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On the Preparation of Textured Surfaces for Cell Adhesion/Differentiation Studies

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Department for **Innovation, Universities** & **Skills**

On the Preparation of Textured Surfaces for Cell Adhesion/Differentiation Studies

J K Lam, L Winkless, A Shard and P E Tomlins
Industry and Innovation Division

ABSTRACT

The behaviour of cells on surfaces is complex and dependent on the surface chemistry, surface topography, the age and type of cell as well as the number of population doublings and the culture conditions. In this report we describe methods of manufacturing surfaces with micrometre or nanometre features in metal, plastic and an inorganic material and describe some of the techniques that can be used to characterise them. These surfaces can be used to investigate adhesion of cells to surfaces or for measurements of protein adsorption.

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1 INTRODUCTION

Fewer than 20 materials are routinely used in the body to restore or repair damaged or diseased tissues due to problems of biocompatibility. The degree of incompatibility varies with material type, application and between individuals, some of whom may be sensitive to, for example, wear debris from hip prostheses or to leaching of nickel ions. The challenge is, and has been for many years, to enhance the degree of biocompatibility thereby extending the durability of the implant and reducing the patient's needs for immunosuppressant drugs. Many attempts have been made to realise this goal using coatings that range from porous matrices to polymer films which contain cytotoxic agents. The efficacy of these different approaches is difficult to quantify unless they fail, due to relatively small patient numbers, differences in patient activity and additional unknown factors such as the influence of the surgical procedure. This lack of quantification makes it difficult to optimise a particular coating and to justify the additional manufacturing costs.

The shape of an adhered cell is used as a measure of behaviour. In vivo cells tend to be polyhedral in shape unlike the typical flattened 'fried egg' structures that are commonly observed in 2D cultures. This shape is formed from the sequential formation of focal adhesions at the edge of an attached cell and it has been shown that this 'spread' configuration has a significant influence on cell behaviour. Cells that don't spread tend to divide less frequently and are more likely to undergo apoptosis [1]. The shape and orientation of cells is influenced by both the chemistry and morphology of the surface. There is a degree of cell type specificity in response to surface morphology but in general cells will align with grooves up to 10 μm depth and bridge deeper features [2]. A significant amount of work has been carried to influence cell attachment by patterning proteins or short peptide sequences onto the surface, where both surface topography and chemistry appear to have equivalent influence [3].

Most metallic implants are manufactured from 316 stainless steels and titanium-based materials. The former with their superior corrosion resistance are used to produce stents, hip and knee joints and internal fixations such as staples. Titanium-based materials can also be used in these applications. Figure 1A shows a double layer of sintered medical grade titanium alloy (90 Ti: 6Al: 4 V) beads that have been applied to an acetabular cup to facilitate bone ingrowth into the implant. A more detailed view of one of the beads is shown in Figure 1B. Even at this magnification it is clear that the surface texture varies significantly across the surface of the structure. The presence of grooves and pits, Figures 1B and 1C may have a significant impact on cell attachment, as could the presence of regular patterns. Figure 1D. Other implants such as stents, femoral stems and bone fixation devices also have heterogenous surface textures on the micrometre scale which appears as fissures, ridges and pits.

Material surfaces that are specifically designed to initiate a specific biological response can be grouped into two classes: those that have a 3-D porous outer layer and those, which are finished in a 2-D coating. Examples of a 3-D porous layer coated materials include bone implants that are designed to integrate with the surrounding bone. Such 'coatings' need to be treated as thin layers of 3-D structures. 2-D coatings are used on stents, catheters and articulating surfaces. Plastics including high density polyethylene, silicone and copolymers of poly(lactic acid) and poly(glycolic acid) are also extensively

used to repair damaged tissues either in their own right or as permanent or temporary coatings. Typically these materials are used to repair soft tissues, such as blood vessels or some of the less load bearing joints such as those found in the fingers as well as degradable bone screws.

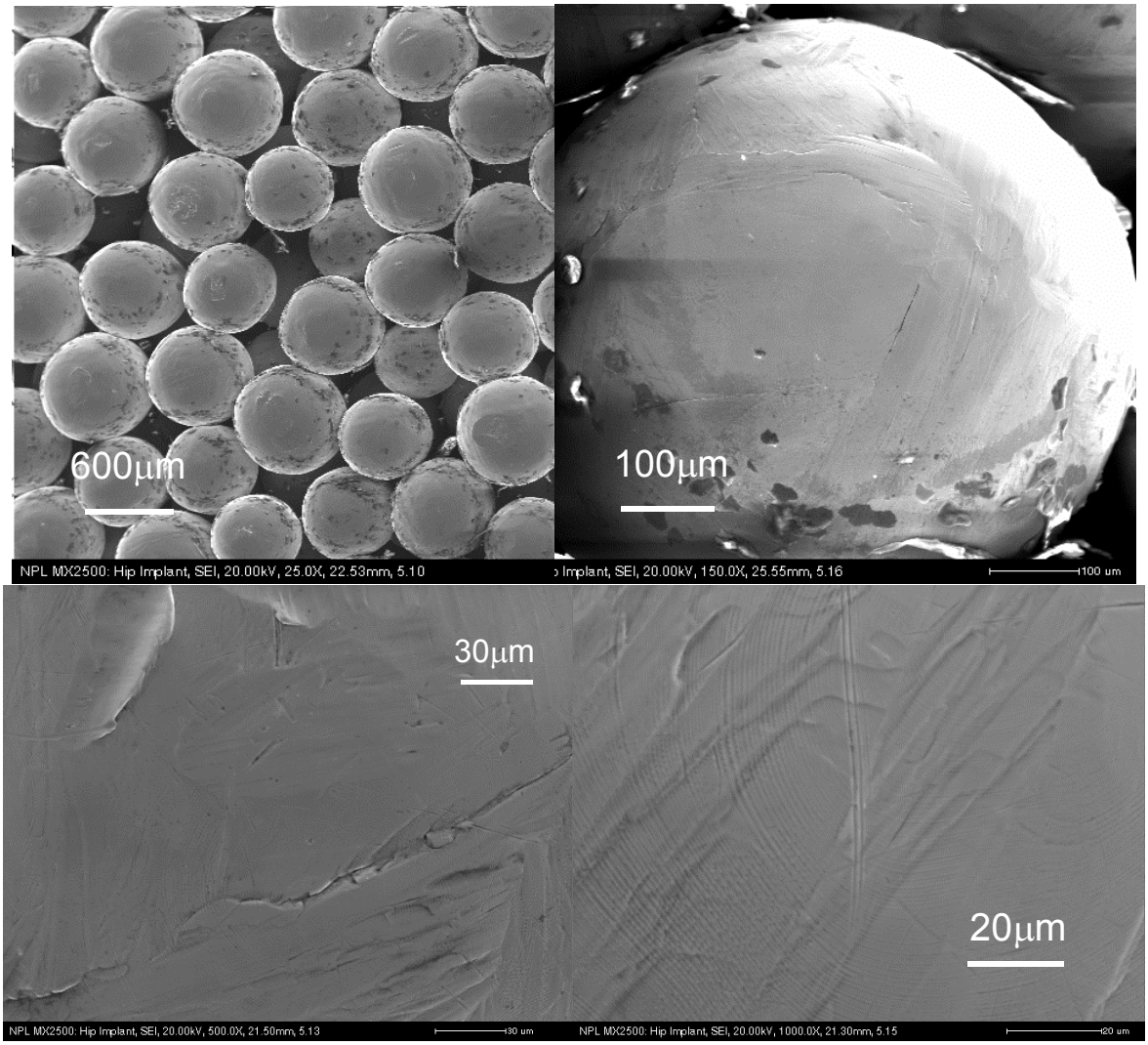

Figure 1: A) A double layer of titanium-vanadium beads on the non-articulating surface of an actetabular cup. B) Detail of a single bead. C) At higher magnification the pits, grooves and ridges that are present on the surfaces of the bead become more evident. D) The beads also contain regions where the surface texture has a pronounced periodicity.

Metal-based surfaces are usually manufactured by machining or casting. Machined surfaces are very controllable in terms of consistency and repeatability, factors that are highly significant in the development of a surface for a particular function. The identification of a suitable polymer based surface is less easily controlled as most machining operations will damage or distort the material through scuffing, pulling or localised heating. These problems can be overcome by using molding, extrusion or casting methods.

