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Curved micromachined surfaces in polymeric material for cell culturing

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Title (English)

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Abstract

Earlier work has shown that human cells react to very small structures with sharp edges. In this Degree project, a method for production of well-defined curved micromachined structures in polycaprolactone (PCL) was developed. Human fibroblasts were cultured on these structures and fluorescence stained for actin. Cells were found to align to and stretch along the grooves.

Keywords

Micromachining, quartz, polycaprolactone, cytoskeleton, fibroblasts, fluorescence

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Sammanfattning

Om man doppar en bit kvarts (glas) i en starkt frätande vätska kommer kvartsen sakta lösas upp. Med ett skyddande mönster av krom på kvartsbitens yta kommer dock bara de områden som inte är skyddade att utsättas för frätning. På så sätt kan man skapa ett mönster av gropar och spår med djup som beror på hur länge man doppar kvartsbiten i vätskan. Pressar man sedan biten mot varm plast kommer groparna och spåren stå upp som toppar och åsar i plastbiten.

Tidigare forskning har visat att däggdjursceller kan reagera olika beroende på hur små, små ojämnheter i ytan de växer på ser ut. Detta är av stor betydelse när man sätter in nya konstgjorda ytor, som tex benimplantat, i människokroppen. Detta arbete beskriver tillverkning av plastytor med mycket små diken tätt tätt tillsammans och hur celler reagerar på dessa.

CONTENTS

INTRODUCTION

Cells respond to topographic features of the surface upon which they are grown [1,2]. The results of previous research have demonstrated that both topography and surface chemistry are important. However, we still have a rather limited knowledge of how these factors influence cellular responses. There are several reasons why we would like to gain a better understanding, e.g. to improve bone implants and scaffolds for cell and tissue engineering. In some cases, it is desirable that cells adhere and proliferate on a certain surface, for instance in tissue culturing, in other applications, such as for intraocular lens insertion after cataract surgery, the opposite is required.

Early experiments on the effects of surface roughness have been confined to random adventitious topographies such as those formed by polishing. Polishing results in structures that are complex, chaotic and, above all, unrepeatable. Fortunately, the advent of various microfabrication methods, originally developed for the microelectronics industry, has provided a range of methods for making precise surfaces and for controlling and assaying each [3]. Furthermore, various replication techniques provide means for the copying of such micro- or nanostructures into different polymeric materials, and give access to low-cost mass production for high throughput cell assay.

The actual range of topographies so far tested has been small. Microfabrication has tended to give structures with vertical sides and the experimentalists have tended to define very simple patterns such as square pits or alternating grooves and ridges. So there is a whole range of different structures that has not been investigated, such as convex and concave sided grooves, undulating grooves, branch structures, etc. The surfaces that have curved features are in many ways much more similar to the type of topography shown by collagens, elastin and cells found within the body.

The aim of this Degree project has been to develop a working process for the production of curved surfaces in a biodegradable polymeric material and study how cells react to these structures. The report is written in a way as to be easily understood by both material scientists and biologists.

Micromachining

Micromachining, or microfabrication, describes one of many precision engineering techniques for creating small three-dimensional structures with dimensions ranging from subcentimeters to submicrometers. Such tiny structures are used in, for example, sensors and integrated circuits. In short, basic micromachining involves lithography and etching.

Lithography

Lithography is the technique used for transferring copies of a master pattern onto the surface of a solid material. The master can then be re-used to make many identical copies rather than being consumed directly. Here the most widely used form of lithography is described: photolithography.

In photolithography, a plane surface covered with a thin layer of a light sensitive polymer, a so-called *photoresist*, is illuminated through a *mask* and then developed (fig. 1).

