OR–II–2. Effects of calcium ion incorporation on osteoblast gene expression in MC3T3–E1 cells cultured on microstructured titanium surfaces

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Background
Surface chemistry and topography are known to be key factors governing osteoblast cell responses and the eventual bone healing of titanium (Ti) implants. It can be expected that the addition of bioactive surface chemistry to the surface of Ti implants may have synergistic effect on osseointegration when combined with optimal microtopographies. The purpose of this study was to evaluate if the surface calcium chemistry, provided by nanostructured CaTiO3 layer, would contribute to the response of osteoblast–like cells cultured on the microstructured titanium surface, by observing cell proliferation and gene expression of the osteoblastic phenotype using MC3T3–E1 pre–osteoblast cells, which subsequently affect the quality of the osseointegration of Ti implants in vivo. An additional aim was to investigate which surface property, chemistry or topography, plays a more dominant role in osteoblast differentiation.

Materials and methods
The surface characteristics of a calcium ion (Ca)–incorporated Ti surface, produced by hydrothermal, and its effects on osteoblastic differentiation were investigated. MC3T3–E1 cells were cultured on machined or grit–blasted Ti surfaces with and without Ca incorporation. The MTT assay was used to determine cell proliferation, and real–time PCR was used for quantitative analysis of osteoblastic gene expression.

Results
Surface treatment produced a crystalline CaTiO3 nanostructure of approximately 100nm in dimension, preserving original micron–scaled surface topographies and microroughness caused by machining, blasting, or blasting/etching treatments. After
immersion in Hank’s balanced salt solution, considerable apatite formation was observed on all surfaces of the Ca-incorporated samples. Significantly more cell proliferation was found on Ca-incorporated Ti surfaces than on untreated Ti surfaces (P < 0.001). Quantitative real-time PCR analysis showed notably higher alkaline phosphatase, osteopontin and osteocalcin mRNA levels in cells grown on Ca-incorporated blasted surfaces than on other surfaces at an early time point.

Conclusion

Ca incorporation, provided nanostructured CaTiO3, may have a beneficial effect on osseointegration of microstructured Ti implants by accelerating osteoblast proliferation and differentiation during the early healing phase following implantation.