Micro and nano structuring of poly(ethylene-2,6-naphthalate) surfaces, via imprint techniques, for use in biomedical applications.

C. A. Mills[1*], J. Escarré[2], E. Engel[3], E. Martinez[1], A. Errachid[1], J. Andreu[2], J. A. Planell[3] and J. Samitier[1]

[1]Centre of Reference for Bioengineering in Catalonia (CREBEC), Laboratory of Nanobioengineering, Parc Científic de Barcelona, C/ Josep Samitier 1-5, 08028 Barcelona, Spain.

Tel. +34 93 403 7185, Fax. +34 93 403 7181, *cmills@pcb.ub.es.

[2]Facultat de Física, Universitat de Barcelona, C/ Martí i Franquès 1, 08028 Barcelona, Spain.

[3]Centre of Reference for Bioengineering in Catalonia (CREBEC), Biomaterials and Biomechanics Division, Dept. Materials Science and Metallurgy, Technical University of Catalonia, Avda. Diagonal 647, 08028 Barcelona, Spain.

Abstract

Here we investigate the forming 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.

Keywords: Poly(ethylene naphthalate), polymer replication, micro/nanostructures, devices.

---

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 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 utilised 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 (Figure 1*b*) is an amorphous, thermoplastic acrylate polymer with excellent optical properties (including an optical clarity which rivals that of glass), that was discovered by Crawford in 1932 [10]. It is commonly used in polymer forming applications due to its highly applicable physical properties, such as a T_g of ~105 °C, a coefficient of thermal expansion of $7x10^{-6}$ K^{-1} and a thermal conductivity of 0.18 W m^{-1} K^{-1} at 23 °C, which means it readily softens at low temperature and, upon cooling, faithfully retains the structure into which it has been formed. It is also biologically inert. However, PMMA has poor solvent resistance and low heat tolerance, with a working temperature of ~90°C, which puts it at a disadvantage when considering some chemical and biological applications. Therefore, there is a need to examine more robust polymer systems for use in device applications.

Here, we examine the imprinting properties of a polyethylene derivative, poly(ethylene-2,6-naphthalate) (PEN), and compare it with PMMA. PEN (Figure 1*a*) is a semi-crystalline, thermoplastic polyester material, available since 1948 [11], with a higher T_g than PMMA (~125 °C), but with a working temperature up to 155°C [12]. It has good mechanical properties, is chemically resistant to most dilute acids and organic solvents, and

has good optical clarity and ultra-violet (UV) radiation absorbance [13]. Due to its 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 sterilisation. 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 [14]. Its inherent strength and dimensional stability (partially due to the presence of the co-joined benzene rings in the monomer [15]) means PEN is also commonly used for fibres and films where low shrinkage and elongation properties are required[12].

2. Experimental

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 Microelectrónica (CNM), Barcelona, fabricated using lithographic techniques from silicon nitride (Si_3N_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 focussed 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_3N_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_3N_4 (180 nm).

The SiO_2/Si_3N_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 1c) 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 I. 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 I. 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 polymers 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. Characterisation

Characterisation 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 characterised 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 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.

2.4. Cell culturing

Osteoblast-like MG63 cells (from ATCC) were used to test the compatibility of the PEN surfaces used in this work towards cell culturing. The cells were maintained at 37°C and 5% CO_2 in complete medium (D-MEM), containing 10% fetal calf serum (FCS) and 1% each of L-glutamine, pyruvate and streptomycin/penicillin. Squares (4 mm^2) of PEN thin film polymer were placed in well plates and immersed in 0.5 ml of the complete medium for 24 hours. After this time, the medium was replaced with fresh medium and the MG63 cells were seeded at a density of $2x10^5$ 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 PLA 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 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 to 800 nm, and compared with that of a 1.5 μm 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 ~80% of the incident light in the near IR/visible region, but its transmission falls rapidly as the UV region is encountered at ~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 r.m.s. roughness (R_q) and the maximum peak to valley distance (R_t) is given in table **I**. 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 μm 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, and there is evidence of replication of the terracing seen in the master. Images of the surface of the PEN polymer, embossed with the same master, reveals that the imprint is just as successful (Figure 4*c*). The roughness values calculated from the interferometric data for both polymers (Table II) 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 $3x10^7$ Nm^{-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 figure 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 optimisation 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 roughness; a conclusion supported by the roughness values in table II. 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 ~13°. 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 micrometers down to the low hundreds of nanometres. This indicates that it has potential for the production of systems containing micro and nanostructures, where the polymer must be formed into the required shape for the application whilst retaining structural stability at sterilisation 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 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.