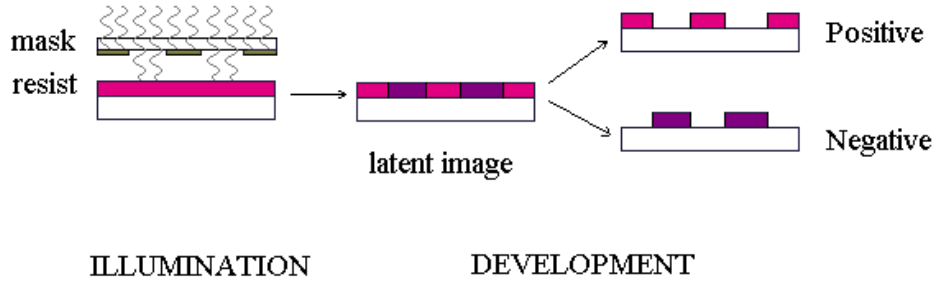

***Figure 1**. In photolithography, a surface covered with resist is illuminated through a mask and developed.*

The mask is a glass or quartz plate with a metal (e.g. a 500 Å thick chromium layer) pattern describing the outlines for the structures to be fabricated. It is placed above the resist surface and as light (of wavelengths depending on the resist used) is shone through it, the pattern is transferred to the surface in the form of a latent image. The image is developed by, for example, immersion of the surface in a developing solution (e.g. acetone). Depending on the nature of the resist, the image can be either the same as the metal mask pattern or its inverse. The resist is either *positive* or *negative* in its reaction to the illumination. Positive resists are weakened (breaks are induced in the main and side polymer chains) by light of the appropriate wavelengths and exposed areas become more soluble in developing solutions. Negative resists, on the other hand, are strengthened (cross-linkage of main and side polymer chains) in the exposed areas and thus become less soluble. Either way, the surface is now covered with a pattern of resist that will protect it from etchant in the etching step [4].

Etching

By different etching procedures one aims at removing material from the resist-patterned surface in different ways to obtain different results. Here I will only briefly describe two of the possible etching processes even though there are many more and much more to be said about the two I've chosen to discuss.

Figure 2. *In isotropic wet etching, the material is removed equally fast in all directions and smoothly rounded edges are obtained.*

Wet etching is done by immersing the surface in an etching solution. The etchant will remove material from the surface and depending on the material of the surface and the etchant in question, the wet etching process can be either *anisotropic* or *isotropic*. Anisotropically etched pits are due to crystal structures in the material (e.g. silicon) and are not discussed here. Isotropic etching is maybe the etching method that is the most intuitively easy to understand. The etchant solution removes material at equal speed wherever it is in contact with the surface. In the beginning, only areas not covered with photoresist are exposed to the etchant, but as material disappears, the etchant eats its way in under the resist layer (fig. 2). This way a smoothly rounded pit can be obtained when remaining resist has been removed.

To obtain more vertically walled pits, *dry etching* can sometimes be used. By using glow discharge techniques to generate plasma in a vacuum chamber containing the resist-coated surface, ions are to some extent directed to bombard the surface from above. More ions will hit the bottom of the pit than the walls, thus yielding pits deeper than they are wide [4].

Polycaprolactone

Polycaprolactone (PCL, fig. 3) is a fully biodegradable, non-toxic polymer that is rending more and more appreciation among biomaterials scientists. Its melting point is quite low (58 - 60°C, [5]) and it is therefore soft and easy to work with at room and body temperature. However, one problem with PCL is its non-transparent milky white colour. It is only transparent when melted but is then, obviously, impossible to work with in most applications.

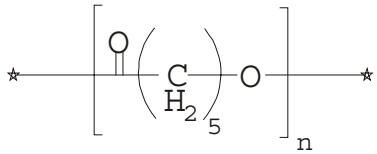

Figure 3. *Structure of ε-polycaprolactone.*

Transparency is desirable mostly because laboratory cell culturing is very much simplified by culture surfaces that enable light microscope analysis of the cells. There are some ways of making pieces of polycaprolactone clearer, though. One is to make extremely thin films of the polymer that will let light pass due to their sheer thinness. This can be done either by dissolving bits of PCL in chloroform, spread the solution and let it dry or by pressing melted polymer. Both these methods result in PCL films that are so thin that they can be quite impractical to work with. An alternative way to obtain transparent PCL is to melt relatively thin pieces and then cool them very fast with, for example, liquid nitrogen. This will prevent the polymer from re-crystallizing (Prof. A. Wirsén, personal communication) and will give almost perfectly transparent PCL pieces. However, it is important that the cooling is extremely fast; cold water or a freezer will not do as cooling agents.

Cell cytoskeleton

Eukaryotic (with nucleus) cells possess an interior network of filamentous proteins to support their inner and outer structure. This flexible and dynamic construction is called the *cytoskeleton* and enables the cell to adopt different shapes and move in a coordinated fashion as well as to organise the many components in its interior. Unlike our own bony skeleton, the cytoskeleton doesn't just constitute the "bones" of the cell but also the "muscles" as it is directly responsible for the cell movements along surfaces.

Some of the most important protein aggregations in the cytoskeleton are the *actin filaments* (F-actin). They are built up of small globular actin molecules (G-actin) arranged in two-stranded helices (fig. 4) that can be either unstable or stable, depending on with what actin-binding proteins they are associated.

