating [24], and ultrathin poly(methyl methacrylate) films, which are deposited by screen printing [25]. However, these flexible materials are not biodegradable, limiting their applicability to bio-platforms. Nafion poly(tetrafluoroethylene-co-perfluoro-3,6-dioxa-4-methyl-7-octene-sulfonic acid), a hydrophilic polymer with sulfonic acid groups as ionomeric components, is a material with considerable potential for flexible, nano- or micro-patterned substrates because of its properties. Specifically, Nafion has good thermal and mechanical properties and superior electrical properties as a proton exchange conductor. Furthermore, Nafion-based electrochemical sensors as drug delivery devices have been developed in the biosensor field [26–28].

Herein, we further investigated Nafion from the perspective of cell adhesion and biocompatibility. We demonstrated flexible, strong, micro-patterned Nafion sheets for the adhesion and orientation of cells. Nafion sheets were micro-patterned using an imprinting process, and polydimethylsiloxane (PDMS), polyurethane–acrylate (PUA), teflon, and acryl were used as molded material. Through an optimization process, we demonstrated that acryl showed the best performance. Furthermore, different substrates were used to optimize the process. By modulating the surface architecture of the Nafion micro-pattern, cell adhesion and spreading can be controlled. The fabrication process in this research is simple, rapid, and highly reproducible. Furthermore, the Nafion micro-pattern is separate from the substrate, making it straightforward to form micro-patterns with different mechanical properties. The proposed Nafion micro-pattern can be applied to study cellular interactions by controlling and directing the adherent cells.

1. Reagents and chemicals
Two commercially available ultraviolet (UV) curable molds (PUA, acryl), one thermal curable mold (PDMS), and one self-made UV curable mold (Teflon) were used. A PUA mold (MINS-311RM; Minuta Technology, Osan, South Korea), an acryl mold (OrmoStamp; Micro Resist Technology GmbH, Berlin, Germany), PDMS (SYLGARD 184 Silicone Elastomer Kit; Dow Corning, Midland, MI, USA), Teflon (Fluolink MD 700; Solvay, Brussels, Belgium); and 1-hydroxycyclohexyl phenyl ketone; Sigma-Aldrich, St. Louis, MO, USA), polyethylene terephthalate (PET) (SH71S; SKC, Seoul, South Korea) were used. In the cell culture process, Dulbecco’s modified Eagle’s Medium (DMEM; Welgene, Gyeongsan, South Korea), fetal bovine serum (Welgene), penicillin-streptomycin (Welgene), glutaraldehyde solution (Sigma-Aldrich), ethanol (Daeyeung, Busan, South Korea), phosphate-buffered saline (Sigma-Aldrich), and osmium tetroxide solution (Sigma-Aldrich) were used.

2. Micro-patterning of Nafion
The flexible Nafion micro-patterned mold was fabricated by a silicon master (linewidth, 800 nm; space, 800 nm; depth, 600 nm; line pattern). Four different molds were also fabricated based on PDMS, PUA, acryl, and Teflon. The PUA, acryl, and Teflon molds were exposed under a 365-nm UV lamp for 90 s at 40 mJ/s for curing. To create the PDMS mold, a PDMS solution mixed with an elastomer and curing agent (10:1) was dropped onto the PUA mold and incubated for 2 hours at 80°C.

After that, 3 substrates (Si wafer, glass wafer, PET film) were exposed to O2 plasma for 3 minutes at 15 sccm (120 mTorr, 50 W, LF-plasmaster 100; JNE, Korea) to make a hydrophilic surface. Then, a thin film of Nafion was deposited by spin coating (Spin 300 A; Midas System, Daejeon, South Korea) with 5% Nafion (Nafion perfluorinated resin solution; Sigma-Aldrich) solution as the substrate. The micro-pattern was fabricated using a mold by thermal nanoimprinting (T-NIL; Midas System) for 10 minutes at 105°C and 10 bar.

3. Cell culture
The sensors were first sterilized by immersion in 70% ethanol (Daejeung), followed by UV light exposure for 20 minutes. Then, MDA-MB-231 (Korean Cell Line Bank; Seoul, South Korea) and MCF-7 (Korean Cell Line Bank) were seeded on each sample in a six-well plate (SPL life Science, Pocheon, Korea) and incubated at 37°C and 5% CO2 in DMEM supplemented with 10% (v/v) fetal bovine serum, and 1% (v/v) penicillin-streptomycin.

Materials and Methods

**Ethics statement:** The Cell-line used for Cell adhesion test in this study was purchased from commercially available vendor, KCLB, and was therefore exempt form institutional review board approval.

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4. Characterization of the Nafion micro-pattern

1) Scanning electron microscopy
Scanning electron microscopy (SEM) micrographs were recorded with a JSM-7610F (JEOL; Tokyo, Japan). The samples were gold-sputtered before the microscopic analyses.

2) Atomic force microscopy
The surface morphology of the gold surfaces was investigated using atomic force microscopy (AFM) (AFM5300E; Hitachi, Tokyo, Japan). A normal tapping mode of the silicon cantilever with an oscillation frequency of 365 kHz and spring constant of 47 N/m (NCH-10V; Digital Instruments, Tonawanda, NY, USA) was used for AFM imaging. No destruction of the sample surface was noticed during imaging. All images are presented in the height mode, where higher parts appear brighter.