Acknowledgements

The authors would like to thank J. Bausells and G Villanueva at the National Centre for Microelectronics (CNM), Barcelona, for providing the ordered, microstructured masters used here, and also to Dr. F. Bessueille and M. Pla-Roca of the Barcelona Science Park for the silanisation of the of the masters. Thanks also go to Dr. G. Gomila and A. Samso, of the Barcelona Science Park, for advice during the completion of this work. CAM and EM acknowledge support from the Spanish Ministry of Science and Technology via the Ramon y Cajal program.

References

1. Chou S. Y., Krauss P. R. and Renstrom P. J. 1995 *Appl. Phys. Lett.* **67** 3114-3116.
2. Hekele M. and Schomberg W. K. 2004 *J. Micromech. Microeng.* **14** R1-R14.
3. Sotomayor-Torres C. M., Zankovych S., Seekamp J., Kam A. P., Clavijo Cedenõ C., Hoffmann T., Ahopelto J., Reuther F., Pfeiffer K., Bleidiessel G., Gruetzner G., Maximov M. V. and Heidari B. 2003 *Mater. Sci. Eng. C* **23** 23-31.
4. Jaszewski R. W., Schift H., Gobrecht J., and Smith P. 1998 *Microelectron. Eng.* **41-42** 575-578.
5. Heidari B., Maximov I., and Montelius L. 2002 *J. Vac. Sci. Technol. B* **18** 3557-3560.
6. Chou S. Y., Krauss P. R. and Renstrom P. J. 1996 *J. Vac. Sci. Technol. B* **14** 4129-4133.
7. Kasemo B. 2002 *Surf. Sci.* **500** 656-677.

8. de Mello A. 2002 *Lab. Chip.* **2** 31N-36N.

9. Becker H. and Locascio L. E. 2002 *Talanta* **56** 267-287.

10. The Plastics Historical Society (http://www.plastiquarian.com/)

11. Jakeways R., Klein J. L. and Ward I. M. 1996 *Polymer* **37** 3761-3762.

12. Higashioji T., Tsunekawa T. and Bhushan B. 2003 *Trib. Int.* **36** 437-445.

13. Ouchi I., Nakai I. and Kamada M. 2003 *Nucl. Instr. Meth. Phys. Res. B* **199** 270-274.

14. Jokinen E. and Ristolainen E. 2002 *Microelectron. Reliability* **42** 1913-1920.

15. Tonelli A. E. 2002 *Polymer* **43** 637-642.

16. Bessueille F., Dugas V., Cloarec J. P., Vikulov V., Cabrera M., Souteyrand E. and Martin J. R. *Biosens. Bioelectron.* Submitted.

17. Eaton P. J., Graham P., Smith J. R., Smart J. D., Nevell T. G. and Tsibouklis J. 2000 *Langmuir* **16** 7887-7890.

18. Speranza G., Gottardi G., Pederzolli C., Lunelli L., Canteri R., Pasquardini L., Carli E., Lui A., Maniglio D., Brugnara M. and Anderle M. 2004 *Biomaterials* **25** 2029-2037, and references within.

19. Zhu Y., Gao C., Liu Y. and Shen J. 2004 *J. Biomed. Mater. Res. A* **69A** 436-443.

Figure captions

Figure 1 Chemical structure of (*a*) PMMA and (*b*) PEN, and the fluoroalkylsilane (*c*) used as an antisticking monolayer on the masters to prevent sticking between the master and the polymers. A schematic diagram of the hot embossing/nanoimprinting procedure used to transfer superficial features to

the polymers is given in (*d*). The polymer is placed in the apparatus in contact with the patterned master and a second unstructured piece of master material or borosilicate glass (1), the temperature is increased above Tg, and the master is forced into the polymer under pressure (2). After reduction of the temperature, the pressure is released, and the polymer containing the added superficial structures can be separated from the master (3).

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.

Figure 3 Optical transmission spectra of glass (solid line), PMMA (dashed line) and PEN (dash/dot line) 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.

Figure 4 SEM [bar = 20 μm] and (inset) white light interferometer images (image area = 94 x 124 μm) of (*a*) the frosted glass master, and hot embossed replicas in (*b*) PMMA and (*c*) PEN. The SEM images are of random areas of each surface respectively, but the interferometric images show the same area in the master and the imprinted polymers.

Figure 5 (*a*) Maximum peak to valley height (R_t) and (*b*) R.M.S. roughness (R_q) of PEN replicas imprinted with the frosted glass master using increasingly forceful imprint conditions, achieved by varying the temperature and pressure at which the PEN is imprinted. The values of R_q and R_t for the

master are indicated as a plane in the graphs. Inset, are optical images of the PEN polymer surface (*a*) partially embossed and (*b*) fully embossed using the frosted glass master. The boxes in each optical image highlight the same area shown in the white light interferometric images in figure 4.