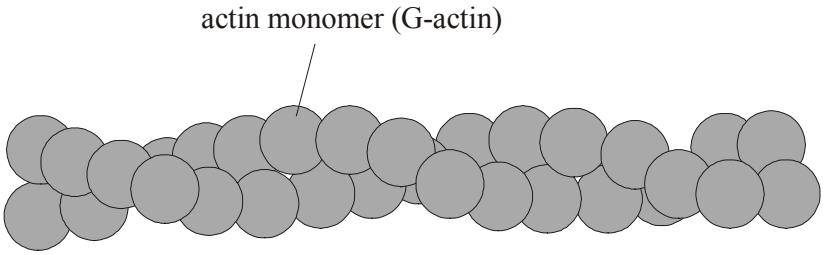

Figure 4. *Simplistic image of actin filament (F-actin) built up of globular actin monomers (G-actin).*

Actin filaments can form, for example, the relatively permanent and brushy structures of *microvilli* on the cells lining the intestine. When working as the muscles of a cell, actin filaments are cross-linked together in *contractile bundles* that are much stronger than the individual filaments on their own. These bundles can contract to change the shape of the cell and direct its movements. [6]

Fluorescent actin staining

The contractile bundles of F-actin in the cells can be specifically marked by antibodies, but are even better labelled by phallotoxins (phalloidin and phallacidin). These latter are bicyclic peptides isolated from the poison mushroom *Amanita phalloides* that bind selectively to the polymerised actin (F-actin) and not the globular monomer (G-actin). Therefore, by using a phallotoxin conjugated to a fluorescent probe (fig. 5), one can make the filamentous fibres of the cell cytoskeleton microscopically visible.

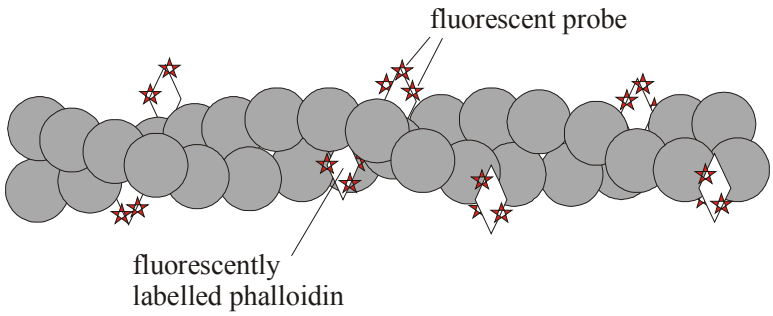

Figure 5. *Actin filaments labelled with phalloidin conjugated with a fluorescent dye.*

Fluorescent probes are large conjugated-system molecules that absorb and emit light at different wavelengths. By illuminating samples containing fluorescent molecules with light of a specific wavelength, the molecules' positions can be observed through a filter permitting the emitted wavelength of the probe. Examples of fluorescent probes are fluorescein isothiocyanate (FITC) and tetramethylrhodamine-5-isothiocyanate (5-TRITC, fig. 6) that absorb - emit light with peaks at wavelengths 495 – 516 nm and 543 - 571 nm, respectively. [7]

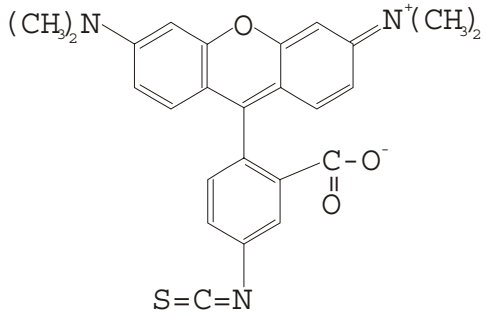

Figure 6. *Structure of tetramethylrhodamine-5-isothiocyanate (5-TRITC). [7]*

MATERIAL AND METHODS

Quartz master

Since the objective of the micromachining process was to etch isotropically to obtain rounded grooves rather than ridges with sharp edges, it was decided to try to work with quartz and wet etching. It so happens that the masks usually used for the photolithography step of micromachining processes often are made of quartz with a pattern of chromium covered with resist. It was concluded that one fabrication step, the photolithography transfer of pattern, could be excluded from the process if the mask itself was used as etching template with the chromium-resist layer as etching pattern specification.

Process development

To develop a working strategy for the quartz etching process, both practical and theoretical investigations were done. The etching speed of buffered hydrogen fluoride, BHF (Ammonium fluoride etchant, VLSI Selectipur®, Merck, Darmstadt, Germany), in quartz was determined by repeated experiments. Chromium/resist-masked pieces of quartz were dipped for different lengths of time (30 s-10 min) in BHF at 20°C. The etched depths were then checked using a Dektak V200-Si stylus profiler (Veeco Instruments Ltd., Cambridge, United Kingdom) and related to the etching durations. A cross-section of an etched pit was examined by scanning electron micrography (SEM, fig. 7) to determine the etching profile.