Results

1. Fabrication and characterization of micro-patterned Nafion films
Mechanically flexible, biocompatible sheets can be used as platforms for applications of biosensors and tissue scaffolds [29]. A micro-pattern can give an opportunity to guide cells or control the cell morphology. In this work, micro-fabrication of Nafion films was performed using various molds and substrates and patterned by photolithographic techniques. Nafion is suitable for use in cell adhesion because it provides a solid support, expressing a high surface charge density and good water wettability. To make a micro-pattern Nafion film, we chose a Nafion solution, not a Nafion membrane, because the Nafion solution has advantages for making patterns on diverse substrates and electrodes. As previously stated, we used different substrates to optimize the micro-pattern, so we chose the Nafion solution. First, a soft
mold was fabricated to make a micro-pattern Nafton film, and we used PDMS, PUA, Teflon, and acryl molds to optimize the micro-pattern of the Nafton film. A soft mold was fabricated using a silicon master, and the micro-pattern was fabricated by thermal lithography using these molds as a base (Fig. 1A). To demonstrate the structural integrity and scalability at the microscale, SEM images were taken. The original dimensions of the pattern were 800 nm linewidth, 800 nm space, and 600 nm height. As shown in Fig. 1B, each mold had diverse dimensions; of particular note, the PDMS mold had reduced linewidth, height, and space. Furthermore, some pattern destruction was observed in the Teflon mold. Of these options, acryl showed the best performance and accurate dimensions (800 nm linewidth, 790 nm space, 590 nm height).

The films were also observed by optical microscopy to visualize large areas (Fig. 2A). In this process, Nafton films were not removed from the base substrate when using a PUA mold. The Nafton films still remained on the base substrate. We suggest that the adhesion force between Nafton and the second substrate was insufficient to remove the Nafton from the base (first) substrate. Some destruction was also observed on the Nafton films when using the Teflon mold. The PDMS and acryl molds showed the best performance on optical microscopy. SEM and AFM images were observed to optimize the mold (Fig. 2B and 2C). Acryl showed a clear and accurate line pattern on the SEM images. PDMS and Teflon showed an accurate linewidth and space, but the height was different from that of the acryl mold. AFM showed the height differences between the PDMS and acryl molds; the acryl mold had an accurate height, whereas the PDMS mold had a height of 150 nm.

**Fig. 2.** (A) Optical morphology of micro-patterned Nafton films on different molds (PDMS, PUA, acryl, and Teflon). (B) SEM images of micro-patterns on PDMS, acryl, and Teflon. (C) AFM images of the micro-patterned Nafton films. PDMS, polydimethylsiloxane; PUA, polyurethane-acrylate; SEM, scanning electron microscopy; AFM, atomic force microscopy.
Optical and AFM images of the films on the different substrates are shown in Fig. 3. An advantage of using a Nafion solution, not a Nafion membrane, is that Nafion could be deposited on various substrates. Nafion was deposited on the silicon wafer, the glass wafer, and the PET flexible film using the acryl mold. Due to the optical transparency of the entire structure, Nafion films were applied in optics for fabrication. The lines had a high structural fidelity and resolution, demonstrating the accuracy of this fabrication process to form micro-patterns over large areas [6]. AFM images are shown in Fig. 3B, with patterns showing a linewidth of around 800 nm and a space of 800 nm. However, the silicon wafer had a height of about 400 nm, while the glass wafer and PET film had a height of 100 nm. Thus, the silicon wafer showed the best performance as a substrate to make micro-patterned Nafion films.

2. Evaluation of cell adhesion
As previously mentioned, the Nafion micro-patterns obtained by the molding process were able to control the cell-adhesive regions, unlike previously reported cell patterning techniques using stamping, enzymes, and other proteins. Fig. 4 shows the cells on films coated with MCF-7 cells (Fig. 4A and 4B) and MDA-MB-231 cells (Fig. 4C and 4D). The cells clearly showed cell adhesion patterns aligning with the designed organization. Most interestingly, although the morphology of cell adhesion of MDF-7 and MDA-MB-231 was different, both MDF-7 and MDA-MB-231 cells migrated from the micro-patterns. These data suggest that the Nafion coating with a micro-pattern organization played a crucial role through its ability to guide and enhance cell growth and adhesion according to a specifically designed order. The cells were guided by the surface morphology and the stiffness differences between the pattern and the substrate. These Nafion films can therefore be used as cell culture sheets for cell-based platforms, where the micro-pattern can be used to guide and align the cells.

Discussion
In this paper, we demonstrate the fabrication of Nafion micro-patterns by molding technology, which permits high resolution, high throughput, and scale. We optimized the micro-pattern using different substrates and mold materials and...
determined that the acryl mold and silicon substrate showed the best performance. The films are mechanically robust, can be formed at various thicknesses, ranging from 100 nm to 500 nm, and have controllable thickness and pattern spaces. Next, cell adhesion was evaluated using both MCF-7 and MDA-MB-231 cells. These micro-patterned Nafion films not only enhanced cell adhesion, but also facilitated cell migration and alignment. These results suggest that micro-patterned Nafion sheets can serve as a valuable tool for flexible cell-based platforms and devices.

**Notes**

**Conflict of interest**
No potential conflict of interest relevant to this article was reported.

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**Authors’ contributions**
Conceptualization: SHL; Data curation: HBP, SKS, HJK;
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Data availability
Please contact the corresponding author for data availability.

References
25. Fujie T, Desai A, Ventrelli L, Mazzolai B, Mattoli V. Inkjet


