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# Investigating filopodia sensing using arrays of defined nano-pits down to 35 nm diameter in size

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**Abstract**

In order for cells to react to topography, they must be able to sense shape. When considering nano-topography, these shapes are much smaller than the cell, but still strong responses to nano-topography have been seen. Filopodia, or microspikes, presented by cells at their leading edges are thought to be involved in gathering of special information. In order to investigate this, and to develop an understanding of what size of feature can be sensed by cells, morphological observation (electron and fluorescent microscopy) of fibroblasts reacting to nano-pits with 35, 75 and 120 nm diameters has been used in this study. The nano-pits are especially interesting because unlike many of the nanofeatures cited in the literature, they have no height for the cells to react to. The results showed that cell filopodia, and retraction fibres, interacted with all pit sizes, although direct interaction was hard to image on the 35 nm pits. This suggests that cells are extremely sensitive to their nanoevironment and that should be taken in to consideration when designing next-generation tissue engineering materials. We suggest that this may occur through nanocontact guidance as filopodia are moved over the pits.


*Keywords:* Nano-topography; Filopodia; Cell sensing; Nanobioscience

## 1. Introduction

There are many chemical, physical and geometric cues within the extracellular environment in vivo that will provide signals to the cells. It has been know for almost 100 years that cells will react to the shape of their environment, and, in 1952, Weiss first used the term contact guidance to describe cell alignment to topography (Carrel & Burrows, 2000; Weiss & Garber, 1952). Due to advances in micro-fabrication, researchers have been able to produce accurate micro-scale substrates upon which to study cells. Thus, the effects of micro-topography on cells are becoming well known and include changes in cell adhesion, contact guidance, cytoskeletal organisation, apoptosis, macrophage activation and gene expression (Britland et al., 1996; Chen, Mrksich, Huang, Whitesides, & Ingber, 1997; Clark, Connoly, Curtis, Dow, & Wilkinson, 1987, 1990; Dalby, Riehle, Yarwood, Wilkinson, & Curtis, 2003; Wojciak-Stothard, Curtis, Monaghan, MacDonald, & Wilkinson, 1996).

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It is possible that cells will also interact with their nano-environment produced by protein folding and banding. There has been some recent research with randomly arranged nano-scale features that have elicited changes in cell adhesion, proliferation, morphology and gene expression. Such techniques include polymer demixing (Affrossman, Henn, O'Neill, Pethrick, & Stamm, 1996; Affrossman, O'Neill, & Stamm, 1998; Affrossman & Stamm, 2000) and colloidal lithography (Denis, Hanarp, Sutherland, & Dufrêne, 2002; Hanarp, Sutherland, Gold, & Kasemo, 2003; Wood, Riehle, & Wilkinson, 2002). In this study, electron beam lithography (EBL) has been used as a method of preparing highly regular nano-topographies. A pattern is generated in a radiation sensitive polymer (resist) by a focused beam of electrons. The achievable resolution for this technique is about 3–5 nm (Cumming, Thoms, Beaumont, & Weaver, 1996; Vieu et al., 2000). The area that can be exposed by an EBL tool without movement of the stage (the field size) is limited by the resolution of feature required. For well-defined 35 nm dots Gadegaard et al. (2003) used a field size of 200 μm × 200 μm. However, larger areas than this are required for cell culture experiments. To make larger areas of pattern, the sample is moved with high precision under interferometric control to allow tiling of the small pattern to fill a larger area (Casey, Monaghan, & Wilkinson, 1997; Wilkinson, Riehle, Wood, Gallagher, & Curtis, 2002).

Fabrication is often a time consuming and expensive process that limits the number of samples available for cell culture experiments. To overcome this hurdle, the nano-patterned samples can be replicated in thermoplastic polymers by hot embossing (Casey et al., 1997; Gadegaard et al., 2003; Heyderman et al., 2001) or injection moulding (Gadegaard, Mosler, & Larsen, 2003). Poly(caprolactone) (PCL), which is easily embossed on a hot plate (Gallagher, McGhee, Wilkinson, & Riehle, 2002), was used in this study. Dimensions as small as 20 nm can be produced in this way (Gadegaard et al., 2003).

One intriguing question is how small a feature can a cell sense? We have set out to start addressing this question using e-beam as a tool to produce ultrafine topography. It seems likely that filopodia are one of the cells' main sensory tools. Gustafson and Wolpert first described filopodia in living cells in 1961 (Gustafson & Wolpert, 1961). They observed mesenchymal cells migrating up the interior wall of the blastocoelic cavity in sea urchins and noted that the filopodia produced appeared to explore the substrate. This led them to speculate that they were being used to gather spatial information by the cells (Wood & Martin, 2002).

Filopodia have been associated with the sensing of chemoattractant gradients (Iijima & Devreotes, 2002; Ueda, Sako, Tanaka, Devreotes, & Yanagida, 2001). Also linked to chemical sensing, osteoblast filopodia have been observed to anchor to hydroxyapatite islands (which is stoichiometrically similar to bone's natural hydroxyapatite) when in a polyethylene (inert) composite matrix (Dalby et al., 2002).

When considering filopodial sensing of topography, fibroblasts have been described as using filopodia to sense and align the cells to microgrooves (Clark et al., 1990). Macrophages have been reported to sense grooves down to a depth of 71 nm by actively producing many filopodia and elongating in response to the shallow topography (Wojciak-Stothard et al., 1996). More recently, fibroblast and endothelial filopodia have been observed to locate to random nano-islands produced by the spontaneous demixing of incompatible polymer blends (Dalby, Riehle, Johnstone, Affrossman, & Curtis, 2002a, 2002b). It was, in fact, noted that as the island size was increased, the filopodia increased in thickness, until fibroblasts appeared almost amoeboid in shape (Dalby et al., 2002a). Whilst distinctly different from the filopodia of the aforementioned cell types, neuronal growth cone filopodia have been described as firstly sensing microgrooves and then aligning neurons to the grooves (Rajnicek, Britland, & McCaig, 1997; Rajnicek & McCaig, 1997; Stepien, Stanisz, & Korohoda, 1999).

Cytoskeletal actin bundles drive the filopodia, and as the filopodia encounter a favourable guidance cue, they become stabilised following the recruitment of microtubules and accumulation of actin in a direction predictive of the future turn if a cell is to experience contact guidance (Lin & Forcher, 1993; Oakley & Brunette, 1993; O'Connor & Bentley, 1993).

Once cells locate a suitable feature using the filopodia presented on the cells leading edge, lamellipodium are formed which move the cell to the desired site. These actions require G-protein signalling and actin cytoskeleton. Rho (involved in stress fibre assembly)