Scanning electron microscopical observation of an osteoblast/osteoclast co-culture on micropatterned orthopaedic ceramics

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Introduction/Purpose: In biomaterial engineering, the surface of an implant can influence cell differentiation, adhesion and affinity towards the implant. On contact with an implant, bone marrow-derived mesenchymal stromal cells (BMSCs) demonstrate differentiation towards bone forming osteoblasts, which can improve osteointegration. The process of micropatterning has been shown to improve osteointegration in polymers, but there are few reports surrounding ceramics. Several implants in forefoot surgery like the Moje metatarsal phalangeal joint replacement were made from smoother ceramic zirconia toughened alumina (ZTA).
The aim was to establish whether the pits were specifically bioactive towards osteogenesis or were generally bioactive and would also stimulate osteoclastogenesis that could potentially lead to osteolysis.

Methods: We established a co-culture of bone marrow-derived mesenchymal stromal cells (BMSCs) with osteoclast progenitor cells and to observe the response to micropatterned ZTA ceramics with 30 μm diameter pits compared to smooth controls. Micropatterned masks were fabricated using a standard photolithography and electroplating technique. Micropatterned ceramic substrates were produced by embossing of visco-plastic green ceramic tapes at room temperature followed by sintering. After informed consent was obtained from healthy patients undergoing routine total hip arthroplasty, bone marrow was aspirated from the femoral medullary canal. At day 3, non-adherent cells were removed within the supernatant and cultured separately until a confluent BMSC layer was identified. The co-culture was maintained up to specific time points (days 3, 7, 14, 21 and 28) with thrice weekly media exchange. At each time point, duplicate samples were fixed and prepared for analysis by scanning electron microscopy (SEM) with immuofluorescence and histochemical staining,

Results: SEM analysis of the patterned materials demonstrated successful micropatterning of the ZTA ceramic with approximately 30 μm diameter, 1.7 μm depth pits due to sintering shrinkage. At the longest time-point (day 28), osteoclast-like cells were visible across the planar surfaces and had strong interactions with the ceramic grains. Podosomes were notable on some of the osteoclast membranes. On the pitted surfaces, we observed significantly less osteoclast-like cells. Also, pit bridging by macrophage-like cells was regularly noted. Nodular clusters of osteoblast-like cells were noted statistically more often on the micropatterned ceramics.

Conclusion: These results demonstrated specific bioactivity of micropatterned ZTA ceramics towards osteogenesis, with more bone nodule formation and less osteoclastogenesis compared to planar controls. In addition, we found that that macrophage and osteoclast-like cells did not interact with the pits and formed fewer full-size osteoclast-like cells on the pitted surfaces. This may have a role when designing ceramic orthopaedic foot implants. We are now using this co-culture for research on other micropatterned biomaterials used in foot and ankle surgery.

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