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Large-area Sub-micron Structured Surfaces Using Micro Injection Moulding Templates of Nanoporous Anodized Aluminum Oxide

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Abstract

This study demonstrates a mass production method using nanoporous Anodized Aluminum Oxide (AAO) templates as mould insert tools that are used to structure large area polymer surfaces by a micro injection moulding process. SEM and water contact angle measurements served to evaluate the effect of nanostructures on surface properties. Human umbilical vein endothelial cells were cultured on nano-structured and ultra-smooth surfaces of polymer parts. Experimental results indicated that nano-pillar features sized from ~100nm to 250nm were easily replicated on these same polymer surfaces. The contact angles varied from 3° to 9°. The nanoporous AAO templates were able to retain their pore integrity very well for ~80 injection moulding cycles: this suggests they could be a potential mould tool for large area patterning of polymer surfaces. The endothelial cell culture analysis indicates that cell growth was not significantly affected by nano-topography compared to the smooth surfaces (baseline $R_a$ ~6nm) and both surfaces had equally good cell adhesion.

Keywords: nanoporous AAO, micro injection moulding, cell adhesion, contact angle measurement.

1. Introduction

Forming micro-nano structured polymer surfaces to control and modify behaviour in the presence of fluids, e.g., hydrophobic/hydrophilic properties, has important applications for cell culture and self cleaning [1-4]. However, limitations in patterning large surface areas are a challenge for many manufacturing technologies. Fabrication methods such as e-beam lithography and nanoimprinting are expensive and unsuitable for mass production [1]. To conquer these challenges, a mass replication method with a low-cost, large-area, nano-structured tool must be developed.

Nanoporous AAO films have become one of the most prominent template materials for preparing nanostructured surfaces for either functional surfaces or tool applications, due to its large area nanoporous nature with pores having high aspect ratios up to several hundred [1,2,5,6]. In order to form high aspect ratio nano-lines, nanoporous AAO has been widely used as a template using nano-imprinting processes [7-9]. However, because the nanolines can break during demoulding, AAO is generally treated as a disposable material and is chemically etched so that high aspect ratio nano-lines can be formed [2]. This method cannot be used for mass production as it is inefficient and the AAO foil is quite expensive. In addition, nanoimprinting is generally used to structure polymer films.

Micro injection moulding is the most efficient production method to fabricate plastic parts with micro/nano scale features and complex geometries. In this work, we show how a commercially available AAO can be incorporated as an insert tool in a mould to repeatedly form large area structured polymer surfaces by micro injection moulding. We evaluate the longevity of the AAO template with moulding cycles and test the formability of nano-features on different polymer materials. The properties of the resulting nanostructured surfaces were evaluated using water contact angle measurements. Endothelial cells were used as a demonstrator example to study the effect of nano-topography on cell adhesion.

2. Experimental details

2.1. Mould and AAO template

AAO is a self-organized nanostructured material containing a high density distribution of uniform cylindrical pores that are aligned perpendicular to the surface of the materials and penetrate its entire thickness. A regular porous structure is formed when aluminium is electrochemically oxidized (anodized) in certain solutions [9]. A thin dense alumina barrier layer separates the pores from the aluminium. The pore diameter can be sized from 5 nm to several hundred nm, with the corresponding pore density varying from $10^{11}$ to $10^{13}$ cm$^{-2}$. In the present study, a specially designed silt cassette mould was used. The cavity was formed by a mould insert, as shown in Fig. 1 (left). AAO on Al substrates manufactured by Synkera Technologies Inc. was used as a template, as shown in Fig. 2. Two type of AAO templates with pore diameters of 55nm and 100nm were used in the present study (c.f. Table 1). High temperature adhesive (epoxy M-Bond 610) was used to glue the templates onto the surface of the mould insert. Template 1 was attached near the gate, while template 2 was far from the gate. It was anticipated prior to moulding that the polymer could be difficult to release from the mould surface, so a release agent,
Frekote 710-NC from Henkel, was sprayed on the template surface, as it is a specific polymer release agent with good thermal stability up to 400°C. In order to compare the effect of nano-topography on cell adhesion behaviour, we also manufactured four Bulk Metallic Glass (BMG) strips, which were polished with average surface roughnesses of 5–8nm [10]. The BMG strips were also incorporated into a stainless steel insert, as shown in Fig. 1 (right).

2.2 Material and processing

We used three polymer materials: COC (8007 X10), PMMA (VS-UVT), and HDPE (HMA 016). COC and PMMA are both transparent polymers with a low heat deflection temperature of ~75°C. Both can be used to fabricate transparent diagnostic devices. HDPE is a conventional semi-crystalline polymer with a glass transition temperature around ~120°C and a heat deflection temperature of 71°C, which is good for replicating nano-scale features. PMMA and COC were both dried in a vacuum oven at 70°C and 55°C, respectively, for more than 2 hours. All experiments were carried out using a Fanuc Roboshot S-2000i 15B reciprocating micro injection moulding machine. The injection moulding process conditions for each material are listed in Table 2.