The attachment of a cell to a surface and it subsequent behaviour is mediated, at least initially, by the properties of the adsorbed layer of proteins that will rapidly coat a surface in an in vivo like environment. This monolayer adsorbs within milliseconds. In a mixture of proteins such as serum, the composition of the monolayer will change with time as those that have a greater binding efficiency displace the more abundant proteins. Proteins bind to surfaces through multi-point interactions, which are dominated by the hydrophobic interaction in physiological media. The hydrophobicity of the surface is therefore plays a key role in determining protein adsorption. A second key factor that needs to be considered is the relative charge of the protein and the surface, an interaction that is strongly influenced by the local pH. Surface charge can, for example, affect the orientation of proteins if they possess a dipole moment. It should however be noted that these electrostatic interactions are strongly influenced by the ionic strength of the medium and under physiological conditions many of these interactions will be screened out.

Despite the importance of morphology on cell attachment it remains a fact that much of the published literature lacks detailed quantification of surface features in terms of both chemistry and topography. Typically, the surface characteristics are either reported qualitatively or in terms of engineering parameters such as *Ra* (Defined in Section 2 below), these are basically measures of the overall surface and are not sensitive to variations in local detail. Parameters such as the highest peak or deepest valley do provide some local information but this is still not sufficiently detailed to map the surface at a scale relevant to the cell.

In this report we describe three approaches to obtaining surfaces with micrometre and nanometre scale textures in both metals, plastics and inorganic materials that can be used to study cell adhesion or differentiation as well as protein adsorption. These include exploitation of commercially available artefacts, injection moulding and etching.

2 QUANTIFICATION OF SURFACE TEXTURE

The ability to mathematically describe a surface[1] is key to identifying any relationships between cell behaviour and surface texture assuming that there are no significant differences in surface chemistry. Various parameters are used to quantify surface texture that provide measures of, for example, the average deviation from a mid-plane and an indication of the number of peaks, the highest peak and deepest valley. These are typically determined from a single line profile that is taken to be representative of the surface. A 2-D profile is defined according to ISO 4288: 1996 (4) in terms of an evaluation length which is the sum of 5 sampling lengths. The parameters that are used to quantify 2-D profiles are determined for each sampling length, *lr*, and then averaged. The details of how the profile is determined and analysed is beyond the scope of this report, but references 5, 6 and 7 will provide the reader with an overview of current practices and terminology. In most cases, the parameter *Ra* is used. *Ra* is the arithmetic mean deviation of the *absolute* ordinate value from a midline given by.

[1] A surface is defined according to ISO 4287: 1997 (5) as something that limits a body and separates it from the surrounding medium. Surface texture can be defined as any repetitive or random departure from a nominal surface. A two-dimensional *profile* of a surface is produced at the intersection of a plane drawn perpendicular to it.

$$Ra = \frac{1}{lr}\int_0^{lr} |z(x)|dx$$

, Figure 2, and as such is not a particularly discriminating parameter. Figure 3 shows that samples with quite different profiles can still have the same Ra value.

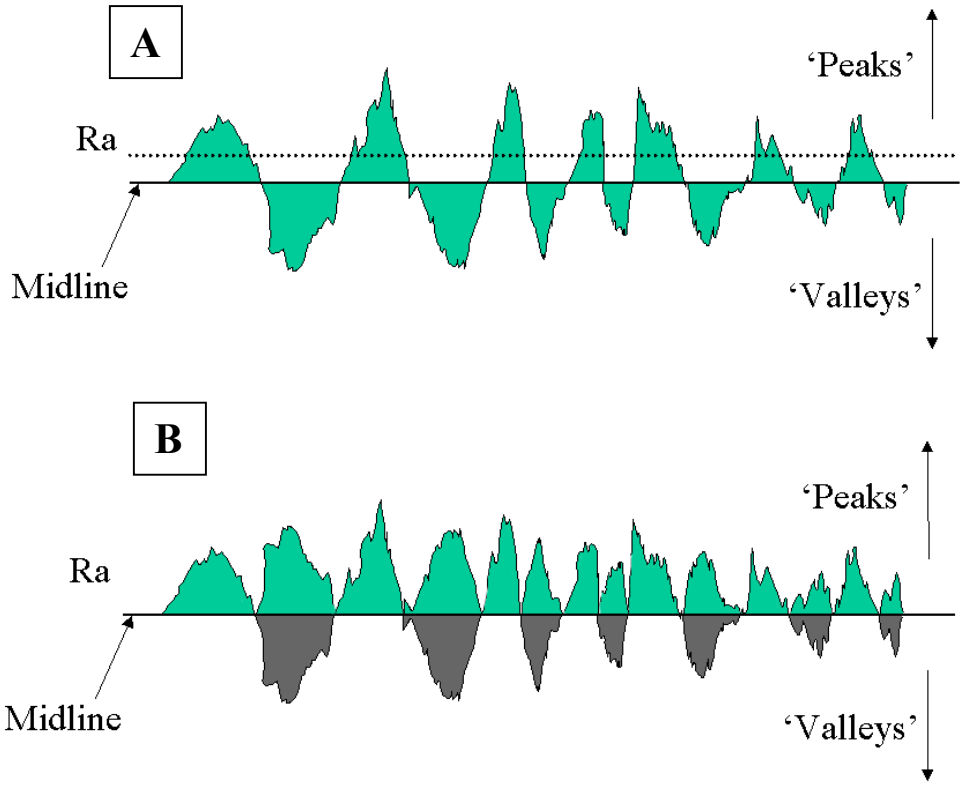

Figure 2: (a) Averaging the peaks and troughs in measured profile data over a given length scale is used to identify a midline. (b) The valleys are inverted to form peaks and averaged with existing peaks to obtain *Ra*, the arithmetic mean deviation from the midline.

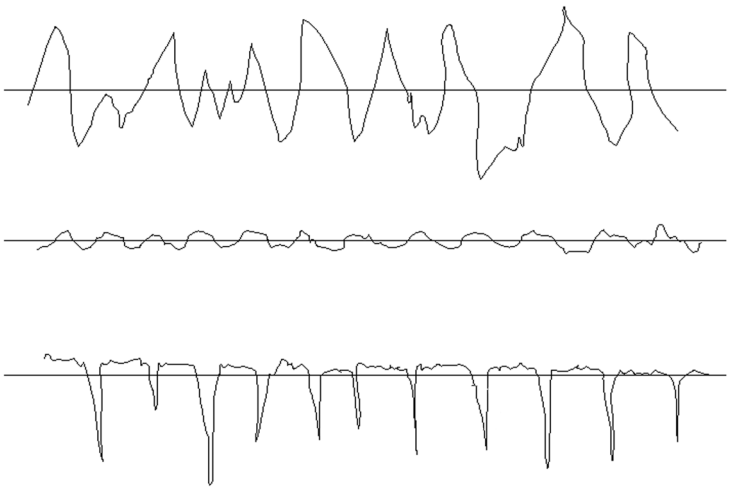

Figure 3: Surface with quite different profiles can have the same RA values.

A similar suite of parameters can be used to describe 3-D profiles, details of which can be found in references 4 and 5. Like their 2-D counterparts the 3-D parameters are not particularly useful in describing the variation in detailed local texture that will be relevant to cell attachment. This limitation can be overcome by using an analytical approach that enables the local roughness over a small area to be calculated. If these are captured in sequence in both the in plane x and y directions then the information can be re-assembled back into a map of variations in local roughness over the entire surface (7).

A number of techniques can be used to obtain a profile of the surface. These include contact methods that use stylus instruments or non-contacting optical techniques such as confocal microscopy and white light interferometers[2]. The approach taken to measure surface textures depends on a number of factors including the sample size, the length scale, the opacity of the material and how soft it is, the latter is a potential concern for stylus instruments that can 'plough' soft surfaces. In this investigation we have used scanning electron microscopy, light microscopy and atomic force microscopy to image surfaces from which 3-D coordinates can be obtained.

3 SURFACE ANALYSIS

It is very easy to believe the surface chemistry of an object manufactured from a material that cells and proteins 'see' is the same as that of the bulk. However, surface oxidation and contamination from other molecular species means that in practice this is rarely the case. The amount and type of contaminant will depend on how and where the object is stored. Storage in 'plastic' bags will potentially expose surfaces to low molecular weight additives that are used to stabilise the material or as a processing aid, these molecules have a tendency to migrate to the surface over time.