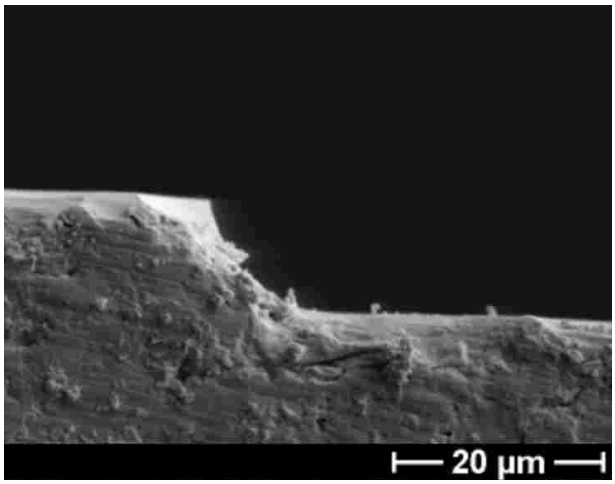

Figure 7. *Scanning electron micrograph of cut quartz etched in BHF and sputtered with a thin (<0.5 µm) film of gold. Sharp flakes are remnants of cross-section sawing.*

Different possible etching processes were evaluated using simulation software (2Dinese, [8]). Dry (directional) as well as wet (isotropic) etchings were considered (fig. 8) even though the process described in this work only involves wet etching.

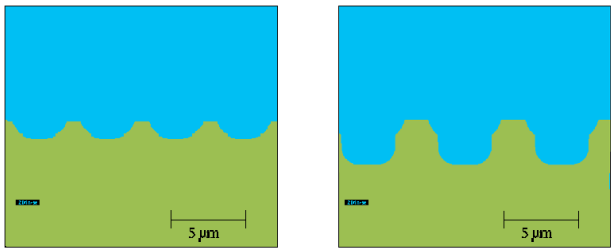

Figure 8. *Structures simulated in 2Dinese.In (a) only isotropic, (b) both directional and isotropic etching used.*

Etching

All etching procedures were performed in the clean room at the Ångström Laboratory, Uppsala, Sweden, at room temperature.

A blank quartz plate (Nanofilm, Westlake Village, CA) with a thin film of low reflective chrome (1000 Å) and photo resist (5300 Å) was used as etching template. The pattern mask (fig. 9) was inscribed with a LRS-18 pattern generator (Micronic Laser Systems AB, Täby, Sweden). The pattern consisted of chromium-resist lines of 200 mm length and 4µm breadth separated by 1 µm, giving a period length of 5 µm.

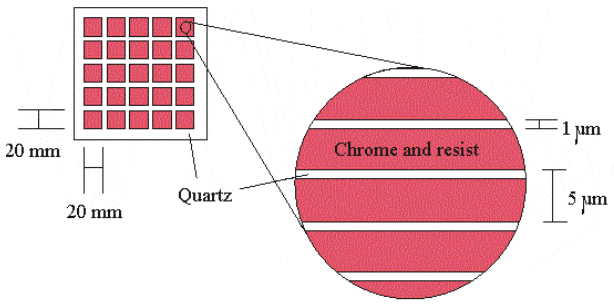

Figure 9. *Quartz plate with inscribed chrome and resist mask with a period length of 5 µm.*

The plate was cut to give 25 (5×5) pieces of approximately equal size (about 23×23 mm). The masked quartz surfaces were thoroughly cleansed in water and blow-dried. They were then quickly dipped in water with a low concentration of Triton to reduce surface tension effects before being transferred to a bath of buffered hydrogen fluoride, BHF. After 120 s, the quartz pieces were cleansed in running water for 30 s and blow-dried. The photo resist layer was removed by 10 min in acetone after which the pieces were again cleansed and dried. The chromium was removed in chromium etchant (LSI Selectipur®, Merck) in <1 min. When the, now transparent, pieces had been cleansed they were again dipped in the Triton solution and transferred to the BHF bath, now for 30 s. The finished surfaces were rinsed in running water for 30 s.

Polymer surface

The polycaprolactone (PCL) used was CAPA® 650 (Solvay Caprolactones, Solvay Interox Ltd, Warrington, United Kingdom) injection moulded to CD format (∅ 120 mm, thickness approx. 1.2 mm) by Åmic AB (Uppsala, Sweden).

Pre-treatment of polymer

Thin circular pieces of PCL were obtained by the aid of a heat press (Pasadena Hydraulics Inc.). Pieces (approx. 50 cm^2) of PCL were heated to 64°C and then pressed between two films (170×170×0.05mm) of brass. The polymer (now approx. 0.6 mm thick) was cooled by running water and separated from the brass films. Circular (∅ 28 mm) pieces were punched from the polymer film and thinned (to approx. 0.3 mm) by another cycle of heating, pressing and cooling.

