Modulation of Cell Motogenic Behaviour Using Biofabrication Techniques to Mimic the Extracellular Matrix.

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ABSTRACT*: The behaviour of cells in vivo is tightly controlled by a wide variety of regulatory factors. These include soluble factors (cytokines, growth factors), immobilised extracellular matrix (ECM) components (e.g. fibronectin, collagen), direct cell-cell "juxtacrine" interactions, and physical parameters (e.g. hydrodynamic shear, matrix stiffness, and topography). These regulatory factors appear to combine to control cell behaviour. Much work has been performed classifying the responses of cells to soluble factors, and there has been a heightened recent interest in the role of the immobilised matrix components in cell-signalling. Similarly, many recent in vitro studies have been performed with the aim of elucidating the responses of cells to micro-scale topographical cues, such as the phenomenon of "ridge-walking" in motile cells. One potential outcome for this work is to properly understand the manner in which cells react to the shape of their substratum, and then design appropriately-textured surfaces for therapeutic implants, to facilitate the process of wound healing around the implants – aiding tissue integration, and preventing implant rejection and extrusion.*

Migrating cells in vivo move through a three-dimensional environment of ECM components, and the composition and stiffness of this matrix regulates cellular behaviour. Our aim is to study the responses of cells in vitro, within such a three-dimensional matrix, to a topographically engineered substratum made from inviscid bioactive polymers, and therefore gain an understanding in optimising the topography and biomaterials for use in vivo.

KEYWORDS*: Microengineering, Rapid prototyping, Biomaterials, Tissue engineering*

1. INTRODUCTION

The natural wound healing response of the body when tissue injury occurs is an extremely complex interaction between cell signalling behaviour and the composition of the underlying extracellular matrix (Schor *et al.*, 1999, 2003). In recent times there have been many advances in understanding the influence on cell behaviour of specific factors such as extracellular matrix material, matrix geometry and soluble regulatory factors such as cytokines. However, much of this work has focused on each of these factors in isolation and so does not fully address the complex nature of the in-vivo biological system

This paper describes the work being undertaken in merging the advances in materials and engineering technologies to allow the development of more complex biological experiments which more closely replicates the natural environment. The research is focused on producing complex 3-D matrices which are cell seeded and contain actual biological extracellular matrix materials and synthetic biocompatible copolymers with tethered cell signalling moieties. As a result of this work, we hope to produce in-vitro assays which mimic the complexity of the in-vivo system and thus improve the understanding of wound healing responses.

2.1 MICROENGINEERING

Photolithographic techniques as developed for the microelectronics industry have been used in the patterning of materials for tissue engineering structures. This process involves a number of stages, namely; (i) the production of a photomask containing a two-dimensional pattern of the microstructure to be created, (*ii*) applying a photosensitive polymer, photoresist, to the surface of the substrate to be imprinted, (*iii*) employing a mask aligner to imprint the pattern contained in the photomask onto the photoresist by exposure to uv radiation and (*iv*) finally, developing the pattern using an appropriate solvent.

Using photolithography, structures can be produced either directly in the photocrosslinkable polymer itself or by using the patterning as a mask to allow surface patterning of the underlying substrate through either etching or deposition processes. Line resolutions of sub-micron scale are easily achieved using these methods and so very accurately defined features can be obtained.

Standard photoresists have traditionally provided pattern depths of no more than a few microns. Newer materials have recently become available for use in microengineering applications which can result in feature heights of greater than 100 microns. These allow the production of High Aspect Ratio Microstructures (HARMs) (Keatch *et al.*, 2000, Li *et al.*, 2000) which offer feature sizes which are more suitable for cell biology research. Examples of HARMS can be seen in Fig. 1 below.

The response of cells to a synthetic matrix is highly dependent on the material chemistry and also on the topographical features. The behaviour of cells on a number of different patterned microstructured surfaces has been investigated. Figures 2 and 3 show the response of 3T3 mouse embryonic fibroblast cells to patterned HARMs produced in SU8-2075 photoresist. In these images the cells can be seen attaching to all surfaces in this synthetic 3D environment invoking a more natural response from the cells.

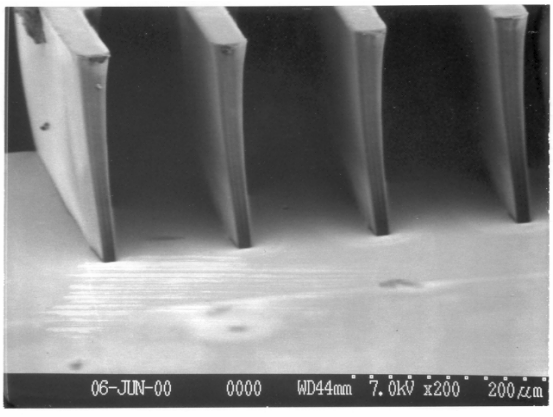

Figure 1. High Aspect Ratio Microstructures (HARMs)