There are many techniques for assessing the chemistry of surfaces including secondary ionisation spectroscopy (SIMS), energy dispersive X-ray analysis (EDX) and X-ray photoelectron spectroscopy (XPS). Most laboratories, except those who specialise in surfaces, use energy dispersive X-ray analysis (EDX) used in conjunction with SEM to assess the surface chemistry. EDX relies on the energy dispersive emission of X-rays excited by the electron beam. Elements in the sample emit X-rays of different energies and thus elemental compositions can be obtained by monitoring the intensities of characteristic emission energies. The detected X-rays are emitted from up to three micrometres within the sample and therefore EDX is not regarded as a surface analytical technique. X-ray photoelectron spectroscopy (XPS) relies upon the energy dispersive detection of core-level photoelectrons, excited during monochromatic X-ray illumination, from a sample. The emission energies of electrons are unique to each element and enable elemental compositions to be determined. The photoelectrons that are analysed originate from within a few nanometres of the surface. This scale length is closer to the interaction length of molecules with the surface (< 1 nm).

[2] A description of these techniques is beyond the scope of this report, further details can be found in references 4 and 5.

4 EXAMPLE 1: MANUFACTURE AND COATING OF METALLIC SURFACE TEXTURES WITH MICROMETRE SCALE FEATURES

The range of textures that can be manufactured in metals is almost infinite. These textures can differ in terms of the size of the out-of-plane features, in the spread of sizes and in the degree of order. For example, surfaces can be produced that consist of a series of ridges and valleys similar to a ploughed field with different peak-to-valley heights and different peak-to-peak distances or a with a random arrangement of hills and valleys that have the same peak-to-valley height as the more ordered structure. Structures can also be produced that have a wide or narrow range of peak-to-valley heights. Techniques such as shot blasting, shot peening, grinding and polishing can be used to manufacture such surfaces with varying degrees of control. Examples of these different surface textures are commercially available as 'comparator plates'. Comparator plates are used in engineering for estimating the roughness of machined surfaces. Table 1, for example, lists a range of surface textures that are typically available together with the *Ra* values that the supplier used to classify them.

Table 1: An example of the commercially available surface textures that are available[3]

Method of manufacture	*Ra* (μm)	Method of manufacture	*Ra* (μm)
Surface Grinding	0.025	Grit Blasting	3.2
	0.05		10.5
	0.1		18
	0.2		25
	0.4	Spark Erosion	0.4
	0.8		0.8
	1.6		1.6
	3.2		3.2
Super Finishing	0.025		6.3
	0.05		12.5
	0.1		25
	0.2		50
Cylindrical Lapping	0.025	Casting	0.8
	0.05		1.6
	0.1		3.2
	0.2		6.3
Horizontal Milling	0.4		12.5
	0.8		25
	1.6		50
	3.2	Honing	0.05
	6.3		0.1
	12.5		0.2
	25		0.4
	50		0.8
Shot Blasting	3.2	Polishing	1.6
	8		0.125
	13		0.025
	18		0.5
			0.1
			0.2

[3] Adapted from Rubert and Co. uk

4.1 IMAGING OF SURFACE TEXTURES AT THE MICROMETRE SCALE

Scanning electron microscopy (SEM), white light interferometry, confocal microscopy and infinite focus microscopy[4] can all be used to image structures at this length scale. In practice white light interferometry can be difficult to use, particularly if the surface is rough or the material is transparent as the interference patterns are difficult to detect. SEM is usually used to provide qualitative data in the form of as images. Although the technique can be used to produce 3-D coordinate files by using image reconstruction algorithms to interpret stereoscopic images (7).

Confocal and infinite focus microscopies can also provide 3-D images. These techniques have an advantage over SEM in that little or no sample preparation is required and measurements are made under atmospheric conditions rather than under vacuum. Overlapping images can be stitched together to gather data over relatively large areas (> 0.5 mm^2) for a range of magnifications. The 3-D coordinates of the structure can be obtained at a resolution of up to 10 nm in the vertical direction for subsequent analysis. Some surfaces do require some degree of preparation, particularly if the material is transparent or only weakly opaque. This involves increasing the degree of reflectivity by coating with lycopodium powder or by sputter coating with gold. Steep sided features or cavities within the structure may not be detectable or give rise to spurious peaks which need to be removed by filtering.

An Alicona Infinite Focus microscope (Alicona Imaging GmbH) was used to capture and reconstruct 3D images of different comparator plates, Figures 4 and 5. From these figures it is clear that surfaces with the same *Ra* can look quite different, as previously described, hence the need to develop quantitative approaches that are sensitive to local variations in topography.

[4] Infinite focus microscopy involves image reconstruction of a series of serial images captured over a defined distance can be used to generate 3-D images.

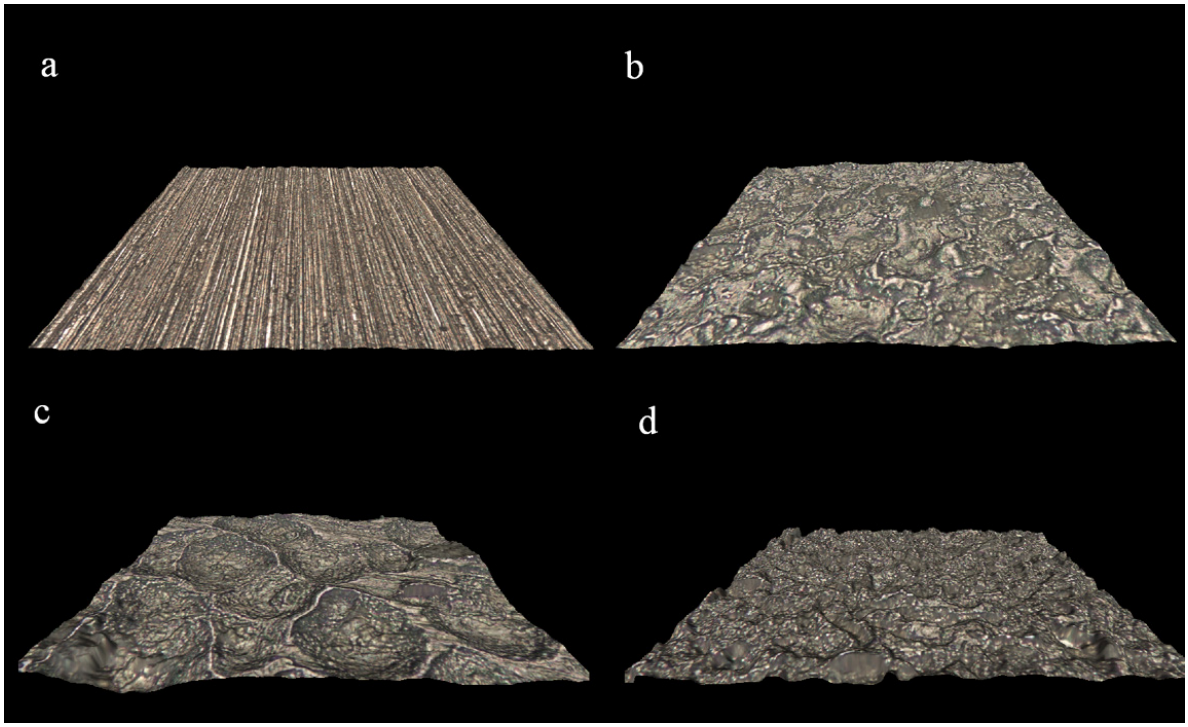

Figure 4: 3-D images of Ti coated comparator blocks that have the same Ra value (3.2 μm). The samples were prepared by surface grinding (a), spark erosion (b), shot peening (c) and grit blasting (d).