To attain transparency, the PCL pieces were treated with liquid nitrogen (N_{liq}). The thin circular films were one by one melted on a small bit of brass film (0.05 mm thick) on a hot plate. They were then quickly transferred on the brass shim to a bath of N_{liq} and kept there until boiling had ceased (about 5 s) after which they were left in room temperature.

Hot embossing

To replicate the structures of the quartz master in the transparent PCL films, hot embossing was carried out with the hot press mentioned above. A sandwich of brass film, PCL, quartz master and silicone pieces acting as planarizing layers was heated without added pressure to 48°C. While continuing heating, pressure was carefully applied in order to reach as high as possible without cracking the quartz master until the temperature had reached 54°C. Pressure and temperature were kept constant for 10 s after which the pressure was dropped and the

sandwich immediately cooled in running water. The sandwich was parted and the master used again in other embossing cycles. The completed replicas in PCL (now about 0.15 mm thick) were kept at room temperature.

Some replicas were analysed in a scanning electron microscope (SEM) in order to verify the transferred structures (fig. 10).

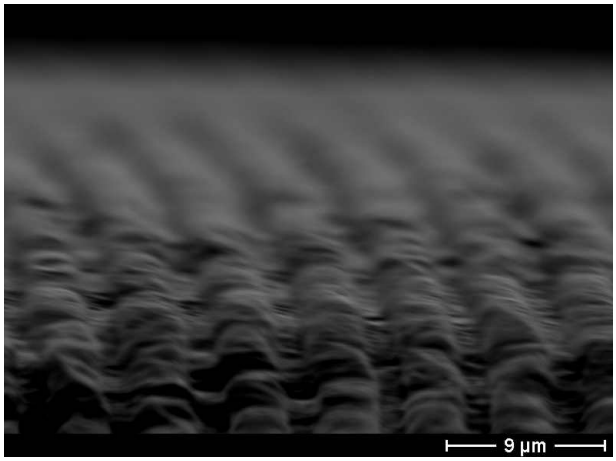

Figure 10. *Scanning electron micrograph of cut edge of PCL replica coated with <0.5 µm layer of gold.*

Wettability

To reduce the strong hydrophobicity that the completed PCL surfaces were found to exhibit the pieces were quickly dipped in a mixture of saturated $KClO_3$, 70% $HClO_4$ and water (ratio 1:3:2) and then rinsed in water.

The water contact angle was determined sixfold for both untreated and treated flat surfaces. Drops of milli-Q (15 µl) were positioned on the test pieces that were placed in a humidity chamber. When the air humidity in the chamber had stabilised (about 70%) the drops were photographed. The contact angle was calculated using computer software (FTA200, First Ten Ångstroms, Portsmouth, VA).

Cell reaction to structures

Cell culture

Cell culturing was done at the *Centre for Cell Engineering* at the University of Glasgow, Scotland. Structured PCL pieces were cleaned in 70% ethanol for about 5 min before being washed in culture medium (Ham's F10 containing L-glutamine, Life Technologies, Paisley, Scotland) supplemented with 3% foetal calf serum. Human HGTFN fibroblasts were seeded on these PCL surfaces (15 thousand cells per cm^2) and cultured for 26 hours (37°C) in fresh supplemented culture medium. Cells were then washed in PBS (37°C) and fixed *in situ* for 15 min in 4% formaldehyde/PBS with 2% sucrose.

Actin staining

Fixed cells were treated with permeabilising buffer (10.3 g sucrose, 0.292 g NaCl, 0.06 g $MgCl_2$ [hexahydrate], 0.476 g Hepes in 100 ml PBS. pH 7.2, 0.5 ml Triton X) for 5 min, 4°C. The cells were then stained with rhodamine phalloidin (Molecular Probes Inc., Eugene, OR) at 1:100 in PBS/BSA (2h, 37°C, in the dark). After subsequent triple washing in RT PBS, the

PCL surface with cells was mounted on a glass slide for analysis. Micrographs were obtained with a Vickers M17 (no longer made) fluorescence microscope (abs. ~540 nm, em. ~570 nm) and a Hamamatsu Argus camera and frame grabber.

RESULTS AND DISCUSSION

Structured surfaces in PCL

The etching speed of BHF in quartz was determined to 10 nm/s through repeated experiments. The quality of the etched surfaces (smoothness of edges) was found to be highly dependent on the cleanness of the etchant solution. If the BHF was more than one week old (BHF bath refilled more than one week ago), it was common to see large crystal-like disturbances in the etched areas. To avoid this, the BHF was refilled before every new etching occasion.

