Generation of microgrooved silica nanotube membranes with sustained drug delivery and cell contact guidance ability by using a Teflon microfluidic chip

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Abstract

Silica nanotubes have been extensively applied in the biomedical field. However, very little attention has been paid to the fabrication and application of micropatterned silica nanotubes. In the present study, microgrooved silica nanotube membranes were fabricated \textit{in situ} by microgrooving silica-coated collagen hybrid fibril hydrogels in a Teflon microfluidic chip followed by calcination for removal of collagen fibrils. Scanning electron microscopy images showed that the resulting silica nanotube membranes displayed a typical microgroove/ridge surface topography with \( \sim 50 \mu \text{m} \) microgroove width and \( \sim 120 \mu \text{m} \) ridge width. They supported adsorption of bone morphogenetic protein 2 (BMP-2) and exhibited a sustained release behavior for BMP-2. After culturing with osteoblast MC3T3-E1 cells, they induced an enhanced osteoblast differentiation due to the release of biologically active BMP-2 and a strong contact guidance ability to directly align and elongate osteoblasts due to the presence of microgrooved surface topography, indicating their potential application as a multi-functional cell-supporting matrix for tissue generation.

Keywords: silica nanotube, microgroove, Teflon chip, drug delivery, cell alignment and elongation

1. Introduction

Cell behaviors in the native microenvironment are highly guided by many nano-/micro-scale structures such as highly aligned collagen fibrils which are the main structural components of the extracellular matrix (ECM) \cite{1}. Mimicking \textit{in vitro} such an organized and fibrous structure is very important in designing ideal biomaterials presenting similar \textit{in vivo} cell behavior. Nanofibrous materials have thus attracted much interest for the construction of artificial cell-supporting matrixes for tissue generation \cite{2}.
Silica-derived forms have attracted considerable attention for the development of artificial bone substitutes since the discovery of bioglass, which consists of SiO₂, CaO, P₂O₅ and Na₂O [3]. Their silanol groups spontaneously induce the deposition of bone-like apatite in the human plasma, promoting osteointegration [4, 5], and exhibit excellent biocompatibility, supporting cell attachment and proliferation [6, 7]. To date, many kinds of silica-derived forms that include spheres [8], membranes [6], xerogels [9], scaffolds [10] and nanotubes [11] have been synthesized and applied to stimulate bone regeneration. Among them, silica nanotubes have attracted special interest since they not only present hollow structural features for delivering various anti-cancer drugs [12], DNA [13] and biological growth factors [14], but also display a typical one-dimensional ECM-like fibrous structural feature to support cell attachment and proliferation [11, 14]. However, despite the prominence of silica nanotubes in biomedical applications, very little attention has been paid to the fabrication and applications of silica nanotubes with highly organized structure.

Currently, several template routes are available for the fabrication of silica nanotubes, including carbon nanotubes [15], vertical silicon nanowires [16] and reverse microemulsion [17]. However, these conventional template routes are not suitable for the fabrication of silica nanotubes with a highly organized structure. Micropatterning is a powerful technique for creating highly organized structures in biomaterials and has been used for micropatterning various materials including polymers and hydrogels [18]. Hydrogels are most popular in the fabrication of micropatterned materials since they are mechanically flexible and can be easily processed with many kinds of highly organized surface morphologies [18, 19]. Microgrooved patterns are one of the most popular micropatterned surface topographies because of their well-defined and organized structure; they are easily directed by soft-/photo-lithographic techniques and strong cell contact guidance ability [7]. Collagen fibrils are one of the organic structural components of extracellular matrices in living tissue and exhibit a strong affinity for silica species via hydrogen bonding and electrical interactions for fabricating silica nanotubes [11, 14]. Therefore, when the collagen-based hydrogels are microgrooved, it is expected that the microgrooved collagen-based hydrogels could serve as an efficient template for in situ fabrication of free-standing and microgrooved silica nanotubes.