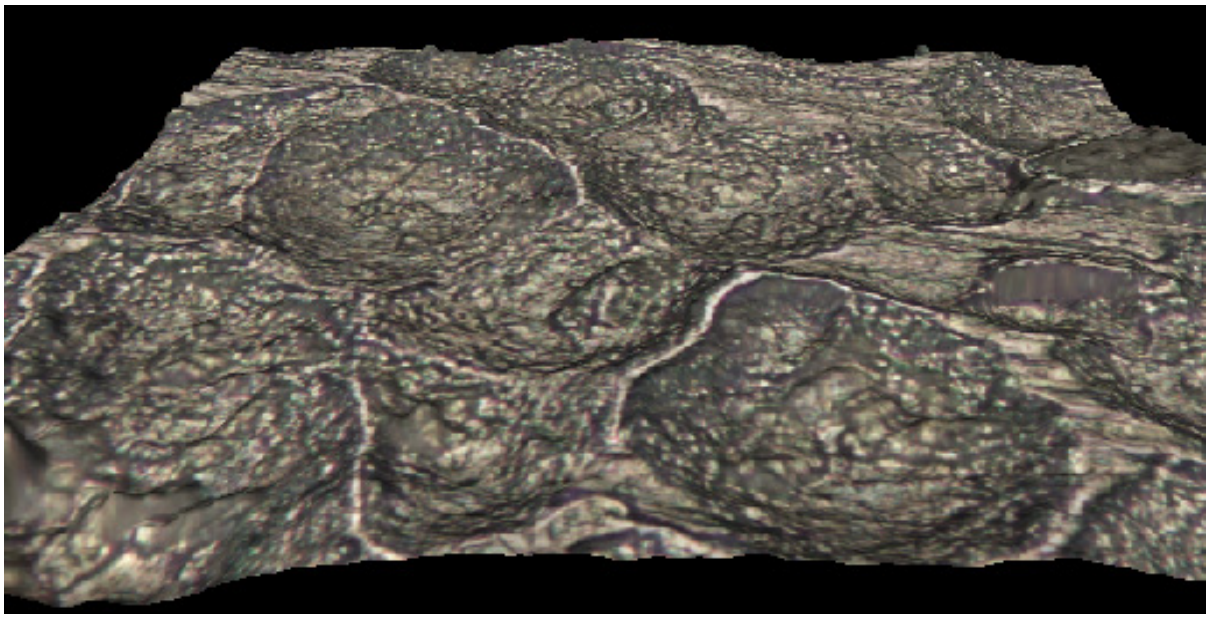

Figure 5: Detailed view of a shot blasted surface shown in Figure 4A.

4.2 ASSESSMENT OF SURFACE CHEMISTRY

Figure 6 shows a SEM image of a drug eluting stent in its unexpanded form. This device consists of a drug-doped polymer coating that covers a 316 stainless steel frame.

Table 2 shows a comparison of EDX and XPS results for this structure. The EDX data is much more sensitive to the underlying components of the steel whereas the XPS barely detects them. Silicon is a commonly observed contaminant on surfaces.

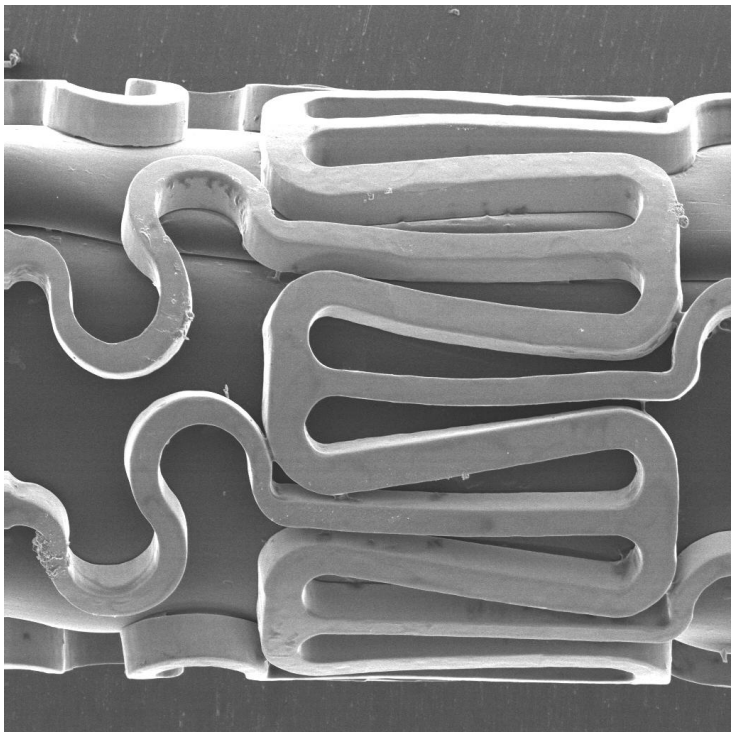

Figure 6: The surface of a drug eluting stent in its unexpanded form.

Table 2: The results of XPS and EDX of the surface of a drug eluting stent show differences in the atomic makeup due to differences in penetration power.

XPS		EDX	
Element	Atomic %	Element	Atomic %
C	76	C	56
O	20	Fe	24
Si	2.5	Cr	7.4
P	1.0	O	6.0
Fe	0.5	Ni	5.2
Cu	0.1	Mn	0.7
Ni	0.05	Mo	0.6

Table 3 compares EDX results for a hip joint ball of the type shown in Figure 1A and the sputter coated comparator plates shown in Figure 4. A sputter coated glass slide acts as a control. From these data it is apparent that there is a significant variation of elemental composition between the different comparator surfaces.

Table 3 shows EDX results for a hip ball joint and Ti 6:4 coated[5] comparator blocks

Surface	Ti (mass %)	Al (mass %)	V (mass %)	C (mass %)
HIP JOINT BALL	92.3	3.7	0.2	**3.8**
Grit blasting/shot blasting (Block 329)	85.2	9.5	**5.3**	
Surface grinding (Block 315)	63.2	36	0.8	
SPUTTER COATED GLASS SLIDE	90.5	5.5	4	

5 EXAMPLE 2: REPLICATING SURFACE TEXTURES IN PLASTIC

The easiest route to producing relatively large (>1 cm^2) controllable surfaces in plastics is to replicate a metal surface. This can be done in a variety of ways using both thermoplastics, such as polyethylene and polystyrene, that melt when heated and re-solidify when cooled, or thermosets which are curable polymers that are extensively used as adhesives, for example, epoxy resins.

A number of different approaches can be used to produce thin sheets of thermoplastic that have a tailored surface texture including:

- Melt casting – pour a melt over a mould surface, allow to cool, separate the replica from the original.
- Compression moulding – replicating a mould using a combination of heat and pressure. (Material is packed into a mould cavity, heated until molten under pressure, cooled, the pressure is then relieved and the moulding ejected.)
- Solvent casting – A polymer solution is poured into a mould and the solvent is allowed to evaporate. The replica is then separated from the original.
- Injection moulding – a combination of high pressure and high temperature is used to inject a melt into a mould cavity. The mould is then cooled and the moulding is ejected.
- Vacuum forming (thermoforming), a sheet of temperature softened material is sucked down on to the surface that is to be replicated.

[5] A coating approximately x 100 nm was applied by sputter coating.

The success of each of these approaches depends on the characteristics of the material, the size of the surface that needs to be produced, the level of detail that needs to be replicated and the size of the production run. The manufacture of an injection moulding tool is both time consuming and expensive and is, therefore, not suited to the manufacture of small batches.

Thermosets can also be moulded on an industrial scale using reaction injection moulding where the thermoset is cured within the mould cavity. On a laboratory scale an uncured thermoset can be used to coat the surface to be replicated and then cured either by application of ultra-violet light or temperature.

In practice, laboratory scale replication of surfaces using melt casting, compression moulding or solvent casting is prone to problems of trapped bubbles, poor reproducibility and problems in separating the replica from the original. Dissolving relatively high molecular weight polymers in organic solvents tends to produce solutions that are viscous and easily trap bubbles that form during mixing. These can lead to imperfections in the replica surface that can also be seriously tarnished if the rate of solvent evaporation is too high. Adding more solvent to the solution to reduce its viscosity generates a new set of problems that centre around deposition of material on the walls of the container which contains the original.

Both solvent cast and melt cast replicas can be subject to bowing after being separated from the mould surface as a result of high levels of residual stress. This problem may be surmounted by slowing down the rate of cooling and annealing of specimens at elevated temperatures.

5.1 INJECTION MOULDING

For this case study two thermoplastics, polycaprolactone and polystyrene, that are widely used in tissue engineering and in the manufacture of tissue culture plates were injection moulded into a specially designed mould cavity. The mould has been designed to accommodate commercially available textures that are used to calibrate surface texture measurement equipment. Incorporating such grids into a mould enables replicas to be made in a variety of materials in a reproducible, relatively cost efficient manner.