The structures obtained through wet isotropic etching of quartz and subsequent replications in polycaprolactone showed to be very much like the ones that had been simulated (compare fig. 8a and 10). The profile of the grooves turned out almost exactly as the simulations and the depth is (by inspection of fig. 10) at least close to the 1.25 μm that had been simulated. Even though the ridges look a bit rough in the SEM micrograph, light micrographs (not included) showed no such coarseness. It is quite probable that the bumps and scratches seen in the SEM micrograph are due to the crudeness of the pre-SEM cutting of the PCL piece.

Pre-treatment of the polymer was aimed to yield transparent pieces suitable for cell culturing. One major problem was that the replication method involved a heating step that could annul the earlier liquid nitrogen treatment by once again melting the polymer structure. To avoid this happening, the hot embossing was carried out at a temperature below the PCL melting point. However, when examining the final cell culturing pieces, one could see that the transparency was no longer as perfect as it had been before the hot embossing step. Considering that the thickness of the polymer pieces had also been noticeably reduced, one could argue that the liquid nitrogen treatment could have been annulled. Contradicting this hypothesis is, though, the fact that earlier embossings at higher temperatures with untreated PCL never showed as good transparency as the treated ones, after all, did.

Wettability analysis

The results of the contact angle measurements are shown in table I.

Table I
Contact angles (degrees) of water droplets on flat PCL surfaces, before and after wettability treatment

Contact angle, untreated	Standard deviation	Contact angle, treated	Standard deviation

76	2	53	2

The contact angles are clearly smaller for the treated surfaces than the untreated ones. The handling is obviously helpful in cases like these where a hydrophobic surface has to be treated quickly.

It should be noted that the untreated surfaces in table I were not as extremely hydrophobic as the pieces that were treated in Glasgow before cell seeding were. Unlike the Glasgow surfaces, the wettability analysis pieces were not at all too hard to submerge in water before treatment. This is corroborated by the contact angle values (table I) that are not extremely high, either. There is no direct explanation to why the pieces were so different. The only difference in the way they had been treated (they were made the same day and transported to Scotland in the same box) is the actual site of investigation. The contact angle measurements were performed at the *Biomedical Centre*, Uppsala, but the first hydrophobicity observations were made at *Centre for Cell Engineering*, Glasgow. Supporting this theory of location importance is the fact that the pieces of PCL used in cell culture trials (results not included in this report) during the first couple of days in Glasgow did not show any sign of extraordinary hydrophobicity that other pieces did later (after about a week). However, the exact reasons to the effect remain to be investigated.

Cell reaction

Human HGTFN fibroblasts showed distinct alignment to the grooves of the PCL surface as soon as they had settled and adhered to the surface (4-5 h). With fluorescent staining (26 h), actin bundles could be seen to stretch along the surface (fig. 11).

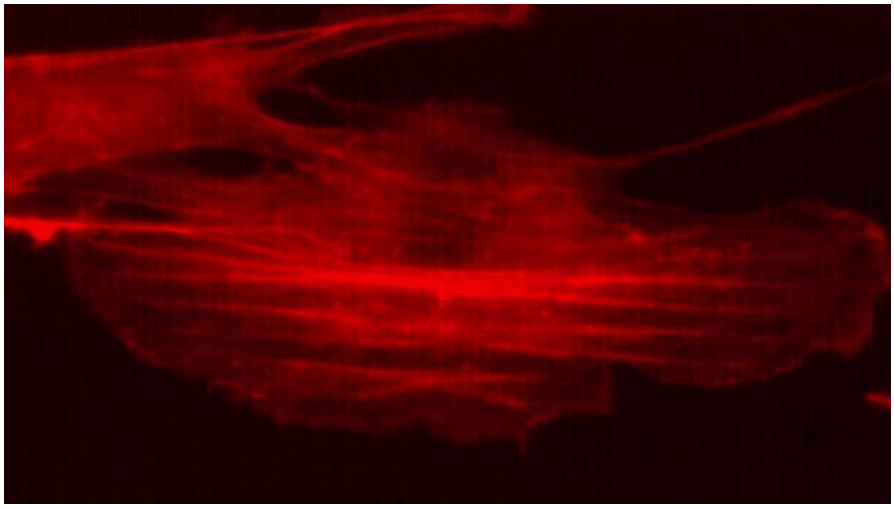

Figure 11. *Fluorescence light micrograph of human HGTFN cells on grooved PCL surface. Cells are stained with rhodamine phalloidin. Groove direction is horizontal.*

On flat PCL, outside the structured areas, the cells showed no alignment (fig. 12a). However, if one part of the cell was outside the structured area and one part inside, the latter showed cytoskeleton stretching correlated to the grooves (fig 12b). If cells were in contact with each other on the structured areas, they were considerably less aligned (fig. 12c) than they were when single.