Subsequent to having processed the samples, they were plasma activated in order to increase their surface energies. Previous work [3,4] had confirmed that this served to positively affect the wettability and effectiveness of cell adhesion to these and other polymer surfaces.

Table 1

<table>
<thead>
<tr>
<th>Template</th>
<th>Pore diameter (nm)</th>
<th>Thickness (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>1</td>
</tr>
</tbody>
</table>

2.3. Surface characterization

Surface characterisation of the polymer and AAO foil was carried out using an FEI Quanta™ Scanning Electron Microscope. The surfaces of the polymer and AAO were gold-coated (thickness c. 10nm) to facilitate these measurements. Water contact angle measurements was carried out using a video capture apparatus (OCA 20 from Dataphysics Instruments) at room temperature to evaluate the effect of surface nanostructures on the surface energy. Contact angle was measured using at least five separate drops on each sample surface by delivering/withdrawing distilled water with a micro syringe. For each polymer material, water contact angle measurements were also made on the smooth surfaces replicated from the BMG insert tool in order to compare the effects of surface topography on surface properties.

2.4. Cell culture

Human endothelial cells (diameter of ~8-12μm) were cultured on both the nano-structured surfaces and the smooth surfaces. Due to the low contact angle and hydrophilic properties of PMMA, parts moulded with PMMA were used initially for cell culture. Both surfaces were placed for coating in a 6-well plate containing 50μl fibronectin (100μg/ml) for 1 hour in an incubator. Then, the surfaces were seeded with a primary endothelial cell suspension of 3mls, containing ~6.5 × 10^5 cells, and placed in an incubator at 37°C.

3. Results and discussion

3.1 Replication of nano-structured surfaces

In our previous work [11], the effects of cavity pressure, melt contact temperature, capillary force, and entrapped air on mould filling were studied. We found that mould temperature is the most important factor that influences the replication of micro/nano features. This is because mould surface temperature determines the polymer melt solidification time and also influences the melt viscosity. However, parts with large surface areas generally have dimensions in the millimetre scale. High mould surface temperatures, especially when higher than a polymer’s glass transition temperature, will extend the injection moulding cycle time. In the some cases, it also induces defects such as flash. Clearly, both the quality of a macro part and good replication of micro/nano features need to be achieved during optimization of the moulding process. In the present work, the mould temperature was set below the heat deflection temperature for PMMA, COC and HDPE.
The process was not optimised. Figure 3 shows one of the moulded parts in PMMA.

![Moulded PMMA part](Image)

**Fig. 3. Moulded PMMA part**

Table 2

<table>
<thead>
<tr>
<th>Process conditions</th>
<th>PMMA</th>
<th>COC</th>
<th>HDPE</th>
</tr>
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<tr>
<td><strong>Process conditions</strong></td>
<td><strong>VS-UVT</strong></td>
<td><strong>8007X10</strong></td>
<td><strong>HMA016</strong></td>
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<td>Hopper(°C)</td>
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<td>40</td>
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<tr>
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<td>205</td>
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<tr>
<td>Zone 2(°C)</td>
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<tr>
<td>Zone 3(°C)</td>
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<td>150</td>
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<tr>
<td>Nozzle(°C)</td>
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<tr>
<td>Injection Velocity (mm/s)</td>
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<td>Shot size (mm)</td>
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<tr>
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<tr>
<td>Holding Time (s)</td>
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<td>1</td>
</tr>
<tr>
<td>Cooling Time (s)</td>
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<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mould temperature (°C)</td>
<td>60°C</td>
<td>60°C</td>
<td>55°C</td>
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Patterned polymer surfaces were successfully fabricated by this micro injection moulding process using a conventional moulding system. Figure 4 shows the nano-scale features replicated in HDPE using the two different templates. The features replicated from the two templates have dimensions of ~100nm and ~250nm. The size of nano features on the PMMA surface is ~200nm when replicated from template 1 and ~250nm using template 2. For COC, the size of the features is ~250nm. From the images of the surface structures, it seems that HDPE replicates better than COC, which, in turn, replicates better than PMMA. However, the size of the replicated features is larger than the pore diameters of both templates. This is because the polymer only fills the entrance of the hexagonal cells on the AAO template, which are shown in Fig. 2. It is possible that the molten polymer would fill into the nano-pores and form some nano-rods. These nano-rods would break easily during the demoulding process. FIB (Focused Ion Beam) milling was used to cut a cross-section on the AAO template after ~80 moulding cycles in order to determine whether this actually happened. By using a wide range energy-dispersive X-ray (EDX) detector on the FEI Quanta™ system, we confirmed that no polymer remained in the porous template. This indicates that the polymer melts solidified before filing any of the nano-pores. In addition, the pore structures on the AAO were found to be irregularly shaped, and non-uniformly sized and distributed. Some hexagonal cells even had two pores, as can be seen in some parts of Fig. 2. This imperfect morphology would prevent the formation of nano-rods. The glued AAO template that was closest to the gate peeled away from the steel cavity surface after ~80 cycles, due to failure of the adhesive under high shear stresses and high temperatures (thermo-mechanical fatigue). Figure 7 shows the condition of AAO template 2 after 80 cycles. Some visible contamination can be seen on the AAO surface. However, it is evident that the pores of the AAO remained intact up to this stage and it was possible to remove this contamination by ultrasonic cleaning with Acetone.