In the present study, microgrooved silica nanotube membranes were generated by microgrooving silica-coated collagen hybrid fibril hydrogels in a Teflon microfluidic chip followed by calcination for the removal of collagen fibrils. Their drug delivery capacity for bone morphogenetic protein 2 (BMP-2) and contact guidance ability for osteoblast MC3T3-E1 cells were evaluated.

2. Materials and methods

2.1. Fabrication of a Teflon microfluidic chip

A Teflon microfluidic chip was fabricated by the method described in our previous study [20]. A polydimethylsiloxane (PDMS) master was fabricated by curing the PDMS prepolymer onto the photoresist patterns produced with standard photolithography and tightly sealed to a glass slide. Subsequently, the semicrystallized polymer perfluoroalkoxy (Teflon PFA) substrate (Yuyisong Inc., China) was sandwiched between the PDMS master and another flat glass slide. The sandwich assembly was placed on a hot compressor (TM-101F, Taiming, Inc., China), embossed at 275 °C for 2 min and cooled to room temperature. The Teflon chip was obtained after the PDMS master and glass slide were removed.

2.2. Fabrication of a microgrooved silica nanotube membrane

Silica-coated collagen hybrid fibril hydrogels were fabricated by the method described previously [11, 14], by coating collagen fibrils with silica in a Stöber-type sol–gel system consisting of tetraethyloxysilicate (TEOS; 1 ml), ethanol (9 ml), water (9 ml) and ammonia (28%, 0.5 ml) at room temperature for 24 h. Subsequently, they were placed on either a microgrooved PDMS microluidic chip or a microgrooved Teflon microfluidic chip (1 cm × 1 cm) and subjected to a loading force of 5 N at 50 °C for 2 h to produce the microgrooved silica-coated collagen hybrid fibril membranes. After calcination at 600 °C for 2 h, the collagen fibrils were completely removed from the silica-coated collagen hybrid fibril membranes and the microgrooved silica nanotube membranes were obtained in situ. The flat silica nanotube membranes were prepared by a similar method using the flat Teflon chips. Microstructures of the resulting silica nanotube membranes were observed under a scanning electron microscope (SEM; JEOL-6500F, JEOL, Tokyo, Japan).

2.3. BMP-2 loading and release in the microgrooved silica nanotube membrane

To evaluate the capability of microgrooved silica nanotube membranes for drug delivery, BMP-2 was selected as the model drug since it is a biological growth factor widely used for stimulating osteoblast differentiation [6, 14]. First, 100 µl of 0.5 μg ml⁻¹ BMP-2 (Peprotech, Rocky Hill, NJ, USA) solution was added to the microgrooved silica nanotube membranes and then freeze-drying was performed to produce the corresponding BMP-2-loaded membranes containing 50 ng of BMP-2. For the BMP-2 release experiments, the BMP-2-loaded samples were soaked in phosphate buffer saline (PBS) and the amount of BMP-2 released from samples was measured via an ELISA kit (ELISA; R&D Systems).
2.4. Cell culture

Osteoblast-like MC3T3-E1 cells (RIKEN, Ibaraki, Japan) were seeded on the resulting samples in a 24-well plate at a density of $2 \times 10^4$ cm$^{-2}$ and cultured in $\alpha$-minimum essential medium ($\alpha$-MEM) containing 10% fetal bovine serum, 100 U ml$^{-1}$ penicillin, and 100 $\mu$g ml$^{-1}$ streptomycin in a humidified atmosphere of 5% CO$_2$ and 95% air at 37°C. To induce osteoblast differentiation, cell-laden samples were cultured in a differentiation culture medium containing 10% fetal bovine serum, 100 U ml$^{-1}$ penicillin, 100 $\mu$g ml$^{-1}$ streptomycin, 2 mM $\beta$-glycerophosphate and 50 $\mu$g ml$^{-1}$ sodium ascorbate.

2.5. WST-1 assay

Cell viability and proliferation were evaluated via a conventional water-soluble tetrazolium salts (WST-1) assay. The samples were taken out and transferred to a fresh 24-well plate. Then, 200 $\mu$l of a WST-1/culture medium (1 : 10) mixture was added to each well and incubated at 37°C. After 3 h, 100 $\mu$l of the liquid was taken out and transferred to a 96-well plate. The absorbance of the liquid at 450 nm was measured using a microplate reader (MTP-880, Corona Electric Co Ltd, Japan).