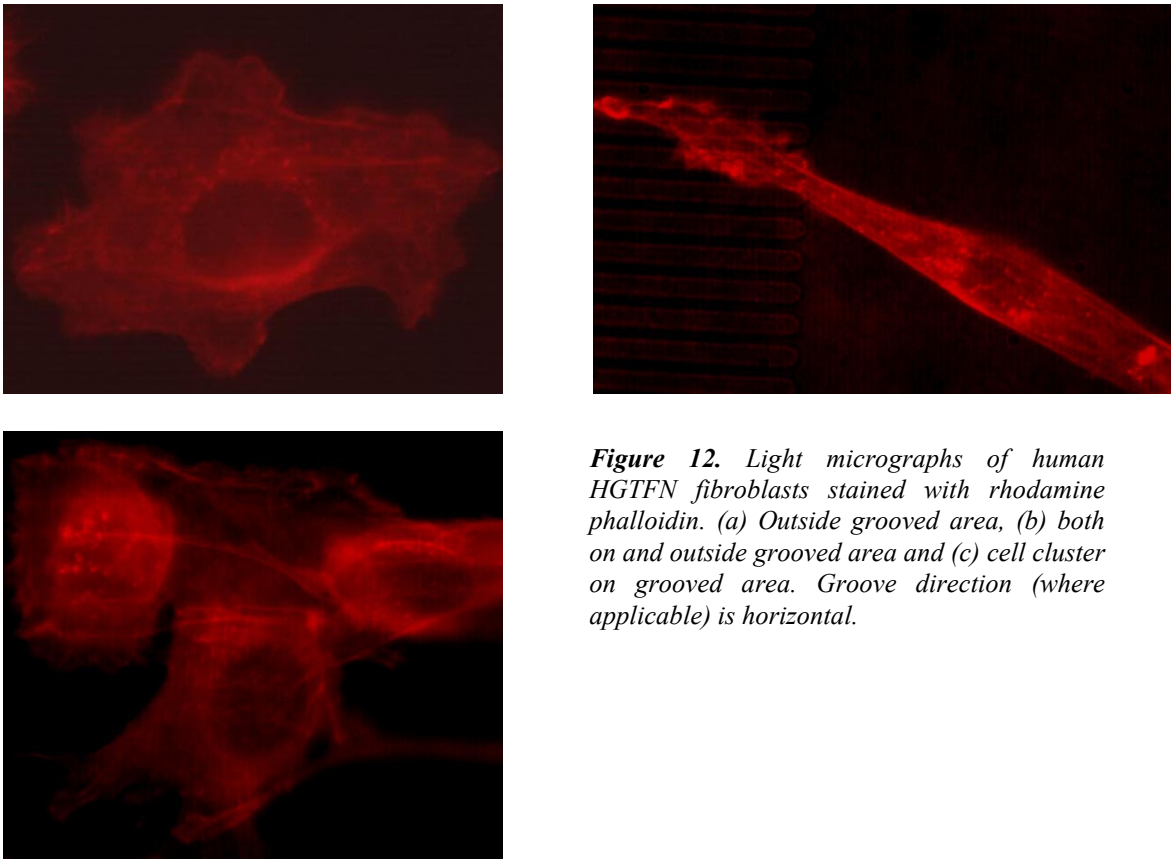

Figure 12. *Light micrographs of human HGTFN fibroblasts stained with rhodamine phalloidin. (a) Outside grooved area, (b) both on and outside grooved area and (c) cell cluster on grooved area. Groove direction (where applicable) is horizontal.*

The latter phenomenon, when cells lose alignment when touching each other, is well known as *contact inhibition*. It is seen in many other situations, such as growth inhibition when a cell culture has reached confluence in a certain restricted area, and is a characteristic of healthy cells. Cancer cells lack this growth checkpoint and can therefore proliferate into huge aggregations of cells, tumours [9]. The consequences of this phenomenon when it comes to cellular reactions to topography are yet to be determined.

The fact that the cells align to the grooves is fully consistent with previous results (for example [2]) found with sharp grooves and ridges of various depths and widths. The orientation of the cells along the structures is called *contact guidance* and has been known since the beginning of the 20th century [10]. Some types of cells have previously been seen to align to ridges only 30 nm in height [11], 1/30 of the groove depth studied here.

Stainings of other proteins than F-actin could have shown different aspects of the cell morphology than the contractile bundles of the cytoskeleton do. For instance, one attempt was made with fluorescent immunostaining with antibodies for the focal adhesion protein *vinculin*. Due to very high levels of unspecific staining, the resulting micrographs are not included in the report. However, a successful staining could have indicated where the cells were in direct contact with the PCL surface. That way it would have been possible to see whether the cells actually went into the grooves or just bridged over the gaps between the ridges.

