Effect of Different CaP Coatings on Different Surfaces of Titanium and Tantalum on Proliferation and Differentiation of Mesenchymal Stem Cells

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INTRODUCTION

Studies suggest biomimetic or electrochemical CaP coatings onto the surface of the implant will encourage more bone formation, as CaP promotes direct bonding with bone tissue through formation of an apatite layer and it is well known that CaP materials promote MSCs differentiation down the osteogenic lineage. The aim of this study was to investigate the effect of CaP coatings with different particle size that were deposited on different topographic surfaces of titanium and tantalum discs on MSCs proliferation and osteogenic differentiation. The hypothesis was that there is no differences in MSCs proliferation and differentiation between biomimetic and electrochemical coatings on tantalum (Ta) and Ti6Al4V (Ti) discs with polished (P) or sand-blasted surface (SB).

MATERIALS AND METHODS

Pure Ta and Ti discs of 10mm diameter x 2mm thickness were used. Half of them were sandblasted by alumina particles (Al₂O₃) to obtain an average roughness of 4.0µm. Discs were coated by biomimetic method1 (BioM), electrochemical deposition2 at 20 mA/cm² (E20) and electrochemical deposition3 at 6.5 mA/cm² (E6.5). CaP coatings were characterised by SEM, EDAX and XRD. Thickness of coating was measured by embedding the samples in hard grade acrylic resin and analysis by SEM. A study of apatite layer formation was carried out by immersion in Simulated Body Fluid (SBF) and analysis by SEM and EDAX. For this study, a pure hydroxyapatite (HA) disc and an uncoated Ti discs were used as controls.

Ovine MSCs were isolated from bone marrow aspirates using Ficoll gradients, expanded in DMEM with 1%antibiotics-10%fetal calf serum (DMEM+) and characterised by demonstrating their multipotency differentiating them down the adipogenic and osteogenic lineages3. Cells were seeded on the discs and cultured in DMEM+ for 4, 7 and 14 days. Four coating groups were analysed: uncoated (control), BioM, E20 and E6.5. Each group comprises PTa, SBTa, PTi and SBTi surfaces (n=3). oMSCs on Thermax discs in either DMEM+ or osteogenic medium acted as controls. Proliferation were analysed by AlamarBlue and DNA assays, differentiation in osteoblasts precursors was measured using ALP assay.

RESULTS

BioM coatings barely covered the discs surfaces and exhibited globular morphology composed of nanoparticles (less than 0.1µm), arranged in globules (Fig. 1A). On the other hand, E20-E6.5 completely covered the discs and had different morphologies and crystal sizes (Fig. 1B). EDAX and XRD results showed that all the coatings were Ca deficient and composed of amorphous phases. Coating thickness was higher for E20-E6.5 than for BioM and slightly higher for E20 than E6.5. Variation in thickness for SB discs was slightly higher than for P discs. A clear change in morphology was observed after immersion of the CaP coated discs in SBF for up to 7 days (Fig. 1C and D). Deposition of a mineral phase over time was observed on the surface of controls. EDAX showed that Ca and P were still the main elements and Na, Cl and Mg were present for all coatings and HA disc. Only Mg was present in the Ti disc apart from Ca and P. The results suggested that the coatings dissolved when immersed in SBF and subsequently mineralised incorporating Na, Cl and Mg.

DISCUSSION

Different particle-sized CaP coatings were deposited on different topographical metal surfaces using biomimetic and electrochemical methods. When MSCs were cultured on these coatings, the nano-sized crystals of the biomimetic coatings provided the best conditions for cell proliferation compared to the electrochemical ones and the uncoated discs. Cells proliferated more on P discs than on SB ones. All the coatings induced osteogenic differentiation of MSCs, which was greater on electrochemical coatings and complex topographies.

REFERENCES

3) Pittenger et al. Science 1999; 284:143-147