![Replicated nano-scale features on the surface of HDPE](Image)

**Fig. 4. Replicated nano-scale features on the surface of HDPE: from template 1 (left) and from template 2 (right).**

![Replicated nano-scale features on the surface of PMMA](Image)

**Fig. 5. Replicated nano-scale features on the surface of PMMA from template 1 (left) and from template 2 (right).**

![Replicated nano-scale features on the surface of COC](Image)

**Fig. 6. Replicated nano-scale features on the surface of COC from template 2.**
3.2 Surface properties

Water contact angles were measured on the smooth and patterned polymer surfaces, as shown in Fig. 7. On the smooth surface, the angles are around 97°, 94° and 68° for the COC, HDPE and PMMA, respectively. This means that COC and HDPE are both slightly hydrophobic while PMMA is a hydrophilic polymer. After patterning, there was a modest change in the measured contact angles: these increased by ~3° and ~7° for COC and HDPE respectively, as shown in Fig. 8. On the other hand, however, the contact angle decreased by ~9° for PMMA. There was no significant difference between the contact angles on the HDPE surfaces which were replicated using the two different AAO templates, i.e., ~101° in both cases. While the patterned surfaces did not lead to significantly different surface properties from their corresponding smooth surfaces, they could clearly provide a potential patterned substrate which could be incorporated into various micro scale features, such as micro channels and micro pillars in typical microfluidic devices.

3.3 Cell culture

Moulded PMMA parts were used for initial cell adhesion because of its hydrophilic surface properties. Human endothelial cells were cultured on both the nano-structured surface and on the smooth surface. Fig. 9 compares cell growth images using phase contrast microscopy at X10 magnification after 18 hours. Clearly, cells grew very well on both the nano-structured and smooth PMMA surfaces. It also can be seen that there is no significant difference between the distribution or density of cells on the nano-structured surface and the smooth surface after 18 hours, as shown in Figs 9 (a) and (b). After 80 hours, cells on the nano-structured surface appeared to be rather more densely concentrated than earlier and than on the smooth surface. However, this simple visual inspection without any random quantitative analysis is not sufficient to say with certainty that the nano-structured surface provides a better quality surface for culture cell. In general though, it is evident that this nano-structured surface does not have any significant or adverse effect on endothelial cell adhesion. This might well be because the dimensions of the nano-structures (c. 100–200nm) are relatively small compared to dimensions of the endothelial cells (diameter ~8-12 µm [12]).

4. Conclusions and outlook

In summary, we demonstrated the use of nanoporous AAO templates as an injection moulding tool to pattern large area polymer surfaces with a micro injection moulding process. Nano-scale features varying in size from ~100nm to ~250nm were successfully patterned. However, the polymer melts did not fill into the pores: this is likely to have been because of the low mould temperature, the uneven distribution of pores and possible air entrapment. The contact angle measurements indicated that the surface properties of three different polymers were changed with contact angles changing by 3–9°, depending on the particular polymers and nanostructures that were used. The pores on the
AAO template retained their integrity very well after ~80 moulding cycles, although some visible contamination was evident. Human endothelial cells were successfully cultured on both nano-structured and smooth surfaces of PMMA moulded parts. It seems that nano-structured surfaces using nanoporous AAO can ensure a moderately better cell adhesion than smooth surfaces (average surface roughness of ~6nm). This modest difference between cell adhesion on both surfaces is probably because the dimensions of the nano-features (~100-250nm) are small compared to those of the cells (~8-12µm).

Future work will include the development of a robust method to incorporate AAO templates into a mould, selection of a good AAO template, and the possible coating of such a template to reduce demoulding friction. Process optimisation and a variotherm mould temperature control system will be used to improve the replication of nano-rods. From a fabrication perspective, the anodization method to fabricate nanoporous AAO templates should be tailored to provide a certain aspect ratio in order to meet the requirements for them to be used as a tool or master. Issues associated with demoulding are equally important for both injection moulding and nanoimprinting processes.

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