Increased Osteoblast Adhesion on Nano-structured Anodized CoCrMo

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Introduction: Cobalt-based alloys are one of the three groups of metallic materials widely used in orthopedic applications. As with any other metallic implants, CoCrMo used in orthopedics require macro-scale roughness to achieve mechanical fixation and possibly nano-scale roughness to optimize bone growth at the tissue-implant interface. It has been reported that compacts composed of nano-particulate CoCrMo increased osteoblast adhesion compared to conventional or micro-particulate counterparts [1]. Electrochemical methods are popular to create nanometer surface features since it is quick, low-costing and versatile. Indeed, recent studies revealed the possibility to use hydrofluoric acid (HF) as an electrolyte to anodize titanium, creating a thick (more than the natural) oxide layer with nanotopographies that increase bone cell functions [2, 3]. For the above reasons, the objective of the present study was, for the first time, to anodize CoCrMo and determine osteoblast adhesion on such materials.

Methods: A home-made electrochemical cell used in this study was described elsewhere [3]. Briefly, to induce nanometer roughness a constant voltage of 10 V was applied to the electrodes and kept for 2 minutes. The original and resulting surface morphology of the CoCrMo was observed using FE-4800 Field-Emission Scanning Electron Microscope (FE-SEM, HITACHI). All samples were cleaned, dried and sterilized before cell adhesion experiments. Etched glass was used as a reference material in cell experiments. Human osteoblasts (ATCC, population number 7~8) at a density of 3500 cells/cm^2 were seeded into a 12-well cell culture plate containing each sample (unanodized and anodized CoCrMo) in 2 ml Dulbecco's Modified Eagle Medium supplemented with 10% Fetal Bovine Serum and 1% penicillin/streptomycin (all chemicals from Gibco). The samples were then incubated under standard cell culture conditions for 4 hours. After that time period, non-adherent cells were rinsed away while the adherent cells were fixed, stained (Hoescht 33258 dye, Sigma) and counted in five random fields under a fluorescence microscope (Leica).

Results / Discussion: After electrochemical treatment, the original CoCrMo surface was covered by a grey oxide layer. This layer was very stable and resistant to attack by nitric acid and hydrofluoric acid. The composition of the newly-formed layer is probably a mixture of chromium oxide, cobalt oxide and molybdenum oxide. Figure 1 shows the SEM micrographs of CoCrMo samples before and after anodization at different magnifications. It can be seen that the original surface had micro-scale scratches and cracks probably due to mechanical processing but was mainly smooth at the nano-scale. On the contrary, the anodized samples possessed a very rough surface in the micro-scale as well as a porous structure within the nano-scale. The results of osteoblast adhesion tests showed significantly ($p<0.01$) increased osteoblast numbers on anodized compared to unanodized CoCrMo (Figure 2). This result agrees with other studies on anodized titanium and aluminum with nano-rough surfaces [3, 4].

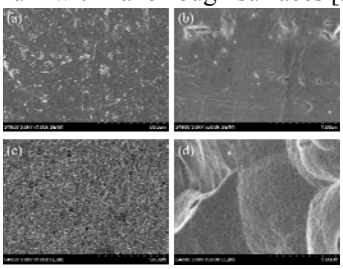

Fig.1 SEM micrographs of unanodized CoCrMo under low (a) and high (b) magnifications and anodized CoCrMo under low (c) and high (d) magnifications. Bars = 50 μm in (a) and (c), bars = 1 μm in (b) and (d).

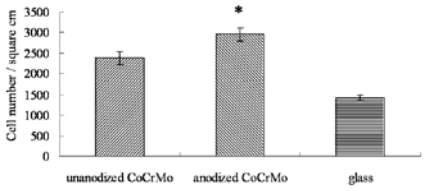

Fig. 2 Increased osteoblast adhesion on anodized CoCrMo compared to unanodized CoCrMo. Data = mean ± SEM, n =3; $p < 0.01$ compared to unanodized CoCrMo.

Conclusions: Results of the present in vitro study add anodized CoCrMo with ananostructured features as another nanophase material which increases osteoblast adhesion. Since osteoblast adhesion is a necessary function before they can deposit calcium, the present results also imply enhanced subsequent functions on anodized, nanostructured CoCrMo.

References:

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