Figure 6 SEM images of (*a*) 500 nm tall, 5 μm^2 square posts, (*b*) a 500 nm deep, 40 μm wide T-channel, and (*c*) 50 nm tall, 500 nm wide and 80 μm long lines, with a period of 1.5 μm, 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 embossed with the frosted glass master [PEN(frost)] and after imprinting to produce the 500 nm tall, 5 μm^2 square posts shown in figure 6*a* [PEN(struct)].

Table captions

Table I Typical hot embossing and nanoimprinting conditions.

Table II Roughness properties of the superficial structure of the masters and the imprinted polymers.

Table I

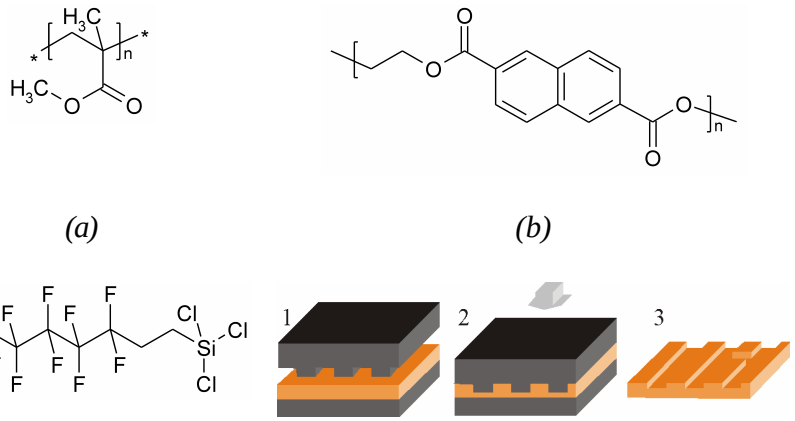
Technique	Polymer	Embossing conditions			Cooling
		T / °C	P / MPa	t / s	T / °C
HEL	PMMA	130	4	600	80
	PEN	200	30	1200	90
NIL	PMMA	130	5	300	80
	PEN	200	5	300	90

Table II

Master	Polymer	R_q / nm	R_t / nm	Method
	PMMA	10	82	AFM
	PEN	6	45	
Frosted glass	-	2890	15810	WLI
	PMMA	2780	15960	
	PEN	2780	15590	

(AFM = Atomic force microscopy, WLI White light interferometry)

Figure 1



(a) *(b)*

(c) *(d)*

Figure 2

(a) *(b)* *(c)*

Figure 3

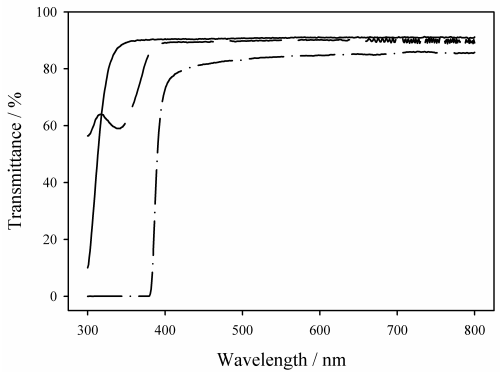
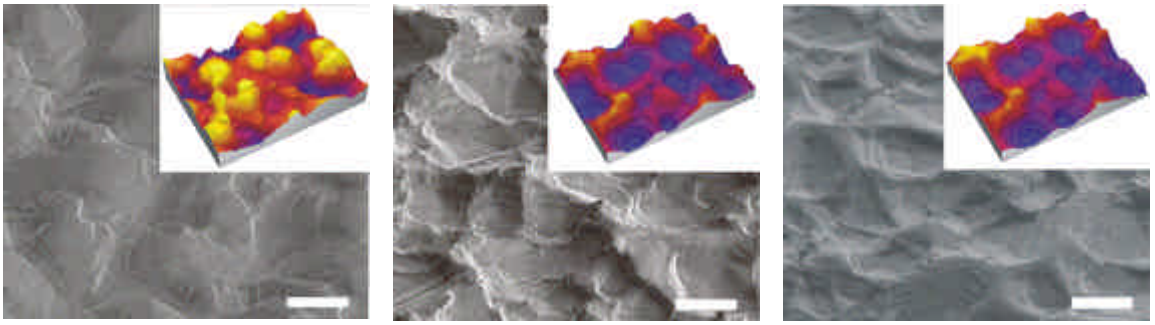
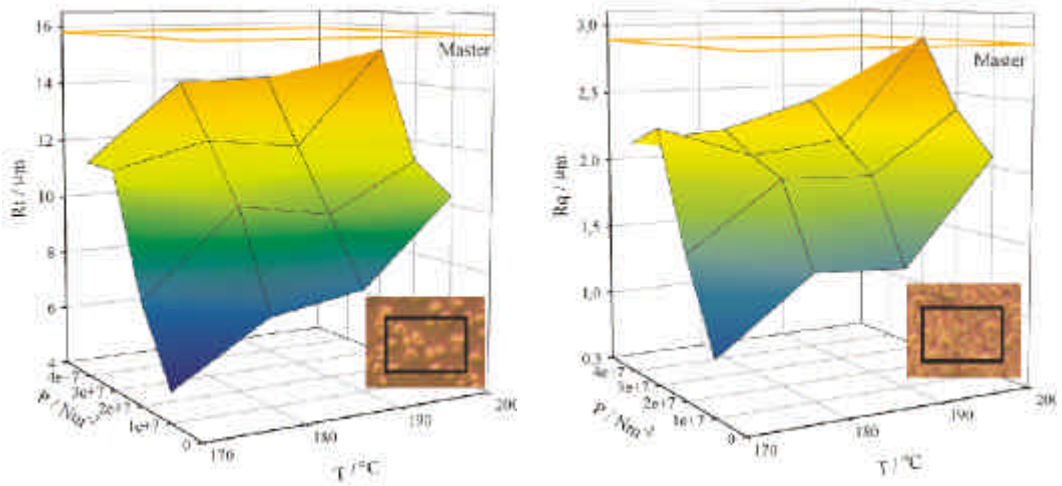


