Micro- and nanostructuring of poly(ethylene-2,6-naphthalate) surfaces, for biomedical applications, using polymer replication techniques

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Abstract
Here we investigate the formation of superficial micro- and nanostructures in poly(ethylene-2,6-naphthalate) (PEN), with a view to their use in biomedical device applications, and compare its performance with a polymer commonly used for the fabrication of these devices, poly(methyl methacrylate) (PMMA). The PEN is found to replicate both micro- and nanostructures in its surface, albeit requiring more forceful replication conditions than PMMA, producing a slight increase in surface hydrophilicity. This ability to form micro/nanostructures, allied to biocompatibility and good optical transparency, suggests that PEN could be a useful material for production of, or for incorporation into, transparent devices for biomedical applications. Such devices will be able to be autoclaved, due to the polymer’s high temperature stability, and will be useful for applications where forceful experimental conditions are required, due to a superior chemical resistance over PMMA.

(Some figures in this article are in colour only in the electronic version)

1. Introduction
Imprint techniques are relatively simple ways of replicating superficial features in a polymer surface with a resolution down to the nanometre range [1]. The most common of these imprint techniques are hot embossing [2] and nanoimprint lithographies [3] (HEL and NIL), which are used to produce structures with super- and sub-micron dimensions respectively. Pattern replication techniques, such as HEL and NIL, are parallel in nature, and tend to side-step some of the disadvantages inherent within other forms of lithography [4]. The advantages of these techniques, over conventional lithographic techniques, include comparatively low running costs and low replication mechanism complexity. The ability to produce repeatable features over a large area [5], and the fact that a given master can be used several times [6], makes these methods appealing for the production of multiple polymeric replicas. Once fabricated, these surfaces can be utilized in a variety of applications; for example as support materials for biomedical experimentation [7], or as fabrication materials for fluidic devices [8, 9].

The most common polymer used in polymer replication techniques is poly(methyl methacrylate) (PMMA). PMMA
inertness and UV barrier properties, PEN has applications in the production of food containers, in particular plastic bottles, which can withstand the temperatures required for sterilization. This high temperature resistance also means PEN is useful as a substrate in the production of flexible printed circuits which can be soldered using conventional tin/lead alloys [13]. Its inherent strength and dimensional stability (partially due to the presence of the cojoined benzene rings in the monomer [14]) means PEN is also commonly used for fibres and films where low shrinkage and elongation properties are required [11]. For biomedical applications, if PEN is found to be biocompatible, structuring of the polymer surface could be used to investigate topography effects on cell growth [15].

2. Experimental details

2.1. Materials

PEN and PMMA sheets (125 µm thick) were used as supplied from Goodfellow Ltd (UK). For each imprinting experiment, the polymer was cut to the approximate size of the master to be used for the imprint. The polymer was rinsed with isopropanol (IPA, Aldrich Chemical Co., UK), to remove any dust particles, and dried using a stream of nitrogen gas.

Two types of masters were used for the HEL and NIL experiments: masters with random, disordered structures and those with regular, ordered structures. A commercially available glass, where the surface has been etched using hydrofluoric acid (HF) to produce a frosted appearance, was used as the randomly structured master.

Masters with an ordered microstructure were designed in house and supplied by the Centro Nacional de Micro-electrónica (CNM), Barcelona, fabricated using lithographic techniques from silicon nitride (Si$_3$N$_4$) or oxide (SiO$_2$) coated silicon. The microstructures were defined in this surface coating to give masters with both positive (where the features are higher than the surface) and negative (where the features are below the surface) structures. Masters with ordered nanostructures were produced by focused ion beam (FIB) milling of a silicon based substrate material. The FIB (Strata DB235; FEI Co., Netherlands) was used to mill superficial structures into the Si$_3$N$_4$ layer of a 1 cm$^2$ piece of the master material, consisting of a silicon wafer coated with successive layers of SiO$_2$ (100 nm) and Si$_3$N$_4$ (180 nm).

The SiO$_2$/Si$_3$N$_4$ layers were used to prevent adherence problems between the master and the polymer. However, to ensure the master did not stick to the PMMA, a monolayer fluoroalkylsilane anti-adhesion layer (trichloro(tridecafluoro-octyl)silane; United Chemical Technologies, USA; figure 1(c)) was also added to the master surface using a previously reported method [16].

