REPLICATION OF BIOMIMETIC NANOSRUFACES AND THEIR APPLICATION TO CELL DIFFERENTICATION STUDY

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ABSTRACT

In this study, we report the influence of the biomimetic polystyrene (PS) nanosurfaces of nanopore and nanopillar arrays on osteoblast differentiation of MC3T3-E1 cells. The nanosurfaces with nanopore and nanopillar arrays were replicated by a hot embossing process with the nickel mold inserts having nanopillar and nanopore arrays, respectively. And the osteoblast differentiation of MC3T3-E1 cells on the nanosurfaces was evaluated by alkaline phosphatise (ALP) staining and RT-PCR analysis of induced ALP and osteocalcin gene expression. We observed that the nanopore arrays were most effective in promoting osteoblast differentiation of MC3T3-E1 cells.

KEYWORDS: Polymer nanosurface, Nickel mold inserts, Hot embossing, MC3T3-E1, Cell differentiation

INTRODUCTION

Recently, the development of nanotechnologies has opened up the possibility of constructing nanosurfaces with a mimicked the extracellular matrix (ECM) which is an interconnected fibrous network with nano-scale architecture [1]. To mimic the nano-scale architecture of the ECM, a variety of nanofabrication methods including traditional photolithography, e-beam lithography, electrospinning, and dip-pen lithography have been suggested [2]. However, they have a disadvantage in terms of productivity over large areas, choice of materials, and variety of geometry types. Among the nanofabrication methods, polymer molding technique (e.g. hot embossing and injection molding) is that it can be mass-replicated thermoplastic surfaces with nanostructures over large areas. Moreover, the thermoplastic have several advantages, such as disposability, high productivity, and biocompatibility, in application to biomedical device [3]. But the cost effective method of metal mold insert for replicating nanosurfaces has not been developed. In this paper, we reported the effective fabrication methods for nickel mold inserts with nanopillar and nanopore arrays and the influence of the biomimetic polystyrene (PS), replicated by hot embossing, nanosurfaces having the nanopore and nanopillar arrays on osteoblast differentiation of MC3T3-E1 cells.

FABRICATION

Figure 1 illustrates the whole fabrication process of the polymer nanosurfaces with nanopopre and nanopillar arryas. Firstly, the nano templates were needed for making metal mold inserts by the electroforming process. To overcome the cost limitation of nanofabrication methods, the two-step anodization process was used for making a nano template having nanopore arrays (Figure 1(a)). It is well known that the two step anodization is very simple and inexpensive technique to obtain a nano template with the ordered nanopore arrays [4]. The optimized process conditions used in this study for making nanopore array of about 200 nm in diameter and 500 nm in depth are listed in Table 1. The other polymer nano template having the nanpillar arrays was prepared by hot embossing process with the AAO template (Figure 1(b)). The polycarbonate (PC) sheet was used as a polymer substrate because it has a good modability for replicating the nanosurfaces. Before the electroforming process, the nano templates were passivated with potassium dichromate. To realize the nickel mold inserts, the electroforming was performed onto the prepared nano templates (Figure 1(c)). Finally, the hot embossing process was carried out, thereby enabling obtain a polymer nanosurfaces with nanopore and nanopillar arrays (Figure 1(d)). In this case, the polystyrene (PS) sheet, used the most widely used materials for cell culture dish or plate. Both the PS nanosurfaces and flat substrates were treated with oxygen plasma for enhancement of cell attachment.

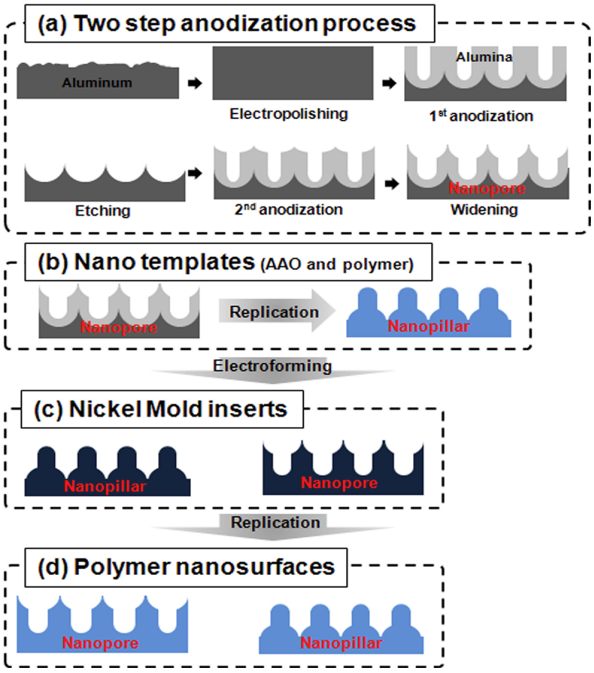

Figure 1. Schematic diagram of fabrication method of the biomimetic nanosurfaces with the nanopillar and the nanopore arrays.