5.1.1 Materials

Polycaprolactone is biodegradable, thermoplastic polyester that has a melting point at approximately 60 ^{o}C. This material is semicrystalline. Polycaprolactone (PCL), due to its crystallinity is soluble in a limited range of solvents that include chloroform, acetonitrile, dioxane and tetrahydrofuran. Polystyrene (PS) is an amorphous thermoplastic that is widely used in the manufacture of cell culture plates that has a glass transition temperature around 100 ^{o}C.

5.1.2 Selection of replica surfaces for injection moulding

Reference surfaces with a range of specially machined surface roughness profiles are

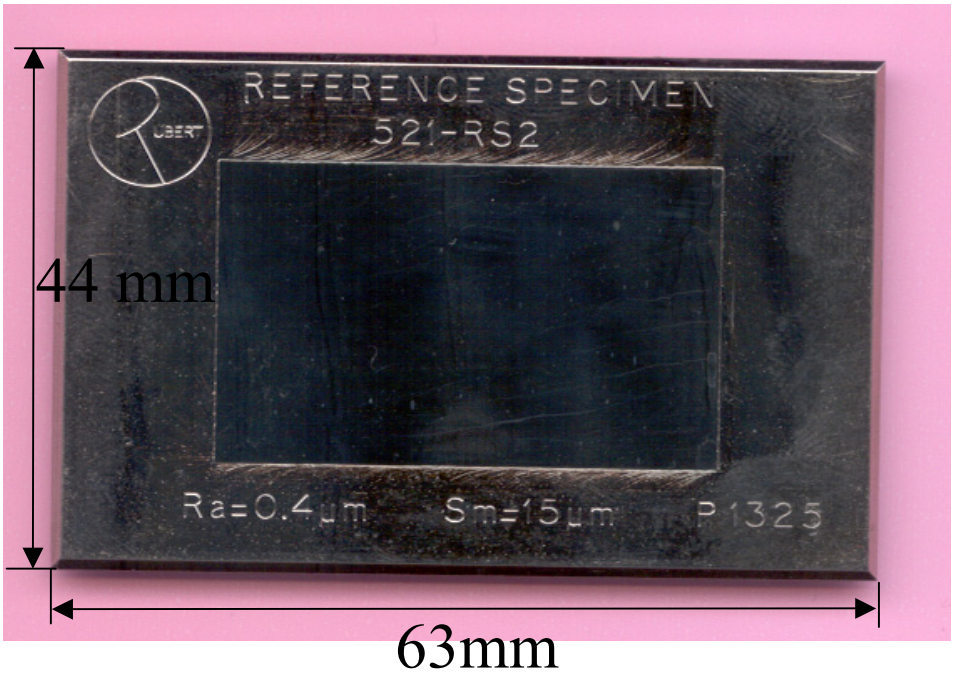

Figure 6: An example of a commercially available machined surface with a well-defined sinusoidal profile.

commercially available (Rubert and Co, UK). These surface profiles are used to calibrate or check the performance of measuring equipment and comply with ISO 5435-1: 2000. However, they can also be used to produce replica surfaces in thermoplastics. The characteristics of the two surface profiles, an example of which is shown in Figure 6, used in this investigation are listed in Table 4 .

Table 4: characteristics of the commercially available textures used in this investigation (as supplied by the manufacturer)

Product code	Profile	Wavelength (µm)	*Ra* (µm)
521	Saw tooth wave	15	0.4
542	Sine wave	8.0	0.06

5.1.3 Tool design

A special housing was designed to house commercially available machined surface profiles such as that illustrated in Figure 6. The housing design is simple and consists of a block of stainless steel with a recess that can accommodate a range of different artefacts. The profile is clamped in place using a small cover and restraining screws. This assembly is then mounted into a larger housing that contains the cooling circuitry required to freeze the melt. Ejector pins are not necessary for moulding this type of replica unless large batches are to be produced.

5.1.4 Processing conditions

The injection moulding parameters used to produce plaques of PCL and PS containing replicas of the machined surface profiles are listed in Table 5. It should be noted that the precision surfaces do suffer some degree of area distortion at the high temperatures and pressures used due to softening of the material used to support the thin metal grating. This distortion takes the form of a shallow depression that has a radius of a round 1 cm. Such distortion doesn't appear to significantly affect the profile of the original grid or that of the replicated surface.

Mould release agents, which tend to be silicone based, should not be used in moulding in order to limit surface contamination. A consequence of this and not using ejector pins in the design the plaques have to be removed by hand at the end of each cycle. Care was taken to ensure that the moulding had cooled sufficiently to avoid bending the plaque during removal or damaging the replica textured surface. The moulding parameters listed in Table 5, were chosen so as to produce the most 'accurate' mouldings for this combination of material and mould design. The mould temperature proved to be the most difficult parameter to set; temperatures that were too high resulted in 'soft' mouldings that were easily damaged as they were removed from the mould at the end of each cycle. When the mould temperature was too low then mouldings were difficult to extricate from the textured surface of the mould due to shinkage. Two moulding temperatures were chosen for PS, 62 ^{o}C and 84 ^{o}C to determine what effect, if any, this would have on the 'quality' of the replica surfaces.

PS, is in many respects much easier to mould than PCL since it is an amorphous plastic that is not prone to water absorption. PCL is both semicrystalline and absorbs a small percentage of water and therefore needs to be dried before moulding. The PCL replicas were still semi-transparent when removed from the mould at 37 ^{o}C, the temperature of the cooling water, becoming increasingly opaque over a matter of minutes as crystallisation continued at room temperature.

Sprues were removed immediately from all the replica plaques after removal from the mould tool and the plaques were allowed to cool naturally in air on a wooden table. The usual precautions were taken to ensure that the moulding conditions were stable before collecting plaques, especially after changes of material.

Table 5: Injection moulding parameters for PCl and PS.

Parameter	Polycaprolactone	Polystyrene
Melt temperature	130 ^{o}C	200 ^{o}C
Mould temperature	37 ^{o}C	62 ^{o}C and 84 ^{o}C
Injection pressure	120 bar	60 bar
Hold pressure	40 bar	25 bar
Hold time	2 s	1.2 s
Cooling time	70 s	20 s
Cycle time	91.5 s	36.5 s

5.2 ASSESSMENT OF SURFACE TOPOGRAPHY

Replica surfaces were investigated using a Taylor-Hobson Coherence Correlation Interferometer (a vertical scanning white light interferometer). This instrument can be used with a range of objective lenses (x10, x20 and x50), which define the field of view *i.e.* the size of the image. Of course, all of these lenses can be used to image surfaces, however, in practice lens selection is constrained by the need to conform with the requirements of ISO 4287: 1997. This standard states that the evaluation length needs to be at least 5 times the sample length.

Figures 7A and B show micrographs of sputter coated replica grids manufactured in PCL. From these images it is evident that the injection moulding process is able to accurately replicate the mould surface although they are easily damaged during removal from the mould. Measures of the periodicity of the structure were estimated from these images and are listed in Table 6.

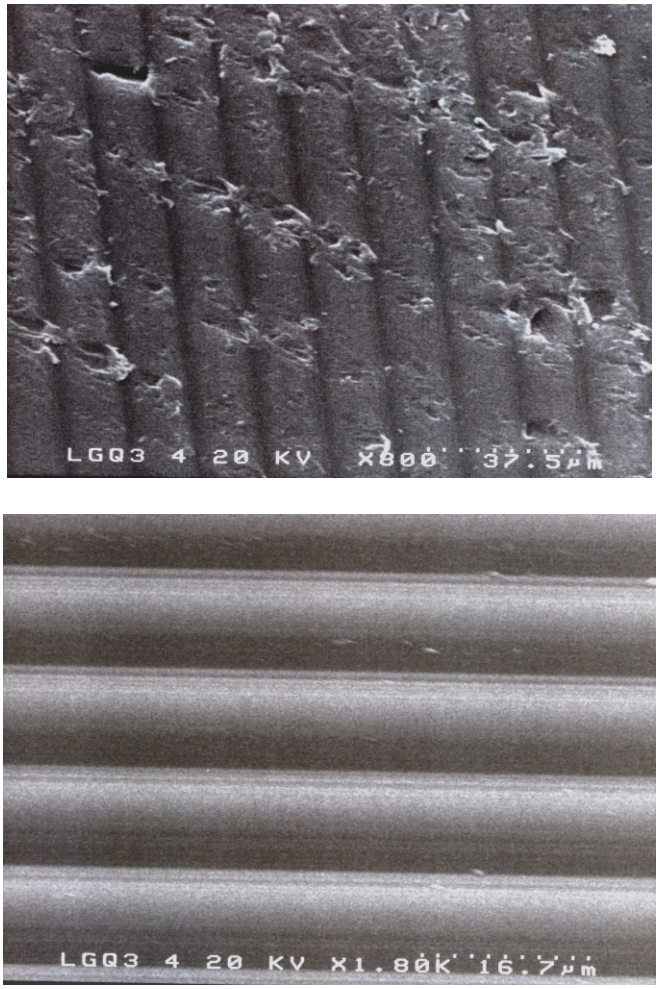

Figure 7. A) SEM of an injection moulded diffraction grating manufactured from PCL, sin wave with λ = 8 μm. B) removing the moulding from the mould can cause damage to the surface.

