Nanogrooved Substrates Influence Osteoblast Behavior and Extracellular Matrix Deposition
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Introduction
To fight bone diseases characterized by poor bone quality like osteoporosis and osteoarthritis, as well as in reconstructive surgery, there is a need for a new generation of implantable biomaterials with an increased life span it is envisioned that the best way to improve implant surfaces is by mimicking the very organized natural extracellular matrix (ECM) of bone tissue. In this study, osteoblast response to nanometric grooved substrates varying in all three dimensions was analyzed. Several aspects of osteoblast response have been studied, namely: 1. alignment of osteoblast-like cells and focal adhesions to nanometric grooves and 2. the interface between osteoblast-like cells and the substrates.

Materials and Methods
A biochip containing 50 different nanometric templates of 250µm² in surface area was created using electron beam lithography. Uniformly nanogrooved substrates of 2cm² were created using laser interference lithography (LIL) and reactive ion etching. Primary osteoblasts were seeded onto polystyrene replicas of the substrates and cell alignment and morphology were assessed by immune fluorescence microscopy and SEM. Osteoblasts were stained for their filamentous (F-)actin cytoskeleton, the nucleus and/or focal adhesions. Mineral/matrix deposition and interfacial analyses were performed by transmission electron microscopy (TEM), SEM and dual-beam cryo SEM.

Results
Using the biochip replicas, a rapid screening of osteoblast response to the nanopatterns by F-actin fluorescent staining showed that osteoblast aligned to nanogrooves with a width down to 75nm and 35nm depth. On basis of these results, parameters were chosen for large area LIL-derived substrates. Osteoblasts responded identical to these substrates in line with the biochip results. Immunofluorescence microscopy of osteoblast focal adhesions in addition to F-actin showed that alignment of focal adhesions to nanogrooves decreased in a similar fashion as cellular alignment.

Dual beam SEM and environmental SEM studies showed that osteoblasts were able to descend into nanometric grooves down to 150nm width. In addition, calcium phosphate (CaP) was deposited inside and controlled by the nanogrooves. CaP deposition was even visible inside 50nm wide grooves. This was confirmed by SEM and elemental analysis via TEM and energy dispersive X-ray analysis.

Figure 1. Differentiated osteoblast cultured for 16 days on a nanogrooved substrate.

Discussion and Conclusions
Nanopatterns are a very promising tool to direct the biological response at the interface between implant and surrounding bone.

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Disclosures
The authors have nothing to disclose.