Table 1. Process condition of the two step anodization.

	Solutions	Temperature (°C)	Time (min)	Voltage (V)
Electropolishing	Perchloric acid, ethanol	7	5	20
1st anodization	0.1 M phosphoric acid	-5	180	195
Etching	Chromic acid, phosphoric acid	65	300	-
2nd anodization	0.1 M phosphoric acid	-5	7.5	195
Widening	0.1 M phosphoric acid	20	150	-

CELL CULTURING

MC3T3-E1, pre-osteoblast cell line, were cultured with osteogenic media on the two kinds of nanosurfaces and flat substrates. The ALP activity was measured by p-Nitrophenyl Phosphate at 7 days. Cell were lysed in lysis buffer. Lysates were incubated with pNPP at 37 °C for 30 min. Absorbance was measured at 405nm using a microplate reader. The ALP staining was performed using an alakaline phosphatase kit (Sigma). Briefly, cells were fixed in 10% formalin and incubated with the substrate buffer at room temperature for 30min. Total RNA was extracted from cultured cells using the Trizol. The cDNAs were synthesized from 1 μg of RNA using the SuperScript synthesis system. Real time PCR (RT-PCR) was performed on ABI StepOnePlus system using the SYBR Green PCR Master Mix assay. The amplification reaction was performed for 40 cycles with denaturation at 95 °C for 10 min, followed by annealing at 95 °C for 15 s and extension and detection at 60 °C for 1 min. All reactions were run in triplicate.

RESULTS AND DISCUSSION

Figure 2 shows the SEM images of the nano template, nickel mold inserts, and the replicated PS nanosurfaces. As shown in Fig. 2(a-i) and (b-i), the two kinds of nano tempaltes (AAO template, polymer template) were successfully fabricated by the two step anodization and the hot embossing. By the nickel electroforming onto the prepared nano templates described above, the nickel mold inserts having nanopillar and nanopore arrays 200 nm diameter and 500 nm height and depth were achieved (Figure 2(a-ii) and (b-ii)). The PS nanosurfaces were successfully replicated on the PS polymer sheet as shown in Fig. 2(a-iii) and (b-iii).