2.2. Polymer replication

Hot embossing was performed using a Jenoptik HEX 01 hot embossing system (Jenoptik Mikrotechnik GmbH, Germany). Typical embossing conditions for each polymer are given in table 1. The polymer was placed onto a piece of borosilicate glass, positioned on the base of the hot embosser, which stopped the polymer from adhering to the base plate of the apparatus. The master was then placed on top of the polymer.
with the surface to be embossed in contact with the polymer and hot embossing proceeded using a typical embossing method [2, 9].

Nanoimprint lithography was carried out in a similar fashion to the hot embossing and was performed using an Obducat nanoimprinter (Obducat AB, Sweden). Again, typical NIL conditions for each of the polymers is given in table 1. The polymer was placed onto an unstructured piece of the material used to produce the master stamp, positioned on the base of the nanoimprinter. The master was placed on top of the polymer, again with the surface to be embossed in contact with the polymer, and imprinting proceeds in a typical fashion [3]. The use of a freestanding piece of polymer, sandwiched between the master and the piece of master material (as opposed to using a polymer film spun down onto the piece of master material, as is usual when nanoimprinting), means that the imprinted polymer can be used in applications where the polymer’s inherent transparency is necessary, such as biomedical applications where optical microscopy is required. As the resolution of the NIL is dependent on the master stamp [3], the production of features with dimensions less than 10 nm should be possible.

A schematic diagram of the hot embossing/nanoimprinting process is given in figure 1(d). With care, the master can be reused a number of times, and in this way imprinting techniques can be used to produce a number of patterned polymer surfaces, containing features with dimensions ranging from millimetres to nanometres.

### 2.3. Characterization

Characterization of the surfaces of the masters and the patterned polymers was achieved using white light interferometry (Wyko NT110; Vecco Metrology, USA), atomic force microscopy (AFM; Dimension 3100; Digital Instruments, USA) and scanning electron microscopy (SEM; Strata DB235; FEI Co., USA). The pristine and structured polymer surfaces were further characterized via contact angle measurements. Ultra-pure water (3 µl, Milli-Q; Millipore, USA) was deposited on the surfaces of the samples using an OCA 20 optical contact angle system (Dataphysics, GmbH, Germany), and the advancing contact angle was measured. The water was then removed in 0.5 µl aliquots until the drop edge receded, and the receding contact angle was measured. Finally, the optical transmission of the polymers was recorded using an ultraviolet/visible spectrometer (UV/2501PC, Shimadzu, Japan) and compared with that of a 150 µm thick glass cover slip.

![Figure 2. Optical microscope images of the proliferation of MG63 cells, cultured in complete medium (D-MEM) on a pristine PLA surface, after (a) 1 day, (b) 4 days and (c) 7 days.](image)

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### 2.4. Cell culturing

Osteoblast-like MG63 cells (from ATCC) were used to test the biocompatibility of the PEN surfaces used in this work. The cells were maintained at 37 °C and 5% CO2 in complete medium (D-MEM), containing 10% foetal calf serum (FCS) and 1% each of L-glutamine, pyruvate and streptomycin/penicillin. Squares (4 mm²) of unstructured, thin film PEN were placed in 24 well plates and immersed in 0.5 ml of the complete medium for 24 h. After this time, the medium was replaced with fresh medium and the MG63 cells were seeded at a density of 2 × 10⁵ cells per well plate. The well plates were cultured in triplicate for periods of 1, 4 and 7 days to evaluate cell proliferation, with the medium being changed biweekly.