2.6. Alkaline phosphatase assay

Alkaline phosphatase (ALP) is a typical marker during the early stage of bone cell differentiation [21]. To evaluate the biological activity of BMP-2 released from the microgrooved silica nanotube membranes, ALP in the cells seeded on the samples was measured via an ALP assay. The samples were taken out, transferred to a fresh 24-well plate and washed twice with 0.9% NaCl. Subsequently, 200 $\mu$l of 0.1% Triton solution in 0.9% NaCl solution was added to each well. After 10 min, 25 $\mu$l of the cell lysis solution was taken out, transferred to a 96-well plate and mixed with 50 $\mu$l of ALP working solution at 37°C for 15 min. Then, 25 $\mu$l of NaOH solution were added to stop the reaction. The absorbance of the solution at 415 nm was read using the above microplate reader.

2.7. Immunochemical assay

To observe nuclei and F-actin in the cells on the samples, the cell-laden samples were fixed in 3.7% paraformaldehyde for 10 min at 37°C, permeabilized in 0.1% Triton X-100 for 3 min, and stained with 4',6-diamidino-2-phenylindole for nuclei and Alexa Fluor 594 phallolidin for F-actin filaments.

2.8. Statistical analysis

All the results in triplicate were expressed as mean and standard deviation (SD) and analyzed using one-way analysis of variance with a significance level of $p < 0.05$.

3. Results and discussion

PDMS microfluidic chips are most commonly used in microfabricating various polymers and hydrogels because of their excellent flexibility in mechanical properties [23]. After collagen hydrogels were soaked in the sol–gel system for a coating of silica, the resulting silica-coated collagen hybrid fibril hydrogels remained soft. Therefore, it is expected that they could be microgrooved using the PDMS chip. When the silica-coated collagen hybrid fibril hydrogels were microgrooved and removed from the PDMS chip, it was found that some silica-coated collagen hybrid fibrils remained in the microgroove of the PDMS chip (figure 1(a)). This indicates that PDMS chips have a strong affinity for the silica species and this affinity resulted in mass loss of samples.

To overcome this problem, the conventional PDMS chips were replaced with novel Teflon chips for microgrooving silica-coated collagen hybrid fibril hydrogels [24]. From
Figure 2. SEM images of silica nanotube membranes produced on (a) a flat Teflon chip, (b) a microgrooved PDMS chip and (c) a microgrooved Teflon chip.

Figure 3. Release profile of BMP-2 from the microgrooved silica nanotube membranes after soaking in PBS for 2 weeks.

Figure 4. Result of the WST-1 assay for osteoblast MC3T3-E1 cells seeded on the flat and microgrooved silica nanotube membranes without and with BMP-2 (error bars: ±SD; *p < 0.05).

Figure 5. ALP activity in the osteoblast MC3T3-E1 cells seeded on the microgrooved silica nanotube membranes without and with BMP-2 (error bars: ±SD; *p < 0.05).

In figure 1(b), no silica-coated collagen fibrils were found in the microgroove of the Teflon microfluidic chip, indicating that the Teflon microfluidic chip was much more suitable for microgrooving silica-based materials.

Figure 2 shows SEM images of silica nanotube membranes produced on (a) a flat Teflon chip, (b) a microgrooved PDMS chip and (c) a microgrooved Teflon chip. It was found that the surface morphology of the samples depended on the type of microfluidic chips. In the case of a flat Teflon chip, the silica nanotube membranes exhibited a similar flat and smooth surface (figure 2(a)) with a porous and fibrous structure (inset) owing to the template role of the collagen fibrils, as was found in the previous studies [11, 14]. In the case of a microgrooved PDMS chip, no clear microgroove structure was found in figure 2(b). This is because PDMS is mechanically flexible and deforms under pressure. Therefore, it cannot effectively maintain its surface microstructure under pressure for further microgrooving silica-coated collagen hybrid fibril hydrogels. In contrast, the silica nanotube membranes in figure 2(c) exhibit a typical microgrooved/ridge surface topography (about 50 µm microgroove width and about 120 µm ridge width), further showing that the Teflon chip is much better than the PDMS chip in fabricating silica-based materials because of their excellent mechanical stability [24]. The inset in figure 2(c) shows that the microstructure of silica nanotubes is not influenced by microgrooved patterns and all of them exhibit a typically fibrous and hollow structure. In addition,
it was found that collagen fibril hydrogels could not be
directly microgrooved in the present case because of their
rapid biodegradation.