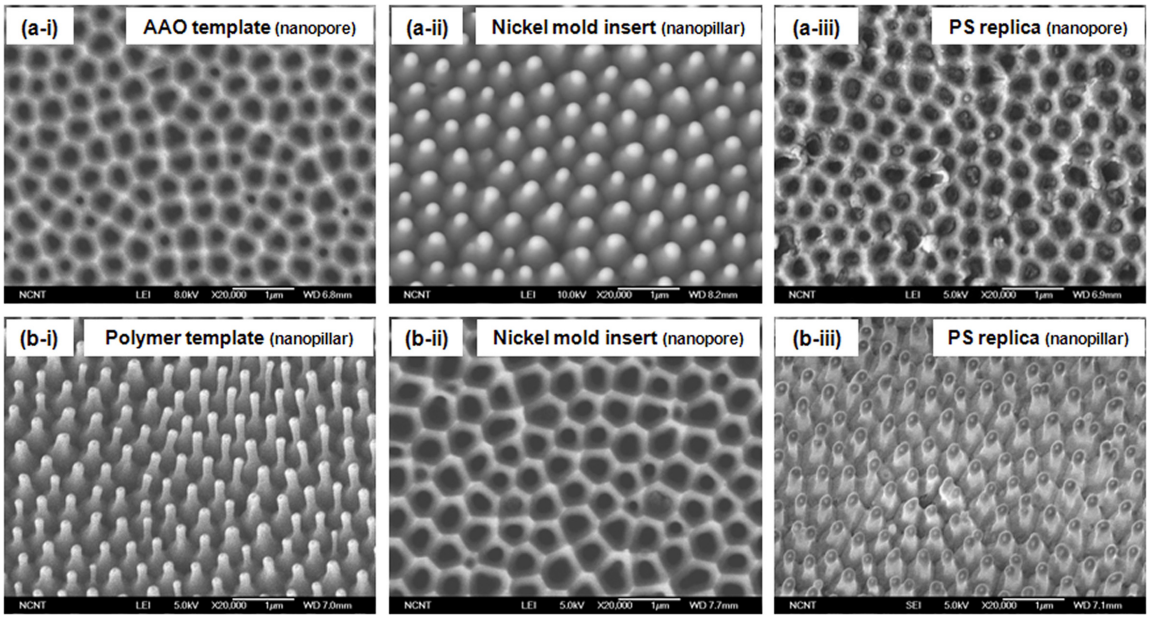

Figure 2. SEM images of the AAO template (a-i), polymer template (b-i), nickel mold inserts (a-ii, b-ii), and replicated PS nanosurfaces (a-iii, b-iii).

To confirm the capacity of osteogenic differentiation on nanoengineered plastic surfaces, the ALP staining and osteogenic marker expression (ALP, osteocalcin) were performed. MC3T3-E1 cells were cultured on flat (control), nanopore, and nanopillar arrays induced osteoblast differentiated. Figure 4 show that cells on nanopore surface was significantly higher than flat and nanopillar surface in ALP staining. Also, significant increases in ALP and osteocalcin gene expression were detected on nanopore surface, as shown fig. 5. These result indicated that cells on nanoengineered plastic surfaces surface accelerated osteoblast differentiation, in particularly nanopore surface.

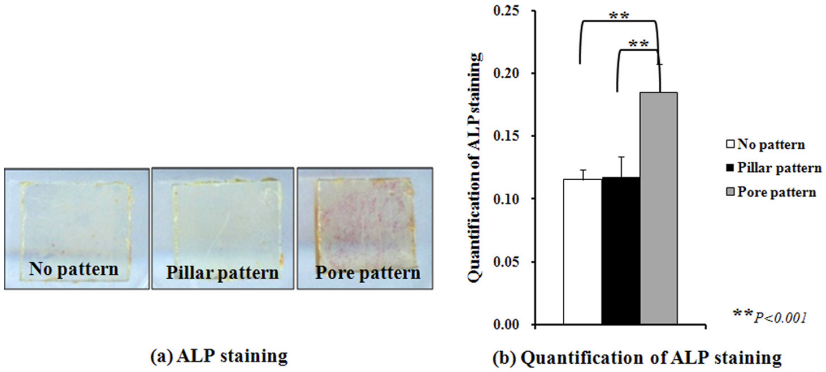

Figure 4. Alkaline phosphatise (a) staining of MC3T3-E1 cells after 7 day and (b) quantification of ALP staining.

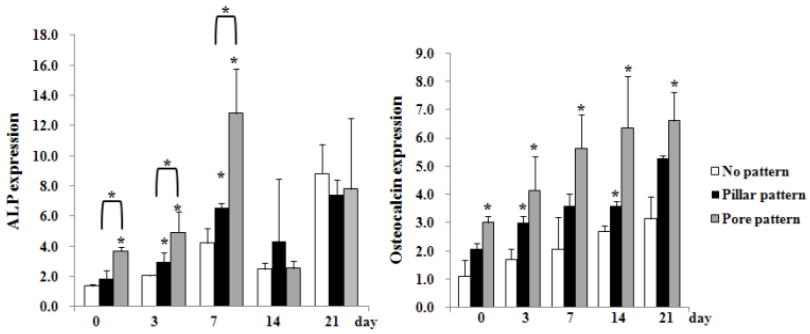

Figure 5. RT-PCR analysis of induced (a) ALP and (b) osteocalcin gene expression.

CONCLUSIONS

The current study has successfully fabricated the nickel mold inserts having nanopillar and nanopore based on the two step anodization and electroforming process, and replicated the biomimetic nanosurfaces with the help of nickel mold inserts by the hot embossing. These results indicate that the fabrication techniques is effective to study of cell behavior on nanosurfaces. Moreover, the nanopore arrays was most effective in promoting osteoblast differentiation of MC3T3-E1 cells. The nanosurfaces enhancing the efficiency of differentiation can be applied to a cell culture dish and plate for the tissue engineering and regenerative medicine.

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