INTEGRATION OF TRACE ELEMENTS INTO CALCIUM PHOSPHATE COATINGS ON TITANIUM AND THEIR CHARACTERIZATION *IN VITRO*

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Introduction

The aim of this study was to combine the trace elements copper and strontium with inorganic surface modification techniques already successful in bone applications. Copper ions released from bone implant coatings are known to enhance vascularization [1] while the incorporation of strontium is intended to stimulate bone forming and simultaneously suppress bone-resorbing cells in the implant vicinity. Calcium phosphate phases (CPP) were utilized as a carrier for the selected trace elements in order to i) provide osteoconductive surfaces and ii) to tune the immobilized amounts and release behavior of the trace elements.

Materials and Methods

Deposition of brushite onto c.p.Ti discs was performed by means of electrochemically assisted deposition (ECAD) from aqueous electrolytes containing $Ca(NO_3)_2$, $NH_4H_2PO_4$ with or without additional $Sr(NO_3)_2$. Cu integration was realized by three approaches: i) galvanostatically prior to the CPP deposition, ii) addition of $Cu(NO_3)_2$ to the electrolyte and iii) adsorption after the ECAD process.
The coatings were characterized by chemical analysis after dissolution in 0.1 M HNO_3. Ion release from coated surfaces was analyzed after incubation in simulated body fluid (SBF) with or without 10% fetal bovine serum (FBS). Human monocytes were isolated from buffy coats by $CD14^+$ labelling [2]. Their ability to differentiate into osteoclasts was studied for cultivation on coated samples differing in their trace element content as well as to cells cultured on tissue culture polystyrene (TCPS) for application of several combinations of trace elements in solution. Impact of the same trace element solutions onto osteogenesis was studied with human bone marrow stromal cells (hMSC).

Results and Discussion

If Cu and Sr ions were added during the ECAD process their deposited amount depended mainly on the concentration of the respective ions in the electrolyte. While addition of 0.5 mM Cu resulted in up to ~100 µg Cu/cm² a comparatively high Sr concentration (40 mM) was necessary to obtain a similarly high Sr deposition. While Cu adsorption was limited to ~50 µg/cm² for coatings composed of ~1000 µg/cm² brushite content, the galvanostatic Cu deposition could be increased up to several mg/cm². No Cu release was detected for incubation of all types of Cu containing coatings in serum-free SBF, while a permanent Sr release was observed in serum free SBF. In contrast, in presence of 10% serum considerable amounts of Cu (50% and 54%, respectively) were released if Cu was adsorbed or deposited as base layer within the first 24 h. Sole incorporation of Cu during the ECAD process (method ii) could reduce initial release to ~13%. However, co-deposition of Cu and Sr by the same method ii resulted in an accelerated release of Cu with 89% being detected within the first 24 h, Sr release on the other hand was nearly unaffected.
For single Sr incorporation the release behavior in presence of serum was comparable to serum free solutions for the first 24 h, but dropped to about one third of the respective amount in serum-free SBF irrespective of the initial Sr content. Continued steady release was monitored for up to 22 d.
In the first cell studies all coatings containing 100 µg/cm² Cu or more prevented the adhesion of monocytes as well as of hMSCs. The presence of Sr did not affect monocyte but clearly improved hMSC adhesion.
The impact of several combinations of Cu and Sr in solution was studied for both cell types. The presence of low concentrations of Cu (beginning from 25 µM) resulted in an increased proliferation of hMSC without osteogenic supplements that was even enhanced for application of both ions together. However, in presence of osteogenic supplements even the lowest concentration of Cu (25 µM), that was only slightly above the serum level, resulted in extreme reduction in cell number.
If monocytes were cultured in presence of Cu these ions provoked decreases in adherent cells for concentrations >50 µM while Sr had no impact on cell adherence. For both ions already at 25 µM a change in osteoclast cell fusion capability was observed (FIG. 1). The activity of tartrate resistant acid phosphatase was not affected by Sr for up to 1000 µM but increased by Cu even for the lowest concentration of 25 µM.

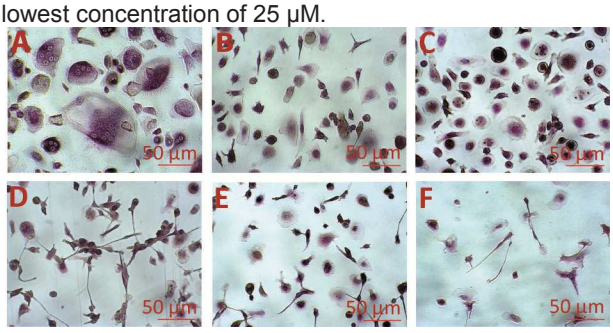

FIG. 1. TRAP staining of human monocytes after 8 d culture on TCPS with addition of A) no trace elements, B) 25 µM Sr, C) 1000 µM Sr, D) 25 µM Cu, E) 25 µM Cu & 25 µM Sr, F) 25 µM Cu & 1000 µM Sr.

It was shown that Cu and Sr can be co-deposited together with calcium phosphate onto metallic implant materials by ECAD. The deposited amount of these ions is tunable in a wide range. The combination of Cu and Sr seems to provoke a suppression of osteoclast differentiation while impact on hMSC depends on the presence of osteogenic supplements. Current investigations focus on fine-tuning of the deposited Cu amount to achieve optimal balance for osteogenic differentiation and osteoclast suppression.

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