Optical microscope images of the cells on the surface of the PEN are given in figure 2. Initially, the seeded cells attach to the PEN surface and start to elongate. After 4 days the cells have elongated further and begin to form microspikes with which they explore the surrounding environment and attach to the polymer surface. After 7 days the cells have

### Table 1. Typical hot embossing and nanoimprinting conditions.

| Technique | Polymer | T (°C) | P (MPa) | t (s) | Cooling T (°C) |
|-----------|---------|--------|--------|------|---------------|
| HEL       | PMMA    | 130    | 4      | 600  | 80            |
| PEN       | 200     | 30     | 1200   | 90   |
| NIL       | PMMA    | 130    | 5      | 300  | 80            |
| PEN       | 200     | 5      | 300    | 90   |
Figure 3. Optical transmission spectra of glass (solid curve), PMMA (dashed curve) and PEN (dash/dot curve) at wavelengths close to the visible region of the electromagnetic spectrum, showing the percentage visible radiation transmission for each, compared to an air blank, and the near-UV absorption of each sample.

Table 2. Roughness properties of the superficial structure of the masters and the imprinted polymers (AFM, atomic force microscopy; WLI, white light interferometry).

| Master          | Polymer | $R_q$ (nm) | $R_t$ (nm) | Method |
|-----------------|---------|------------|------------|--------|
| PMMA            | 10      | 682        |            | AFM    |
| PEN             | 6       | 645        |            |        |
| Frosted glass   | PMMA    | 2780       | 15 960     | WLI    |
|                 | PEN     | 2780       | 15 590     |        |

proliferated successfully and completely cover the surface area of the polymer in the image. This proves that the PEN used here is culture compatible, and non-toxic towards MG63 cells.

3. Results and discussion

The optical transmission of the polymers is given in figure 3, in the range 300–800 nm, and compared with that of a 1.5 $\mu$m thick glass cover slip. PMMA is seen to have an optical transparency rivalling glass throughout the near IR/visible region of the electromagnetic spectrum. Although this transmittance decreases in the UV region of the spectrum, the polymer still transmits some 60% of the incident light. PEN in comparison transmits $\sim$80% of the incident light in the near IR/visible region, but its transmission falls rapidly as the UV region is encountered at $\sim$400 nm, due to the presence of the UV-adsorbing naphthalate moiety in the polymer matrix.

Optical and SEM images of the superficial structure of the masters and the polymers used in this work are presented, along with white light interferometric or AFM images, depending on the size of the features on the sample surface. In the case of the irregular structures, the rms roughness ($R_q$) and the maximum peak to valley distance ($R_t$) is given in table 2. AFM images of the surfaces of the pristine polymers (not shown) reveal a relatively smooth surface. In each case, the roughness of the polymer is less than 10 nm and, hence, the inherent surface structure was not expected to affect the production of the imprinted micro/nanostructures.

The masters for hot embossing and nanoimprinting (see later) were chosen to provide ordered and random features, of various sizes, for transfer to the polymer surface. The images in figure 4 show the surface of the irregular microstructured master and the subsequent HEL embossed surfaces of the polymers for comparison. The SEM images are of random areas of each surface, but the white light interferometric images are of the same area. The frosted glass master is seen, in figure 4(a), to contain a crystalline structure, due to the etched glass, with feature diameters of up to 50 $\mu$m at the base. The geometrical shapes in the crystalline structure are due to the action of the HF etchant on the glass surface. Finer detail is observed on some of the surfaces of the crystals in the form of terracing, probably due to the etching of the SiO$_2$ structural matrix. Embossing of the PMMA using this master produces a pitted polymer surface due to the replication of the master’s crystal structure in the polymer (figure 4(b)). The pits conform to the peaks in the master in size and shape.
PEN, due to the use of insufficiently forceful embossing conditions. The inset in figure 5(a) shows an optical image of the surface of the PEN polymer, embossed with the same master, reveal that the imprint is just as successful (figure 4(c)). The roughness values calculated from the interferometric data for both polymers (table 2) are also similar to those of the master (∼15 μm), confirming that the polymer has imprinted to its full extent.