By virtue of the highly organized and fibrous ECM-like
structure, microgrooved silica nanotube membranes
(figure 2(c)) have potential application as a cell-supporting
matrix. Biological factors released from the cell-supporting
matrix strongly stimulate cell differentiation in the tissue
regenerative field [1]. We evaluated the microgrooved
silica nanotube membrane for delivery of BMP-2. Figure 3
shows a time-course release profile for BMP-2 from the
microgrooved silica nanotube membranes after soaking
in PBS up to 2 weeks. The amount of BMP-2 released
from the sample was 13 ± 2% at day 1, 28 ± 1% at day 3,
45 ± 3% at day 5, 57 ± 4% at day 7 and 75 ± 3% at day 14.
The BMP-2 exhibited a sustained release behavior, and its
amount increased as the soaking time increased over 2 weeks,
indicating that the microgrooved silica nanotube membranes
could serve as a sustained drug delivery system, as was
found for the flat silica nanotube membranes in previous
studies [14].

In vitro biocompatibility of biomaterials is critically
important in supporting cell attachment and proliferation.
To evaluate in vitro biocompatibility and bioactivity of
BMP-2, osteoblast MC3T3-E1 cells were seeded on the
flat silica nanotube membrane and microgrooved silica
nanotube membrane with and without BMP-2. Figure 4
shows the WST-1 assay result of osteoblast MC3T3-E1
cells seeded on those samples after 1 and 7 days of
culturing. As the culturing time increased, the absorbance
value increased for each sample, indicating that all samples
supported cell attachment and proliferation and exhibited
good biocompatibility. However, cell viability depended on
the type of sample. At day 1, the cell viabilities of all samples
were similar, despite the difference in surface topography
and presence of BMP-2. At day 7, the cell viabilities on both flat
and microgrooved samples without BMP-2 were very similar
and higher than that on microgrooved samples with BMP-2,
indicating that the difference in surface topography had no
significant effect on cell viability and proliferation and the
presence of BMP-2 inhibited cell proliferation with increasing
culturing time.

Figure 5 shows the ALP activity of osteoblast MC3T3-E1
cells seeded on microgrooved silica nanotubes without and
with BMP-2 after 1, 7 and 14 days of culturing. As the
culturing time increased, the absorbance value for each
sample increased, indicating that both samples supported
osteoblast differentiation. At day 1, both samples exhibited a
similar ALP value and no significant difference was found.
At day 7, the ALP activity was much higher due to the
presence of BMP-2, indicating that the osteoblast MC3T3-E1
cells started to differentiate and their proliferation was slowed
down. This is consistent with the results in figure 4. At day
14, a significant difference in ALP activity was observed and
this confirmed that BMP-2 induced cell differentiation. That
is, the BMP-2 released from the silica nanotube membrane
exhibited in vitro bioactivity and the microgrooved silica
nanotube membranes maintained the bioactivity of BMP-2.

To evaluate cell contact guidance ability, the alignment
and elongation of an osteoblast on the flat and microgrooved
silica nanotube membranes were evaluated. To observe
the cell size and morphology, the viable osteoblast
MC3T3-E1 cells were stained with calcine-acetoxyethyl
ester (calcine-AM) and excited green fluorescence. Figure 6
shows fluorescence microscopy images of MC3T3-E1 cells
on the samples after 1 and 3 days of culturing. Note

