Sugar-fiber Imprinting to Generate Microgrooves on Polymeric Film Surfaces for Contact Guidance of Cells†

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Anisotropic surface topography is known to induce the contact guidance of cells, and facile and biocompatible approaches of the physical modification of the pertinent matrix surfaces are thus meaningful for biomaterials. Herein, we put forward a sugar-fiber imprinting technique to generate microgrooves on hydrophobic polymers demonstrated by the poly(lactic-co-glycolic acid) (PLGA) films. Microgrooves were conveniently generated after removing sugar fibers simply by water. The resulting locally anisotropic microgrooves were confirmed to elongate the cells cultured on the surface.

Keywords   polymers, biomaterials, surface modification, contact guidance of cells, poly(lactic-co-glycolic acid) (PLGA), sugar fibers

Introduction

Biomaterial surfaces play an important role in tissue engineering and regenerative medicine. It has been realized that an appropriate chemistry of an ideal biomaterial surface refers to not only chemical composition, but also surface topography. Compared to the former, the latter has been paid less attention, and new insight to design biomaterial surfaces and facile techniques to carry out surface modification are strongly desired.

The design of an excellent biomaterial surface is stimulated by the fundamental research of various interactions between cells and materials.[1-5] Among studies of cell-material interactions, the contact guidance is a classic topic. This phenomenon was first reported by Harrison in 1912, and the term “contact guidance” was put forward by Weiss in 1945.[6] The contact guidance leads to the alignment and elongation of cells along anisotropic topographic structures.[7-16] Besides orientational adhesion, the microgrooved substrates have been found to influence the deposition of extracellular matrix, and even the cell communication and differentiation.[17-21]

With much progress on the rapid development of regenerative medicine, more and more interest and concern are focused on studies of the contact guidance, and some methods including forming regular micropatterns on substrates have been developed.[20-24] While the regular micropatterns are very useful in fundamental research to reveal cell-material interactions,[25-28] it seems not always necessary to generate regular patterns as the potential applications of biomaterials are concerned. The important issue comes from that the approach is facile and “biocompatible”. For instance, if a kind of templates are used to generate microgrooves, the templates themselves should be very easily removed, and the residues, if any, cannot lead to any negative influence of the biocompatibility.

We suggest that sugar fibers serve as such an ideal kind of templates, if the matrix to be physically modified is water-insoluble. Our fabrication principles are schematically presented in Figure 1.

Figure 1 Schematic presentation of fabrication of a 2D microgrooved surface via sugar-fiber imprinting technique.

Most of polymers are water-insoluble, and thus this preparation principle is potentially extended. We select poly(lactic-co-glycolic acid) (PLGA), a widely-used biodegradable polymer, as the model matrix.[29,30] The sugar fibers will be prepared using the machine producing “cotton candy”, which is popular for children. This
time, we tried to employ the machine to serve science. The present report is aimed to confirm the feasibility of this idea in the cases of two dimensional films.

**Experimental**

**Materials**

PLGA (lactide/glycolide molar ratio of 85/15) obtained from Purac Corporation were used as received, with the number-average molecular weight $3.59 \times 10^5$ and the polydispersity index 1.72. Dichloromethane (CH$_2$Cl$_2$, Shanghai Wulian Co.) was employed as the solvent of PLGA, and it is not a solvent of sugar fibers. We bought an electric drawbench (Guangdong Hongxing Food Machine Company) to prepare sugar fibers, and the commercially available white granulated sugar (sucrose $\geq$99.5%) was used as raw materials.

**Fabrication of 2D PLGA films with microgrooves**

1.5 g PLGA dissolved in 16 g CH$_2$Cl$_2$ was poured into a glass mould of 6 cm diameter. After the solvent was evaporated, a translucent PLGA film was formed. Then sugar fibers wetted by CH$_2$Cl$_2$ solvent was placed on the surface of the PLGA film. Pressure was maintained at 272 Pa for about 0.5 h, then the PLGA film dipped by sugar fibers was put into the deionized water. The sugar was easily dissolved in water. After leaching the sugar fibers by water, microgrooved PLGA films were obtained.

**Physico-chemical characterizations of the PLGA films**

The chemical structures of PLGA films and sugar fibers were confirmed with a Nicolet Magna 550 Fourier transform infrared spectrometer (Thermo-Nicolet, Madison, WI) in the mode of attenuated total reflectance (ATR-FTIR). The static contact angles of water were measured in a JC2000XP apparatus (Shanghai, China). The pictures were taken when the droplets released from the delivery system 80 ms later.

The optical images of the films were captured by a Canon digital camera in an inverted microscope (Axiovert 200, Zeiss). After sputtering gold, the films were observed in a scanning electron microscope (SEM, Philips 30XL, Netherlands). The images of the microgrooved surface of a PLGA film were observed in an atomic force microscope (AFM, NanoScope IV).

**Cell experiments on the PLGA films**

MC3T3-E1 osteoblasts were cultured on the film surfaces. The films were put into the wells of 12-well plates, and soaked in ethanol (70%) 2 times (30 min each). Then the ethanol was removed by rinsing in phosphate buffer saline (PBS) solution 6 times (15 min each). The films were placed in low-glucose Dulbecco’s modified Eagle medium (DMEM, Gibco) containing 10% fetal bovine serum (FBS, Biochem S0615), 1% L-glutamine (Gibco) and 1% antimicrobial agents (Gibco) 2 times before seeding (30 min each). The cells were seeded onto the films with a density of 15000 cells per cm$^2$ and incubated for 24 h in a humidified incubator with 5% CO$_2$ and 95% air. Four replicates were used for each group.

