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Orientation of human osteoblasts on hydroxyapatite-based microchannels

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ABSTRACT

The effect of calcium phosphate-based microchannels on the growth and orientation of human osteoblast cells is investigated in this study. As substrates, hydroxyapatite-based microchannels with high contouring accuracy were fabricated by a novel micro-moulding technique. Microchannels obtained by this method featured widths ranging from 16.0 ± 0.7 to 76.6 ± 1.4 µm and depths from 7.9 ± 0.8 to 15.5 ± 1.3 µm. Surface and contour characterization was carried out using X-ray diffraction analysis, scanning electron microscopy imaging and 3D-confocal profilometry. Cell activity and alignment on microchannels with different widths were determined after 1 and 3 days by photometric spectroscopy and fluorescence microscopic imaging and statistically analysed by Tukey's multiple comparison test. On days 1 and 3 for microchannels of width 16 and 30 µm, 70–80% of the osteoblasts oriented within an angular range of 0–15° relative to the microchannel direction. Interestingly, only 20% of the cells grew inside the microchannels for channel widths of 16 and 30 µm. Substrates with channel widths of 45, 65 and 76 µm allowed ~40% of the cells to grow inside. The depth of the microchannel showed hardly any significant impact. All micropatterned surfaces provoked a good cell attachment, as flat and spread cell morphologies with lamellipodiae and filopodiae could already be observed after 1 day. The effect of the microchannels on osteoblast activity was determined using the colorimetric WST-1 assay. In addition, the cell differentiation was assessed by collagen type I staining. The cell activity obtained by WST-1 assay differed insignificantly for all micropatterned samples of various widths and depths. The assessment of collagen type I yielded the same amounts for all micropatterned samples after 1, 3 and 7 days. In summary, the microchannel width of HA-based patterns has a distinct effect on the directed growth of human osteoblast cells, allowing novel design strategies for surfaces such as dental implants.

1. Introduction

Substrate surfaces featuring well-defined micropatterns are of great interest and relevance for biosensor applications [1–3], fluidic systems [4,5], antibacterial surfaces [6] and medical implants such as neural cell stimulating implant surfaces [7–9] and bone implants [10,11]. The use of micropatterned surfaces could be helpful for the improvement of the bone growth into dental implant surfaces, which was shown by Sammons et al., for example, who reported that microporous surfaces on commercially available titanium implants enhanced the spreading of primary calvarial rat osteoblasts during a 30-min attachment period [12]. Further, Hallgren et al. reported significantly more bone-to-implant contact and significantly greater peak removal torque values for micropatterned implants compared with non-patterned control implants *in vivo* [13].

Ceramic materials are suitable for many of these biomedical applications and are well established as implant materials nowadays. The non-oxide ceramic hydroxyapatite (HA) is bioactive and, owing to its relative low hardness, is not used as a load-bearing implant material. However, owing to its similarity to human bone apatite, HA is used in medical implants such as bone grafting materials or coatings for dental implants for increased bone ingrowth [14,15].

It was found in different studies that chemical and topographic modifications of polymers and metals affect cell proliferation, depending on pattern geometries and size. For example, Reichert et al. [16] demonstrated the impact of topography on the attachment of Swiss albino mouse fibroblasts (3T3). Cells do not usually show any adherent contact to polytetrafluoroethylene (PTFE) surfaces, but after micropatterning of the PTFE the fibroblasts attached to the surfaces. In particular, alignment of cells with reference to microchannel formation was reported by several groups [17–24]. For instance, Walboomers et al. [18] reported increased rat dermal fibroblast (RDF) alignment with decreasing groove widths from 10, 5, 2 and 1 µm (depths of 0.5 µm) on polystyrene substrates.

In contrast to polymer and metals, the effects of ceramic surface topographies have not been reported often. A randomized calcium phosphate surface texturing, e.g., roughening of HA by grinding

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