Figure 6. Fluorescence microscopy images of osteoblast MC3T3-E1 cells (green) seeded on the flat and microgrooved silica nanotube
membranes.
that viable cells were attached to all samples, and they proliferated, indicating that the resulting silica nanotube membranes had good biocompatibility, despite the difference in surface topography, and could serve as substrates to support cell attachment and proliferation. This result is in agreement with the results of figure 4. However, a significant difference in surface morphology was found. At day 1, the cells on the flat ones were randomly distributed, while that on the microgrooved ones were highly aligned and elongated, indicating good contact guidance ability of the microgrooved silica nanotube membranes. At day 3, the number of cells on all samples significantly increased and the cells grew on the whole surface including the ridge and microgroove, further demonstrating that the samples supported cell proliferation (figure 4). Moreover, the cells maintained a similar difference in size and morphology as on day 1, i.e., randomly distributed on flat ones, and highly aligned and elongated on microgrooved ones. This was evidence of the good contact guidance ability of microgrooved silica nanotube membranes.

Cell alignment is highly correlated with the alignment of F-actin in the cells [22]. Figure 7 shows representative images of the F-actin distribution in cells on the flat and microgrooved silica nanotube membranes after 1, 7 and 14 days of culturing. F-actin is strongly associated with cell morphology and was observed on both samples, indicating that there was a strong interaction between cells and silica nanotubes. However, there was a significant difference in F-actin distributions between both samples. At day 1, F-actin was randomly distributed on flat ones, but highly aligned and elongated on microgrooved ones. At day 7, much more F-actin was produced in the cells for both samples; it was widely distributed on flat ones and highly aligned and elongated on microgrooved ones. At day 14, F-actins exhibited a similar distribution to those at day 1 and day 7. These results indicated that the microgrooved silica nanotube membranes maintained their alignment for more than 2 weeks, which is further evidence of their strong contact guidance ability.

Figure 8 also shows the aligned degree and circularity of the nuclei in the cells on flat and microgrooved silica nanotube membranes after 1 day of culturing. The alignment degree (figure 8(a)) was 10 ± 1% for the flat ones and 45 ± 4% for the microgrooved ones, while the circularity (figure 8(b)) was 0.67 ± 0.01% for the flat ones and 0.45 ± 0.05% for the microgrooved ones. In contrast, the presence of the microgrooved structure resulted in a significant increase in cell alignment and elongation. Figures 8(c) and (d) display histograms of cell alignment angles in 10° increments.
Figure 8. Alignment and elongation of osteoblast MC3T3-E1 cells seeded on the flat and microgrooved silica nanotube membranes after 1 day of culturing. (a) Mean percentage of aligned cell nuclei (within 10° of the preferred nuclear orientation); (b) mean nuclear shape index; (c) and (d) histograms of cell alignment angles in 10° increments (error bars: ±SD; **p < 0.01).

These histograms further demonstrate that the microgrooved silica nanotube membranes exhibited better cell alignment and elongation in comparison with the flat silica nanotube membranes.

Microgrooved patterns have been widely used to direct cellular alignment and elongation. It is found that the dimension of the microgrooved patterns could significantly affect this behavior. Nagamine et al [25] reported that cellular alignment and elongation could be directed on the microgrooved fibrin hydrogels with the microgroove width ranging from 100 to 250 µm. In our previous study, we have demonstrated that microgrooved patterns consisting of 100 µm groove width and ridge width ranging from 50 to 100 µm could direct the cellular alignment and elongation [26]. The dimension of the microgrooved silica nanotube membranes belongs to those dimensions and the cellular alignment and elongation could be thus observed on both the ridge and the microgroove. Although the specific mechanism for such a cellular behavior is still unclear, it is reported that the microgrooved patterns provided a mechanical restriction on the formation of certain linear bundles of microfilaments to push cellular alignment and elongation [27, 28].

4. Conclusions

In summary, microgrooved silica nanotube membranes were successfully fabricated using a Teflon microfluidic chip. Silica-coated collagen fibrils were synthesized, microgrooved in a Teflon microfluidic chip, and then calcined for in situ fabrication of the microgrooved silica nanotube membranes due to the removal of collagen fibrils. Compared with the conventional PDMS microfluidic chip, the Teflon microfluidic chip was much better in producing microgrooved silica nanotubes. The resulting microgrooved silica nanotube membranes exhibited not only a sustained release behavior for BMP-2, but also a strong contact guidance ability to induce alignment and elongation of osteoblasts. Our results indicated that these microgrooved silica nanotube membranes can potentially be used as multifunctional biomaterials.