Micropatterned lines etched into quartz slides have also been used to investigate the thigmotropic behaviour of cells to such topographical features (Curtis and Wilkinson, 1998, Wojciak *et al.*, 1995). Figure 4 shows cells grown on 50micron wide lines etched to a depth of 2microns and clearly shows the behaviour of the cells as they try to align themselves along the direction of the channels

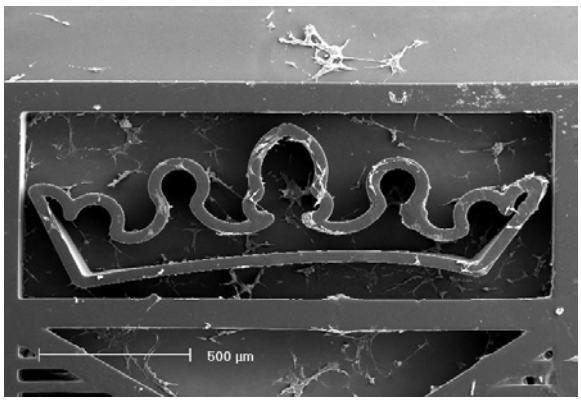

Figure 2. 3T3 Cells cultured on University Crest patterned HARM

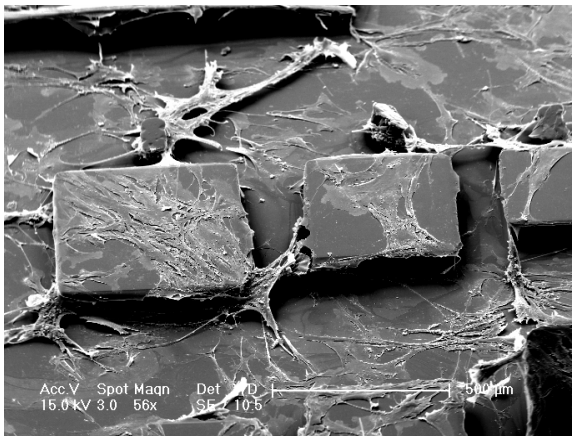

Figure 3. 3T3 Cells cultured on square patterned HARM

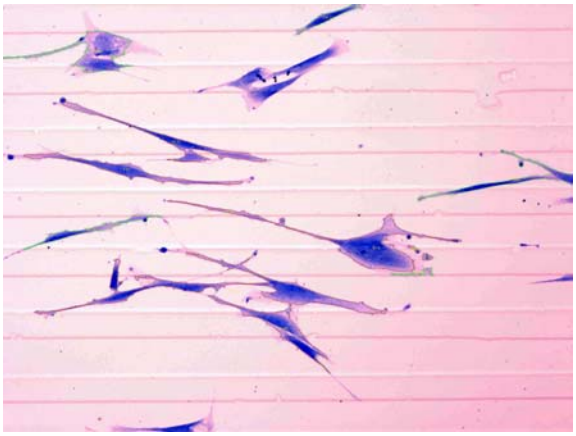

Figure 4. Cell alignment along 50micron etched channels in quartz slide

2.2 COMPLEX 3-D STRUCTURES

In promoting angiogenesis, tissue engineering scaffolds are required to be complex structures which are porous enough to allow cell penetration. We have therefore investigated the feasibility of using the photolithographic techniques with SU-8 photoresist to produce multilayer patterns which form complex 3-D matrices. Three-layer structures have been produced which show a number of different types of shapes formed by sequential patterning using the photolithographic process previously described. One modification to this process, however was that development of the patterned layers did not occur until after the final layer had been exposed. The reason for this was that when previous layers were developed first, subsequent coatings of resist would fill the voids which had been developed out. When this thicker resist layer was soft baked, the viscosity of the surface would drop as the solvent was removed. As solvent was still being driven off from the lower parts of the resist, this had the effect that once it reached the upper levels, it would deform the surface. Since the resist was now quite a viscous material, the smooth spun coating became warped and pitted and so was unsuitable for further patterning.

Figure 5 shows a scanning electron microscope photograph of a three-layer structure produced using this process. It can be seen from this image that in many areas there exists an extension of the second and third layers down to the substrate, indicating that exposure of these areas is occurring due to the highly sensitive nature of the material and the penetration depth of the uv radiation.

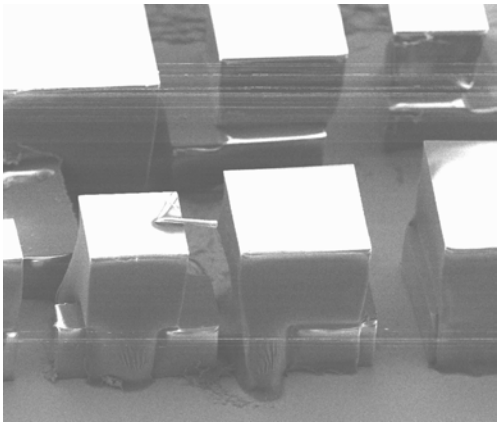

Figure 5. Two layered structure produced using SU-8 photoresist

From these results we have concluded that the production of porous complex 3-D microscaffolds for cell-seeding is difficult using standard photolithographic techniques without the addition of sacrificial inter-layer masks which would avoid the problems of excessively deep exposure.