5.2 WHITE LIGHT INTERFEROMETRY

Figure 8a shows a height map for one of the surfaces used as mould (Rubert 542). This image was obtained after the mould had been used to injection mould several batches *i.e.* approximately 200 replicas. Figure 8b shows a photo-simulation of the same grid. The repeating sine wave can easily been seen from both these figures.

Table 5: The mean and standard deviation of 2D parameters determined for 5 profiles each containing 5 sample lengths per profile.

Sample	Material	*Ra* (μm)	*Rt* (μm)	*RSm* (μm)	*Rq* (μm)
542rubert_no6	Grid 542	0.170±0.004	0.477±0.006	8.436±0.715	0.183±0.003
542_20_62_no1	PS (84 °C)	0.141±0.005	0.461±0.008	8.611±0.909	0.159±0.005
542_20_62_ no2	PS (62 °C)	0.149±0.004	0.468±0.008	7.784±0.335	0.165±0.003
542_20_37_ no3	PCL (37 °C)	0.158±0.003	0.502±0.023	7.768±0.605	0.173±0.003
Grid 521 521rubert_no5	Grid 521	0.377±0.012	1.784±0.111	13.412±0.989	0.447±0.010
521_20_82_no 4	PS (84°C)	0.501±0.066	3.743±0.324	14.528±0.411	0.638±0.100

These data can be quantified either by deriving areal (3-D) parameters or in 2-D by selecting a range of profiles. For this investigation the 2-D approach was adopted. The orientation of the profiles was normal to the lay. The sample length of the profile was extended to 40 μm as per the requirements of ISO 4287: 1997. Figure 9 shows a typical profile extracted from the data shown in Figure 8A. The profile shows a well-defined sine wave that can be easily analysed, Table 5.

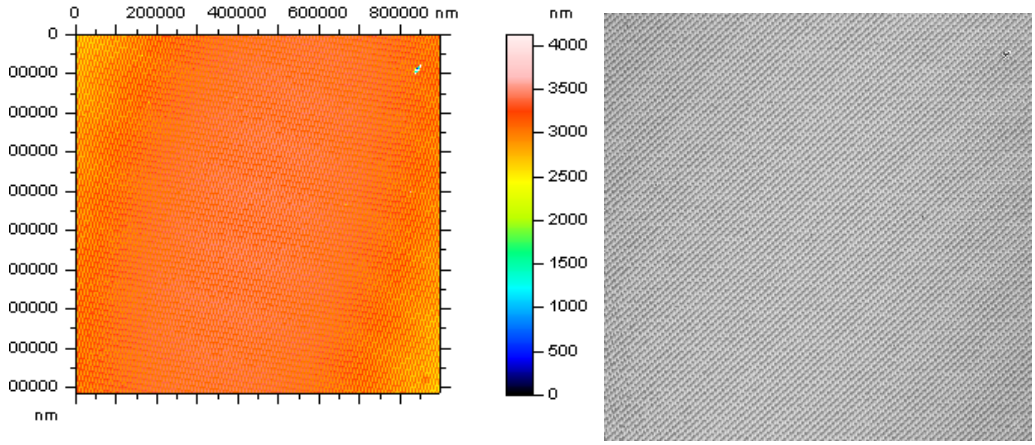

Figure 8: (A) Levelled height map of Rubert grid 542.

(B) Photosimulation of the grid based on the height map shown in (A).

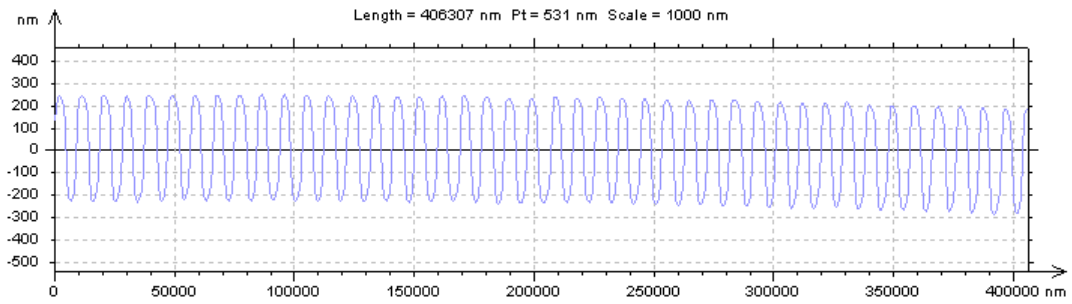

Figure 9: A profile normal to the lay for grid 542.

From Table 5 it is apparent that the replica profile characteristics are different to those for the mould profile *i.e.* smaller. This reduction in peak-to-peak separation (*RSm*) and in height (*Ra*, *Rt* and *Rq*) is not unexpected and is likely to be caused by material shrinkage, an observation endorsed by the fact that the parameters appear to be associated with the mould temperature; the lower mould temperature producing a flatter profile with a reduced peak-to-peak separation. This material shrinkage is probably due to the material prematurely freezing out, *i.e.* solidifying during processing so that it either does not adequately fill the textured surface of the mould or that it is too solid for any compensation to occur during the packing phase of the moulding cycle. Despite this shortfall the parameters compare reasonably well with the manufacturer's quoted values for a grid that has not been subjected to the harsh environment of injection moulding regardless of the mould temperature used.

6 EXAMPLE 3: CHARACTERISATION OF POLISHED GOLD COATINGS AT THE NANOSCALE

It is practically difficult to produce large areas of textures that are uniform on the nanoscale using technologies such as lithography. A more pragmatic approach is to exploit 'naturally' occurring textures that are produced as a result of sputtering potentially followed by polishing. Of course this approach does not allow surfaces to be tailored to meet a specific requirement but it does offer an opportunity to see the effect that different nano-textured surfaces have on protein adsorption and subsequent cell attachment.

Gold and titanium coated quartz crystals are readily available. These substrates are used to assess protein-substrate and protein-protein interactions amongst other applications, based on changes in the resonant frequency of the crystal. This change in resonant frequency can be interpreted to provide a measure of the adsorbed mass of material and a measure of the coating 'stiffness'.

A Park Systems Atomic Force Microscope, model XE-100 was used to image the surface of coated quartz crystals. The Z-scanner of the XE-100, which controls the vertical movement of the AFM tip, is completely separate from the XY-scanner, which moves the sample in X-Y horizontal plane. This means that non-linearity problems, intrinsic to conventional piezoelectric tube-based AFM systems, are effectively eliminated. All software analysis was carried out with XEI – a software product produced by Park Systems. Contact Mode AFM was used to image the samples and MikroMasch™ - NSC36/Al BS, produced the tip used for analysis

Cantilever Type	Cantilever Length, l ± 5, µm	Cantilever Width, w ± 3, µm	Cantilever Thickness, (µm)			Resonant Frequency (kHz)			Force Constant (N/m)		
			min	typical	max	min	typical	max	min	typical	max
C	130	35	0.7	1.0	1.3	50	75	105	0.15	0.60	1.5

A calibration sample was used to 'tip-check' - Model 150-2D — Very High Reference and Traceable Standard for Resolution Calibration AFM, SEM, Auger, and FIB