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References

[1] Langer R and Vacanti J P 1993 Science 260 920
[2] Liu X H, Jin X B and Ma P X 2011 Nature Mater. 10 398
[3] Hench L L 2006 J. Mater. Sci.: Mater. Med. 17 967
[4] Chen S, Osaka A, Hayakawa S, Tsuru K, Fujii E and Kawabata K 2008 J. Sol-Gel Sci. Technol. 48 322
[5] Chen S, Hayakawa S, Shirosaki Y, Fujii E, Kawabata K, Tsuru K and Osaka A 2009 J. Am. Ceram. Soc. 92 2074
[6] Chen S, Osaka A, Ikoma T, Morita H, Li J, Takeguchi M and Hanagata N 2011 J. Mater. Chem. 21 10942
[7] Chen S, Chinnathambi S, Shi X T, Osaka A, Zhu Y F and Hanagata N 2012 J. Mater. Chem. 22 21885
[8] Chen S, Osaka A, Hayakawa S, Shirosaki Y and Tsuru K 2010 J. Mater. Chem. 20 7337
[9] Lee E J, Shin D S, Kim H E, Kim H W, Koh Y H and Jang J H 2009 Biomaterials 30 743
[10] Ren L, Tsuru K, Hayakawa S and Osaka A 2003 J. Sol–Gel Sci. Technol. 26 1137
[11] Chen S, Osaka A and Hanagata N 2011 J. Mater. Chem. 21 4332
[12] Kapoor S and Bhattacharyya A 2009 J. Phys. Chem. C 113 7155
[13] Chen C C, Liu Y C, Wu C H, Yeh C C, Su M T and Wu Y C 2005 Adv. Mater. 17 404
[14] Chen S, Shi X, Morita H, Li J, Ogawa N, Ikoma T, Hayakawa S, Shirosaki Y, Osaka A and Hanagata N 2011 Sci. Technol. Adv. Mater. 12 065003
[15] Yin Z H, Liu X and Su Z X 2010 Bull. Mater. Sci. 33 351
[16] Kovtyukhova N I, Mallouk T E and Mayer T S 2003 Adv. Mater. 15 780
[17] Jang J and Yoon H 2004 Adv. Mater. 16 799
[18] Khademhosseini A and Langer R 2007 Biomaterials 28 5087
[19] Peppas N A, Hilt J Z, Khademhosseini A and Langer R 2006 Adv. Mater. 18 1345
[20] Shi X T, Chen S, Zhou J H, Yu H J, Li L and Wu H K 2012 Adv. Funct. Mater. 22 3799
[21] Dieudonné S C, van den Dolder J, de Ruijter J E, Paldan H, Peltola T, van’t Hof M A, Happonen R P and Jansen J A 2002 Biomaterials 23 3041
[22] Aubin H, Nichol J W, Hutson C B, Bae H, Sieminski A L, Cropek D M, Akhyari P and Khademhosseini A 2010 Biomaterials 31 6941
[23] Xia Y N and Whitesides G M 1998 Annu. Rev. Mater. Sci. 28 153
[24] Ren K M, Dai W, Zhou J H, Su J and Wu H K 2011 Proc. Natl Acad. Sci. USA 108 8162
[25] Nagamine K, Kawashima T, Ishibashi T, Kaji H, Kanzaki M and Nishizawa M 2010 Biotechnol. Bioeng. 105 1161
[26] Hosseini V, Ahadian S, Ostrovidov S, Camci-Unal G, Chen S, Kaji H, Ramalingam M and Khademhosseini A 2012 Tissue Eng. 18 2453
[27] Dunn G A and Heath J P 1976 Exp. Cell Res. 101 1
[28] Ohara P T and Buck R C 1979 Exp. Cell Res. 121 235