Figure 5 shows the effect of incomplete embossing of PEN, due to the use of insufficiently forceful embossing conditions. The inset in figure 5(a) shows an optical image of the surface of a PEN replica imprinted with the frosted glass master at 10 MPa and 170°C for 1200 s. The embossing conditions were not forceful enough to drive the polymer into the master to its fullest extent and hence only the highest peaks of the master have imprinted in the surface of the polymer. Increasing the embossing conditions to 30 MPa and 200°C for 1200 s produces a PEN polymer surface in which the master has been fully embossed (inset figure 5(b)). By measuring the values of $R_q$ and $R_t$ for a series of polymer replicas, and comparing them to those of the master, the extent of the embossing can be followed (figure 5). The measurements of both of the characteristics were made using the same area of the master/polymer replicas as highlighted in the inset images, and both are seen to increase towards the values for the master as the embossing parameters are increased to $3 \times 10^7$ N m$^{-2}$ and 200°C. At this point the master can be assumed to be embossed to its full depth, at least locally. Confirmation of these measurements at a number of points on the master/polymer replica surface will confirm that the embossing across the full surface of the master has been successful.

Figure 6 shows regular micro- and nanostructures imprinted in the surface of PEN using NIL. The structures all have sub-micron vertical dimensions and have horizontal dimensions that range from microns down to hundreds of nanometres. The lines and posts in figures 6(a) and (c) have potential for use in the structured culturing of cells, whereas the T-shaped channel in (b), when sealed, could be used in fluidics applications. In all cases, the polymer adequately replicates the master, although in the case of the channel system there is evidence of some sticking of the polymer to the master near the edges of the channel. This may be rectified by optimization of the anti-adhesion techniques and imprinting conditions used for the replications. It is possible that smaller structures may be produced using PEN, but it is unlikely to rival PMMA in its minimum resolution, mainly due to the size of the ethylene naphthalate monomer unit, and the structural rigidity it imparts to the polymer chain. However, for many biological and fluidics applications, structures with dimensions similar to those given in this work will be sufficient.

The results of contact angle measurements on the surface of the PEN replicas are given in figure 7. The pristine PMMA surface is found to have an advancing contact angle of ∼73° and a receding contact angle of ∼54°, values in close agreement with those reported in the literature [17, 18], and consequently produces a wetting hysteresis of ∼19°. The pristine PEN surface, on the other hand, produces values of ∼89° and ∼71° respectively, producing a wetting hysteresis of ∼18°, similar to that for PMMA. This suggests that the PMMA surface is slightly more hydrophilic than the PEN, but that both the surfaces have similar roughnesses, a conclusion supported by the roughness values in table 2. Upon patterning the PEN surface using the frosted glass master, the surface characteristics are seen to change. The surface becomes slightly more hydrophilic, and, as expected due to the increase in the roughness of the surfaces, the wetting hysteresis is seen to increase to ∼43°. Interestingly, the microstructured surface presented in figure 6(a) produces a still more hydrophilic surface, although with a much lower hysteresis than the other microstructured surface. This could be useful for biomedical applications as some cells proliferate more easily on a hydrophilic surface [19].

4. Conclusion

Compared to PMMA, the physical properties of PEN make it more resistant to softening, and therefore more forceful conditions are required for polymer replication techniques. However, PEN is shown here to be capable of replicating structures with dimensions ranging from tens of micrometres down to the low hundreds of nanometres which are structurally
be formed into the required shape for the application whilst retaining structural stability at sterilization temperatures. With an optical transmission only slightly less than that of glass, and a UV resistance which is useful for packaging applications, where, for example, biological specimens require UV protection, PEN’s optical properties make it particularly useful for biomedical applications, such as cell biology, which require transparent structural materials. Finally, the inherent hydrophilicity of the polymer surface, which is retained after structuring, means PEN can be safely used as a structural material for cell culturing experiments.

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Figure 6. SEM images of (a) 500 nm tall, 5 \( \mu \)m\(^2\) square posts (bar = 5 \( \mu \)m), (b) a 500 nm deep, 40 \( \mu \)m wide T-channel (bar = 10 \( \mu \)m), and (c) 50 nm tall, 500 nm wide and 80 \( \mu \)m long lines, with a period of 1.5 \( \mu \)m (bar = 5 \( \mu \)m), imprinted in PEN using NIL.

Figure 7. Advancing (●) and receding (○) contact angle measurements for PEN and PMMA in their pristine state and for PEN after embossing with the frosted glass master (PEN(frost)) and after imprinting to produce the 500 nm tall, 5 \( \mu \)m\(^2\) square posts shown in figure 6(a) (PEN(struct)).

Figure 374
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