The live/dead viability assay of the cells on the surfaces of the films was then tested. After seeding cells for 24 h, the DMEM was removed and PBS was added. The films were washed twice with PBS for 10 min each time, and then 7.5 mL prepared 2 μmol/L calcein AM and 4 μmol/L ethidium homodimer-1 were added. An inverted microscope (Axiovert 200, Zeiss) was used to observe the cells. The live cells were visualized in green and the dead cells in red after stained by the live/dead viabilty kit.

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich) assay of the cells was tested. The samples were washed twice with PBS and incubated with fresh culture medium containing MTT (0.5 mg•mL$^{-1}$ medium) for 24 h at 37 °C under 5% CO$_2$ atmosphere. Then the unreacted dye was removed and DMSO was added to dissolve the intracellular insoluble purple formazan product into a colored solution. The absorbance of this solution was quantified at 490 nm by photospectrometry. The results were reported as mean±standard deviation (S.D.). For each group, three independent tests were made ($n=3$). To test the difference between two groups, a Student t-test was carried out. A value of $p<0.05$ was considered to indicate a statistically significant difference.

**Results and Discussion**

The sugar fibers were made by squeezing a sugar melt via a nozzle. An as-prepared cotton candy is displayed in Figure 2. The candy is composed of sugar fibers of dispersed diameters around 10 μm. An optical micrograph of some fibers taken from the cotton candy is shown in Figure 3a.

![Figure 2](image-url) Gross view of cotton candy produced in our lab.

The surface of the PLGA film was observed by SEM, and an image is presented in Figure 3b. The microgroove width is basically consistent with the size of their template (5—15 μm, roughly around 10 μm), if the bright borders due to an optical phenomenon in Figure...
Figure 3  (a) A typical optical micrograph of some sugar fibers taken from the cotton candy; (b) an SEM image of a micro-grooved surface of a PLGA film.

3b are not taken into consideration. The AFM imaging gives a similar result and further shows the surface roughness (Figure 4).

Figure 4  A typical AFM image of a microgroove on the PLGA film imprinted by sugar fibers.

No noticeable sugar residue was observed, as illustrated by comparison of the IR spectra of sugar fibers, an initial PLGA film, and an imprinted film after leaching sugars (Figure 5). Yet, the topographic surface modification led to a decrease of contact angles of water from 92±1 to 84±1 degrees, with typical images shown in Figure 6.

The MC3T3-E1 cells were confirmed to be elongated on the microgrooved surface, as shown in Figure 7a and 7b in comparison with Figure 7d and 7e on a smooth PLGA film. Most of the adherent cells were alive, as indicated by the live/dead assay (Figure 7b and 7e), and only few dead cells were observed (Figure 7c and 7f).

The viabilities of the cells on the microgrooved surfaces were quantified by MTT measurement, with the results shown in Figure 8. While the average viabilities of cells cultured on the imprinted surfaces are higher than that on the smooth film, no statistically significant difference was found because $p > 0.05$. Nevertheless, the results illustrate that our fabrication process to
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Figure 7  Optical micrographs of PLGA films cultured with MC3T3-E1 cells. Both microgrooved (a—c) and smooth (e—f) films were observed. (a and d) Bright field micrographs; (b, c, e and f) fluorescent micrographs after stained by live-dead kit, which led live cells in green (b and c) and the dead cells in red (c and f).

Figure 8  Optical density (OD) at 490 nm from the MTT measurements of the viabilities of the MC3T3-E1 osteoblasts on the PLGA films. The amounts of sugar fibers used for modifying the surface topologies of PLGA films of 28.26 cm² are 0 g (a), 12 g (b), 35 g (c) and 105 g (d). n=3. The value of p > 0.05 in the t-tests in comparison between any two groups.

Conclusions

In this study, we put forward a sugar-fiber imprinting technique to generate microgrooves on a polymeric surface. This structure was demonstrated on the surfaces of 2D PLGA films, and the contact guidance of cell orientation by the microgroove topography was confirmed on the resulting surface. Our method using sugar fibers as an imprinting template is valuable with three main advantages: first, sugar fibers are biocompatible; second, sugar fibers are easy to be removed by water; third, compared to the common water-resolvable template (salt), sugars are ready to be processed into the form of fibers besides particles. The feasibility of this idea to use the cotton candy to physically modify the surface topography of water-insoluble material encouraged us to extend this technique into three dimensional PLGA porous scaffolds, and the results would be published elsewhere. Our facile technique to generate topological surfaces via water-soluble sugar might stimulate modification of other water-insoluble materials such as other hydrophobic polymers, crosslinked hydrogels and appropriate inorganic materials.

Note: After submitting our manuscript, we found a pertinent publication in Nature Materials appeared in the web form in July 2012, [31] which used carbohydrate fibers including sugars to generate porous networks of both PLGA and hydrogels after removing the sacrificed template by water. Their template fibers were produced by 3D printing instead of our “cotton candy”.

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References

[23] Yan, C.; Sun, J.; Ding, J. Biomaterials 2011, 32, 3931.
(Zhao, X.)