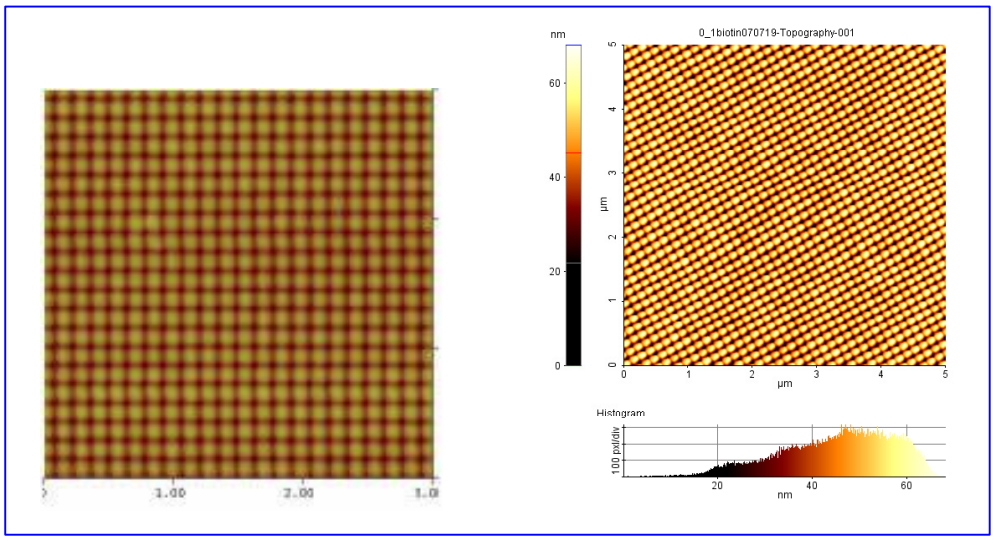

Figure 10: 150 2D Calibration sample images (1) From manufacturer's website) and (2) as taken using NSC36 tip subsequently used for quartz crystal analysis

3D images of the titanium-coated crystal and the chromium/gold-coated crystals are shown below in Figures 11 and 12. These images were obtained under the same conditions. From these figures it is apparent that there is a significant difference in the surface texture of the two materials: the gold-coated surface appears as globular in contrast to the titanium coating. This difference in texture will have a direct impact on the surface area available for protein attachment, that of the titanium surface will be greater than that of gold. Typically the textures are

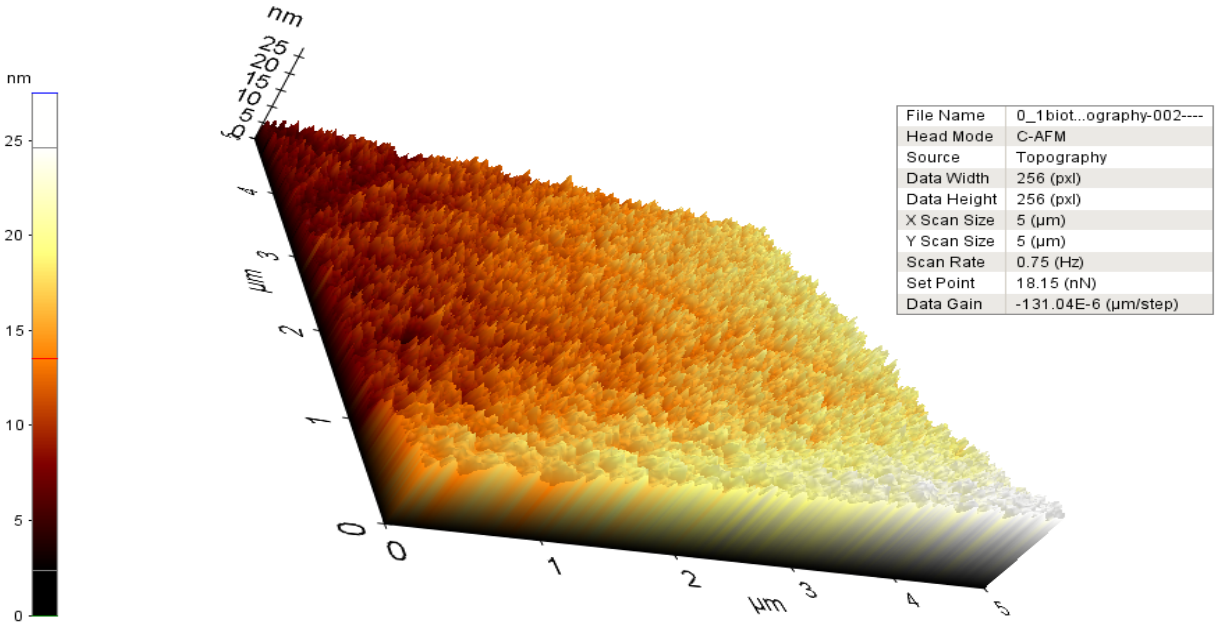

Figure 11: The surface of a titanium coated quartz crystal shows a needle-like texture

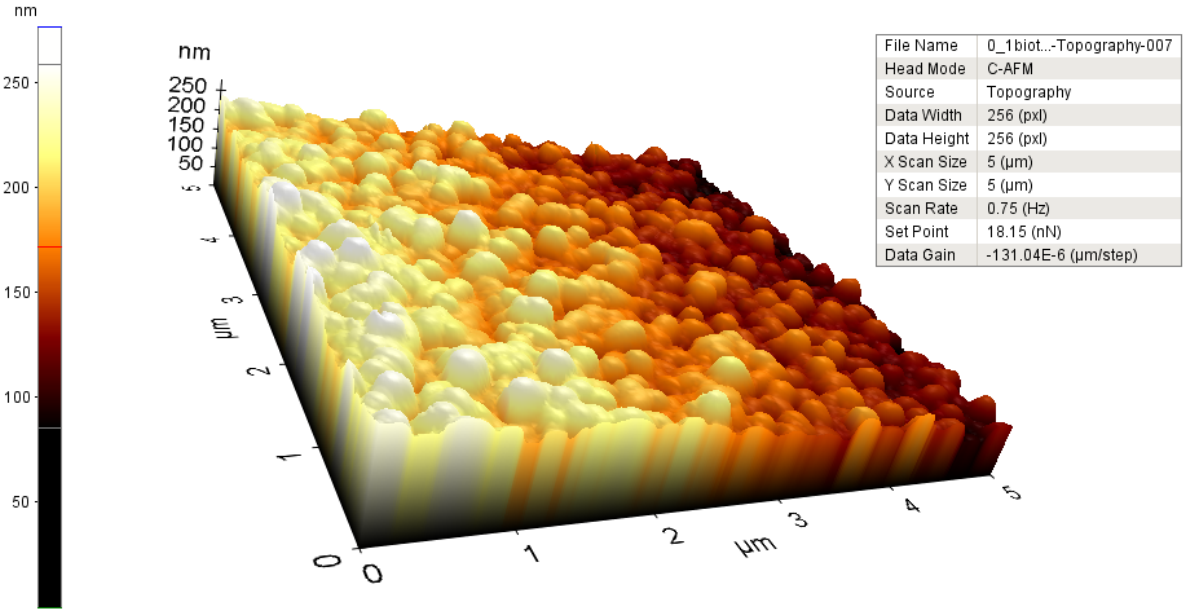

Figure 12. The globular structure of a gold coated quartz crystal.

7 EXAMPLE 4: CHARACTERISATION OF ETCHED WAVEGUIDES AT THE NANOSCALE

Etching a surface rather than polishing can also be used to generate a range of nanotextured surfaces. Again this approach doesn't offer much control of the process but it provides a pragmatic approach to producing different textures from the same

starting material. Examples of etched surfaces are shown in Figures 13. 14 and 15 for silicon waveguides that are used in dual polarization interferometry. This technique, like the quartz crystal microbalance provides information on the interactions of proteins with surfaces. The atomic force microscope and operating conditions are the same as those reported above for imaging polished sputter coated quartz crystals (Example 3). The effect of increasing the etch time is to reduce both the width and height of the crystalline structures. Changes in the morphology of the structure appear to happen over a short time scale based on the differences observed between the un-etched sample and one that has been etched for ten minutes and the difference between 10 minutes etching and 27 hours.

