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Measurement of cell motility on proton beam micromachined 3D scaffolds

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Abstract

Tissue engineering is a rapidly developing and highly interdisciplinary field that applies the principles of cell biology, engineering and material science. In natural tissues, the cells are arranged in a three-dimensional (3D) matrix which provides the appropriate functional, nutritional and spatial conditions. In scaffold guided tissue engineering 3D scaffolds provide the critical function of acting as extracellular matrices onto which cells can attach, grow, and form new tissue. The main focus of this paper is to understand cell behavior on micro-grooved and ridged substrates and to study the effects of geometrical constraints on cell motility and cell function. In this study, we found that BAE (Bovine Aortic Endothelial) cells naturally align with and are guided along 3D ridges and grooves machined into polymethylmethacrylate (PMMA) substrates. Average cell speed on micro-grooves and ridges ranged from 0.015 μm/s (for 12 μm wide and 10 μm deep ridges) to 0.025 μm/s (for 20 μm wide and 10 μm deep ridges). This compares with the cell motility rate on a flat PMMA surface where the average cell speed is around 0.012 μm/s. In this work we used scaffolds which were directly written with a focused proton beam, typically 1 MeV protons with a beam spot size of 1×1 μm^2.

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1. Introduction

The new field of tissue engineering can be described as "you start with some building material

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(e.g. extracellular matrix (ECM), biodegradable polymer), shape it as needed, seed it with living cells and bathe it with growth factors''. When cells multiply, they fill in the scaffold and make up new three-dimensional (3D) tissues, and once implanted in the body, cells can recreate their intended tissue functions. With blood vessel attachment to the new tissues, the scaffold dissolves, and the newly grown tissues combine with their surroundings. Tissue engineering can also utilize naturally derived or synthetic, engineered biomaterials to replace injured or defective tissues, such as skin, bone, cartilage and even organs. Today, thanks to this realization and the improving research in this area, some of the simpler tissues, for example skin and cartilage, have already been used in clinical applications [1].

The formation of new capillary branches from pre-existing blood vessels is known as angiogenesis. Endothelial cells (ECs) form the inner surface of a blood vessel; they are surrounded with a layer of extracellular matrix (ECM) and an outer layer of smooth muscle cells (SMCs). The efficient control of the angiogenesis process is essential to help organs build up vessels in bioreactors and cure many diseases caused by either the excessive or insufficient blood vessel formation. Therefore it is necessary to understand the behaviour of ECs which line the blood vessels, not only through biochemical signaling, but also the behaviour of cells constrained in a three-dimensional physical environment.

In tissue engineering, 3D scaffolds act as extracellular matrices onto which cells can attach, grow, and create new tissues. The fabricated scaffolds are usually synthetic polymers. It is believed that in the tissue micro-environment, geometric constraints (i.e. a scaffold) can play a key role in determining the endothelial cell behaviour, such as growth, migration, or death [2]. However, very little work has been carried out on the effect of micro-substrate geometry on cell behaviour because of the general unavailability of precise 3D substrate fabrication techniques. The work that has been performed previously was, in the main, dependent on optical lithography in order to pattern surfaces. Optical lithography is a masked surface machining technique that fabricates a shallow pattern in the substrate (usually silicon). In order to extend the pattern to a 3D structure, reactive etching is required. The whole process is relatively complicated, and therefore not widely available to biomedical researchers. Potentially proton beam (p-beam) writing, as the only direct-write 3D micro-machining process, does not need an intermediate masked step or subsequent reactive ion etching. P-beam writing uses a focused MeV proton beam to write structures directly which have precise 3D geometry and vertical side walls [3,4]. We have previously observed that fibroblast cells attach and grow very well directly on the surface of PMMA, and we have also shown that simple ridges and grooves of different sizes in the micrometer range produced by proton beam writing on PMMA substrates can have significant effect on fibroblast cell behaviour [4].

The aim behind this current project is to fabricate various 3D micro-patterns to investigate the effects of different substrate geometry and matrix material on the behaviour of endothelial cells, in particular migration rates and alignment.

2. Experimental

2.1. Scaffold manufacture

All the micro-machining work presented in this project was carried out using the p-beam writing

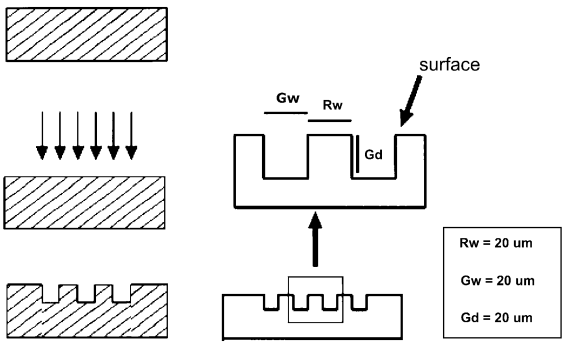

Fig. 1. Schematic representation of the production process of micro-pattern: the dimensions of micro-grooves and ridges are described in terms of groove width (Gw), ridge width (Rw) and groove depth (Gd).