Figure 4



(a) *(b)* *(c)*

Figure 5



(a) *(b)*

Figure 6

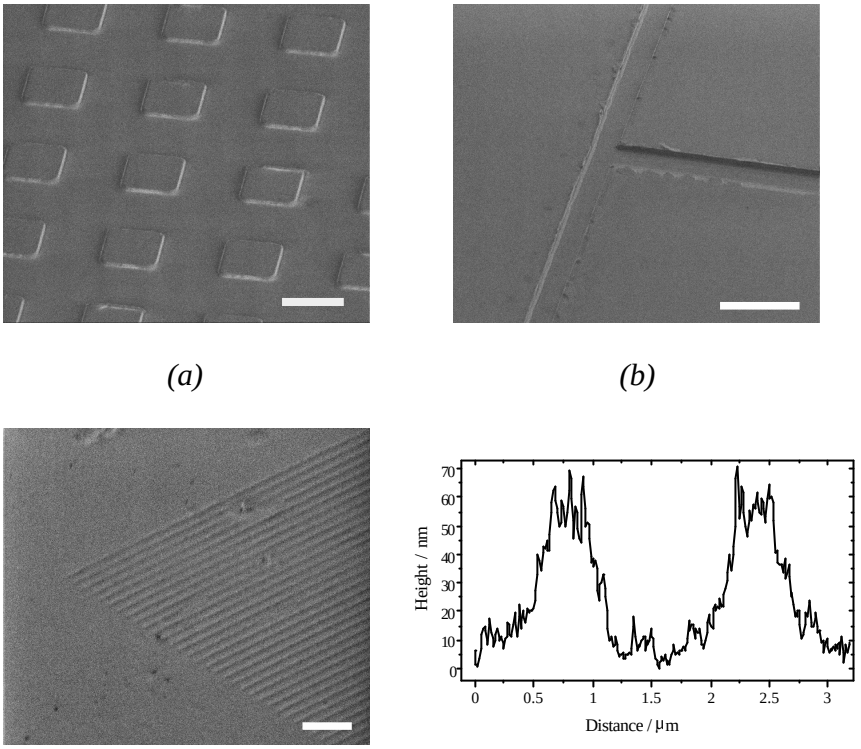
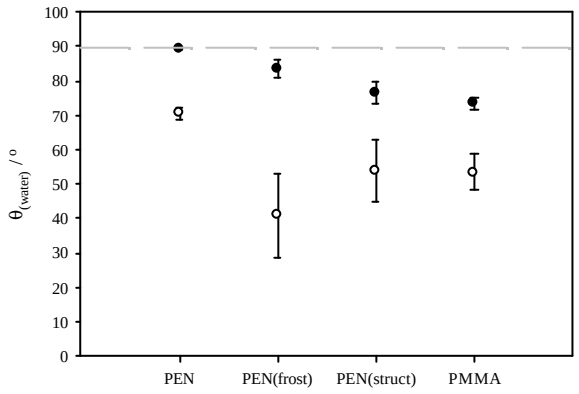

(a)
(b)
(c)
Height / nm
Distance / µm
0
10
20
30
40
50
60
70
0
0.5
1
1.5
2
2.5
3

Figure 7


θ(water) / °
0
10
20
30
40
50
60
70
80
90
100
PEN
PEN(frost)
PEN(struct)
PMMA