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Biomaterials 26 (2005) 4985–4992
Biomaterials
www.elsevier.com/locate/biomaterials

# The fibroblast response to tubes exhibiting internal nanotopography

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Received 12 October 2004; accepted 17 January 2005

**Abstract**

The use of three-dimensional scaffolds in cell and tissue engineering is widespread; however, the use of such scaffolds, which bear additional cellular cues such as nanotopography, is as yet in its infancy. This paper details the novel fabrication of nylon tubes bearing nanotopography via polymer demixing, and reports that the topography greatly influenced fibroblast adhesion, spreading, morphology and cytoskeletal organisation. The use of such frameworks that convey both the correct mechanical support for tissue formation and stimulate cells through topographical cues may pave the way for future production of intelligent materials and scaffolds.


*Keywords:* Actin; Cell adhesion; Cell morphology; Cell spreading; Nanotopography; Vinculin

## 1. Introduction

The interaction of cells with the surface of laboratory-designed materials is of great importance to progress in implant technology and tissue engineering, and is thus of great relevance in biomedical research [1]. It is becoming clear that the role of biomaterials in this field is extending from mere mechanical support to actually using intelligent material surfaces capable of providing chemical and physical cues to guide cell attachment, differentiation and thus aid the eventual assembly of cells to form functional tissue. One possibility that shows promise is that of adding defined topography to a material [2,3].

Micro-topography has been shown to present powerful cues to cells, altering adhesion, movement, morphology, apoptosis, macrophage activation and gene expression [4–6]. Patterning methods developed by the electronics research sector such as photolithography, electron beam lithography and laser holography are now allowing cell engineers access to well-defined nanotopographies [7]. Indeed, there is growing evidence from recent research that cells do react to nanoscale surface features [8–10].

Whilst it is possible to produce nanotopographies with 5 nm resolution using the aforementioned techniques, such methods are costly and time consuming, particularly if large areas of pattern are required. Recent work within our group has also concentrated on the production of rapid and cheap nanotopography by polymer demixing. In this technique, blends of polymers, for example, polystyrene (PS) and poly(4-bromostyrene) (PBrS), spontaneously undergo phase separation during spin casting onto silicon wafers [11]. By controlling the polymer concentration and the proportions of the polymers, different topographies can be produced; these range from pits to islands or ribbons of varying height and depth. X-ray photoelectron spectroscopy (XPS) and static secondary ion mass spectrometry (SIMS) have been used to determine the surface composition of the blends [12,13], showing that PS segregates to the surface on annealing the films. This

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doi:10.1016/j.biomaterials.2005.01.046

means that despite the topography being formed by polymer blends, the cells only interact with a single chemistry on annealed substrates. Another polymer blend that gives topography in cast films is polystyrene-poly(*n*-butylmethacrylate (PS/PnBMA). In this system, the PnBMA segregates spontaneously to cover the surface at ambient temperature, producing a single chemistry without annealing [14].

To date, there have been studies observing the influence of nanoisland topography with various heights created using polymer demixing on a variety of human cells in culture, in particular endothelial and fibroblast cells, indicating a range of responses [15–18]. Interestingly, the islands of smaller heights (e.g. 14 nm) tended to increase adhesion/proliferation/cytoskeletal development/gene expression, while those of larger height (e.g. 95 nm) induced a stellate cell morphology with a poorly formed cytoskeleton [15,16].

It is noted, however, that the vast majority of research utilising topographical cues is two-dimensional (2D) on the macro-scale, and can thus be criticised for being a poor representation of most topographies presented to cells in the body. We are thus currently trying to capitalise on our wealth of information on topography fabrication and to transfer this knowledge to a 3D situation. Current research on the production of 3D scaffolds exhibiting topography tends to rely on pre-fabrication of the topography on a flat surface, followed by subsequent shaping of the material, for example rolling into a tube. Earlier this year, our group presented the first paper indicating successful nanopatterning inside a glass tube with an internal diameter (ID) of 1 mm. There are currently many shapes of scaffold under investigation; however, the successful production of a tube exhibiting internal topography would provide very useful insight for a wide range of cell and tissue engineers, encompassing, for example, vascular, bone and tendon/ligament research, and also benefit stent design, providing support during and after surgical anastomosis. This paper also reported an initial observation of fibroblast behaviour on culturing inside the nanopatterned tubes, resulting in the cells adopting a stellate morphology and forming large clusters [17].

The method of internal patterning using polymer demixing is shown here to be applicable to and has more recently been adapted for use with, standard commercially available nylon tubing. Nylon tubing is organic and flexible and transparent and thus is a step towards using a more physiological material. The present study focuses on the response of human fibroblasts (as a cell type representative of those a material would contact in vivo) to islands formed from a PS/PnBMA blend and presented in two tube sizes with IDs of 0.5 and 1.5 mm, respectively. Qualitative and quantitative adhesion and morphological observations were made using light microscopy, scanning electron microscopy (SEM) and fluorescent observation of the cytoskeleton (F-actin, $\beta$-tubulin and vinculin).

## 2. Materials and methods

### 2.1. Nylon tube preparation

Surgical, non-toxic, standard grade nylon tubing (Jencons), 0.5 or 1.5 mm ID, was used as obtained. The purification of the polymers has been described previously [24]. A blend of 20% PS and 80% PnBMA was dissolved in toluene at a concentration of 2% w/w. The polymer blend solution was introduced into the tube, length 50–100 cm, via a syringe and the solution was then blown through the tube by nitrogen at a pressure of 1−1.5 p.s.i. The process is similar to spin casting and leaves a thin layer of solution on the substrate surface that loses solvent rapidly to give a polymer film. With a suitable blend of polymers, the components phase separates and the polymer surface exhibits topographical features. There is less control over the film deposition conditions than with spin casting, so the topography is less uniform than on a flat substrate. Accordingly, the samples used for the cell studies were selected from the middle of the tubing.

A control sample was made by passing a toluene solution of the homopolymer, PnBMA, through the tubing. Cells seeded into the tubing will contact a relatively smooth PnBMA surface in the control tubing or a rough surface covered by PnBMA in the blend-coated samples.

For ease of comparison the four samples will be referred to as indicated in Table 1.

### 2.2. Cell culture

Infinity™ Telomerase Immortalised human fibroblasts (h-TERT-BJ1, Clonetech Laboratories, Inc., USA) were seeded via injection ($1 \times 10^5$ cells per ml medium) inside 3 cm sections of both 0.5 and 1.5 mm diameter tube. Cells were also seeded into sections of

Table 1
Details of sample acronyms and average nanofeature heights for further reference

| Sample type | Sample acronym | Average feature height |
|---|---|---|
| 1.5 mm ID control | 1.5 C | NA |
| 0.5 mm ID control | 0.5 C | NA |
| 1.5 mm ID internal topography | 1.5 IT | ~90 nm |
| 0.5 mm ID internal topography | 0.5 IT | ~40 nm |

ID denotes internal diameter.