2.3 RAPID PROTOTYPING

Rapid prototyping techniques have long been used in biomedical applications. These techniques have primarily been used to produce such items as medical prosthetics using polyurethanes or silicones or bone implants using ceramics (Yang *et al.*, 2002, Wicker *et al.*, 2001). Recent developments in rapid prototyping technologies have allowed the emergence of new equipment designed specifically for use in biomedical applications. In particular, one system, the Envisiontec Bioplotter, has been developed to allow the production of complex 3-dimensional structures by the multi-layered build-up of material. In addition, it allows the use of biologically relevant materials such as agar, collagen, gelatine and even living cells in the manufacturing process. The process involves the pressure extrusion of the plotting material through a thin nozzle onto a substrate using a 3-axis plotting arm. The pattern is built up using a boustrofudonic method as shown in Fig. 6 and thus porosity of tissue engineering scaffolds can be determined by both the material type and also the line spacing during the plotting process.

Figure 6. Boustrofudonic plotting pattern of Bioplotter

The Bioplotter process can also be adapted to plot multiple materials in one structure. This can be achieved either by changing the plotting cartridge after each layer, thus having each layer being produced in different materials or by careful meshing of two separate plotting patterns into a single layer. Figure 7 shows an example of the latter method, where, for clarity, instead of each pattern being produced in different materials, the two different patterns have been highlighted using different plotting directions.

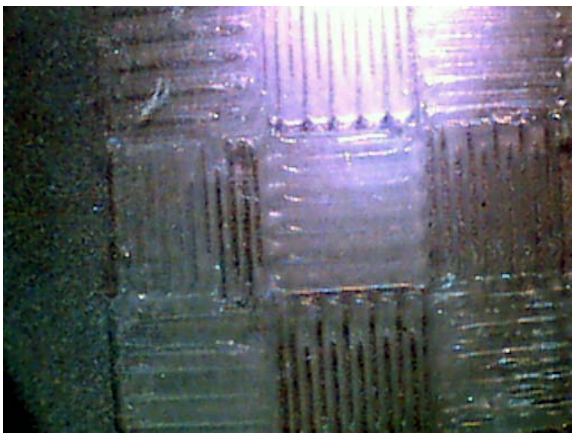

Figure 7. Structure produced from two seperately plotted patterns

2.4 BIOMATERIALS

Recent advances in synthetic polymer materials have allowed the development of new biomaterials which can be tailored to meet the requirements of tissue engineering implants for properties such as mechanical strength, biodegradation rates, biocompatibility and the ability to promote or inhibit cell growth. Additionally, in some cases, synthetic polymers can be copolymerised with peptides such that specific cell signalling functionality is introduced. One of these systems, based on polyethylene glycol diacrylate materials (PEGDA) (Hern and Hubbell, 1998, Lutolf *et al.*, 2003) has been adopted for use in our research due to its photocrosslinkable nature. This feature allows us to produce patterned gels of the material using our photolithography techniques as shown in Fig. 8. In turn, these structures can be incorporated into 3-dimensional matrices using the rapid prototyping equipment.

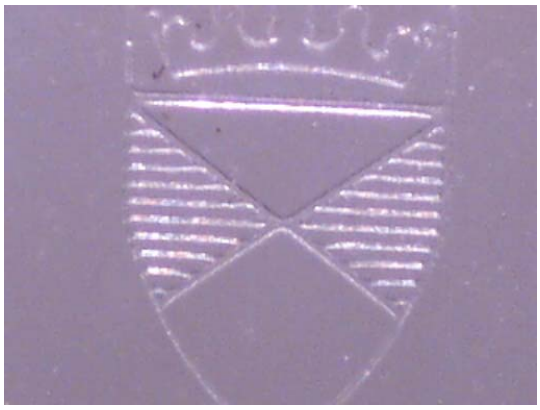

Figure 8. Micropatterned university crest structure produced in PEGDA

2.5 CONCLUSIONS

This research has investigated the suitability of a number of engineering techniques for use in producing 3-D scaffolds for tissue engineering. It can be seen that each technique has unique advantages i.e. edge definition and resolution for the microengineered structures, compared the structured 3-dimensional capabilities and the ability to use biomaterials of the rapid protyping systems. In conjunction with the development of new biomimetic polymers, we are intending to integrate these technologies such that we can produce tissue engineering scaffolds which more accurately reflect the complex nature of the biological system into which it is being placed. This should allow us to better understand the wound healing process that is initiated when tissue damage occurs and thus further the development of biocompatible tissue engineered implants.

3. REFERENCES

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4. Responsibility Notice

The authors R.P. Keatch, K.P. Donnelly, M.S. Pridham, C. Gribbon, S.L. Schor, and A.M. Schor are responsible for the printed material included in this paper