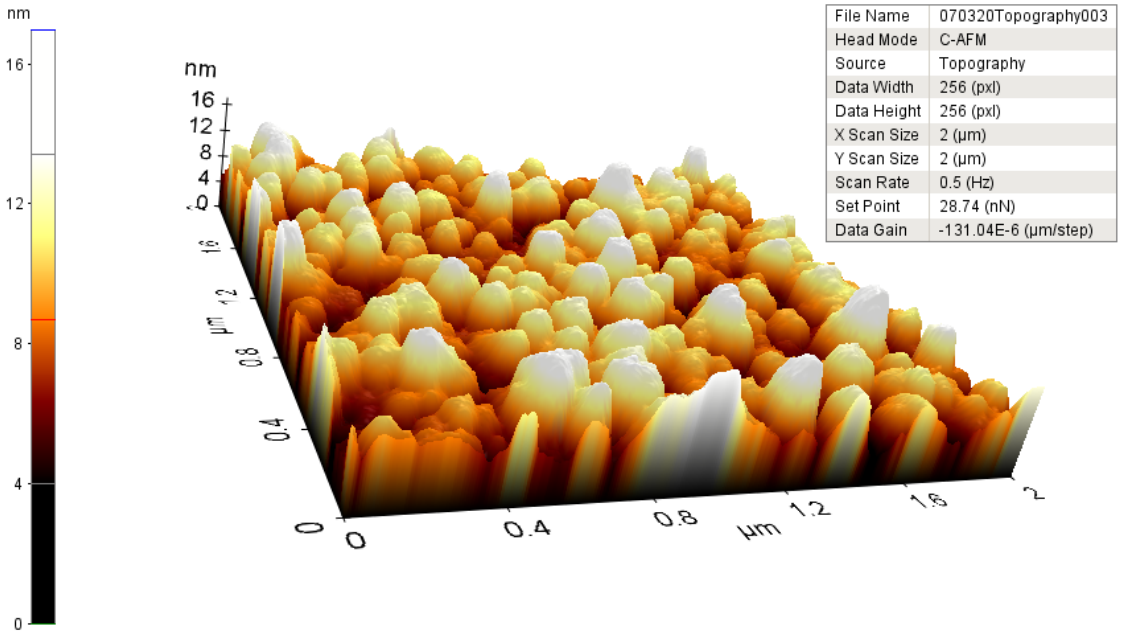

Figure 13. The appearance of a silicon waveguide before any etching treatment.

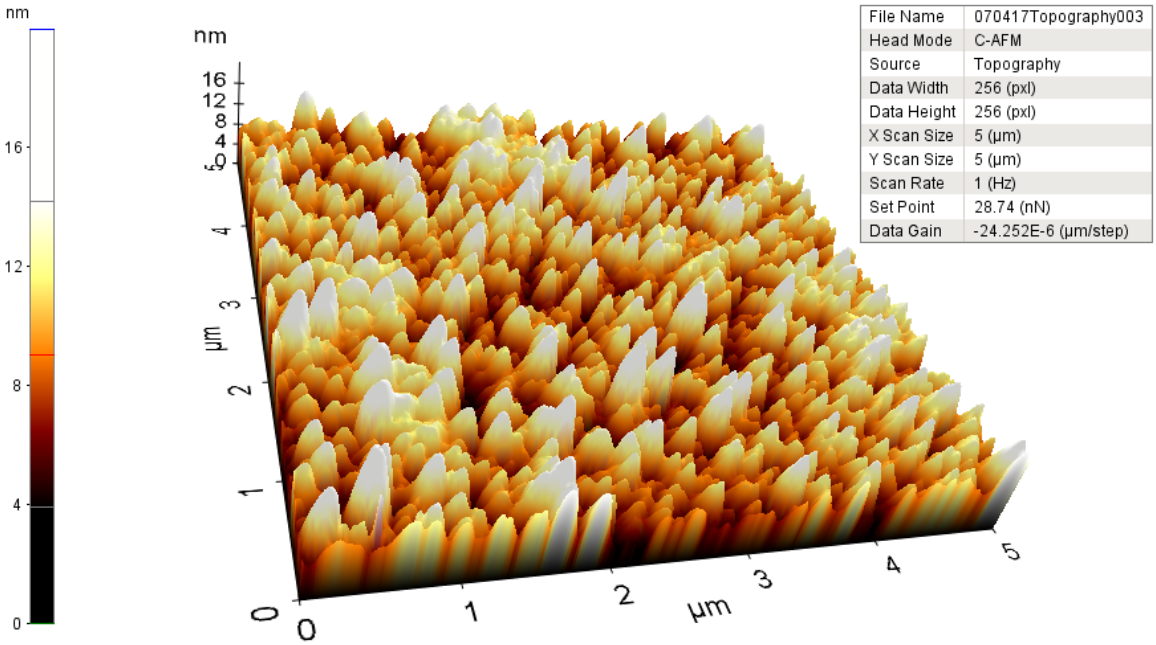

Figure 14. The appearance of a silicon waveguide after being etched for 10 minutes.

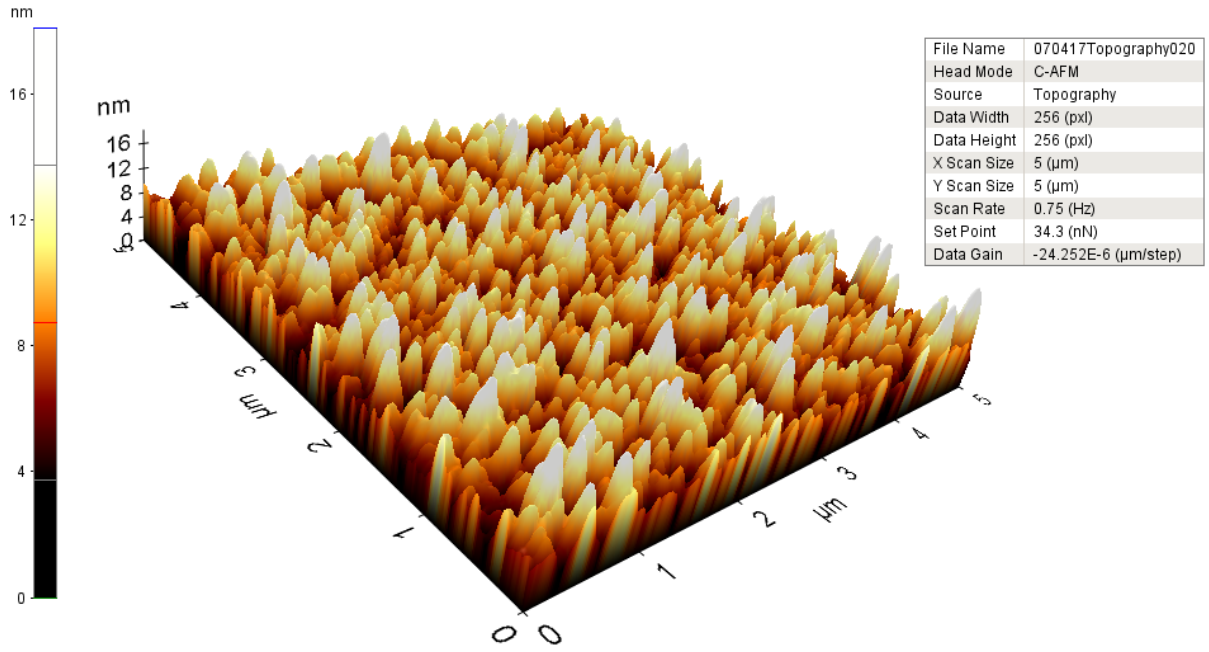

Figure 15. The appearance of a silicon waveguide after being etched for 27 hours.

8 DISCUSSION

In this report we have demonstrated a number of methods for manufacturing surfaces in a range of materials that have reproducible surface topographies at both the micrometre and nanometre scales. These include utilisation of existing, relatively low cost, commercially available surfaces that can be re-coated to change the chemistry of the surface without significantly changing the topography, at least for micrometre scale features. Etching of silicon wafers offers a route for producing nanoscale textures on a scale that is required for both protein and cell adhesion studies i.e. areas that lie between 0.5 cm^2 and 1.0 cm^2, exploiting textures that are found as a result of plasma deposition.

A number of different techniques are available for characterising both the chemistry and topography of the surfaces once they have been created. Care should be taken to choose techniques that are appropriate to the length scale under investigation and that they are compatible with the surface features. Similarly it is advisable to choose a technique that is only sensitive to the surface such as XPS or SIMS to identify the chemical species present and to avoid the influence of the underlying material.

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