CONCLUSIONS AND FUTURE WORK

The cell culturing and staining done in this work show that human fibroblasts align in much the same way to smoothly grooved substrata as they do to ridges with sharp corners of the same dimensions. This was suspected. However, what would be interesting to investigate in future work is if the cells in any aspect react *differently* to smooth grooves. The genes expressed, the proteins produced and the strength of adhesion to the structures could very well be functions where the reactions may differ. Intuitively one would guess that the surfaces that the cells encounter in the human body are more similar to smooth groves than to sharp corners and that the reactions would therefore be more "body like" on smooth grooves.

Apart from comparing reactions to smooth grooves and sharp ridges of the same dimensions, smooth grooves of different lengths of period and amplitudes should be compared to each other. It has earlier been shown [12] that cells react differently depending on how high the ridges are and their frequency. This should therefore be thoroughly investigated for smooth grooves as well. For instance, the smallest influencing groove depth could possibly be different with rounded corners instead of sharp ones.

Another aspect that should be studied is if cells differentiate differently on different structures. The implications for such reactions could be very important for implant surface structuring in the future. For instance, bone implants could maybe be incorporated better in the body if cells in contact with the implant developed into bone cells. Alternatively, differentiated cells in laboratory cultures could be kept on structured surfaces suited especially for their kind. As it is today, laboratory culture dishes used for *in vitro* experiments are the only flat surfaces that cells encounter. They are also the only kind used to study how cells react to various treatments, even though they cannot provide a biologically normal environment for the cells.

Many properties of polycaprolactone are often coveted enough to compensate for its lack of transparency. However, for PCL to become widely used in laboratories better methods to make it clear have to be developed. Even though working with opaque materials is possible, it is definitely more time consuming. Commercially produced Petri dishes for laboratory work are most often made in other, transparent, polymeric materials such as polystyrene (PS) or polypropylene (PP). These plastics do not possess the biodegradability of PCL, though, and are therefore not suitable for, for example, tissue engineering.

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REFERENCES

1. CURTIS, A. S. G. AND WILKINSON, C. D. W. (1998) Reactions of cells to topography, *J. Biomaterials Sci. Polymer*, **9**, 1313-1329

2. CURTIS, A. AND WILKINSON, C. (1997) Topographical control of cells, Review, *Biomaterials* **18**, 1573-1583

3. SCHWEITZ, J. –Å., LARSSON, K., THORNELL, G., BJÖRKMAN, H. AND NIKOLAJEFF, F. (2000) New materials and new proceses for MEMS applications, *Mat. Res. Soc. Symp. Proc.*, **605**, 57-72

4. MADOU, M. J., (1997) *Fundamentals of microfabrication*, CRC Press LLC, Boca Raton, FL

5. SOLVAY CAPROLACTONES. "Safety information, CAPA® Polycaprolactone" Nov 1999. http://www.caprolactones.com/SafetyInfo/SafetySheet/CAPA-650_s.htm (22 May 2001)

6. ALBERTS, B., BRAY, D., JOHNSON, A., LEWIS, J., RAFF, M., ROBERTS, K. AND WALTER, P. (1998) *Essential Cell Biology: An Introduction to the Molecular Biology of the Cell*, Garland Publishing Inc., New York, NY

7. MOLECULAR PROBES INC. "Handbook of Fluorescent Probes and Research Products, probes for Actin" May 2001. http://www.probes.com/handbook/sections/1101.html (22 May 2001)

8. KATARDJIEV, I. V., CARTER, G., NOBES, M. J., BERG, S. AND BLOM, H. O. (1994) Three-dimensional simulation of surface evolution during growth and erosion, *J. Vac. Sci. Technol. A*, **12**, 61-68

9. COOPER, G. M. (1997) *The cell: a molecular approach*, ASM Press, Washington, DC, Sinauer Associates, MA

10. VON RECUM, A. F., SHANNON, C. E., CANNON, C. E., LONG, K. J., VAN KOOTEN, T. G. AND MEYLE, J. (1996) Surface roughness, porosity, and texture as modifiers of cellular adhesion, *Tissue Engineering*, **2**, 241-253

11. WOICIAK-STOTHARD, B., CURTIS, A., MONAGHAN, W., MACDONALD, K. AND WILKINSON, C. (1996) Guidance and activation of murine macrophages by nanometric scale topography, *Exp. Cell Res*., **223**, 426-435

12. WALBOOMERS, X. F., MONAGHAN, W., CURTIS, A. S. G. AND JANSEN, J. A. (1999) Attachment of fibroblasts on smooth and microgrooved polystyrene, *J. Biomed. Mat. Res*. **46**, 212-220