MULTIFUNCTIONALIZATION OF INERT CERAMIC SURFACES USING IN SITU CAP NUCLEATION

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Introduction

Bioinert ceramics such as alumina or zirconia have been commonly used in the field of orthopedics and dentistry due to its excellent mechanical properties, esthetic look, good biocompatibility and chemical inertness in biological environment. Activation of its bioinert surface could bring additional advantages for better implant-integration with surrounding tissues *in vivo*. Therefore, the aim of the present study was to develop an innovative biomimetic co-precipitation technique by using modified Simulated Body Fluid (SBF) to obtain a composite coating made of organic and non-organic components enhancing a bioactivation/functionalization of this inert biomaterial.

Materials and Methods

Zirconia samples were biomimetically coated by immersion in double-concentrated SBF-solution prepared according to Tanahashi *et al.* and kept at a body temperature for 3d [1].
Bovine Serum Albumin (BSA) has been chosen in 5 different concentrations (0.01, 0.1, 1, 10, 100 gL^{-1} and 0 gL^{-1} as a control) as a standard protein to be incorporated into the CaP-coating during the precipitation process. The incorporation of BSA into the SBF solution occurred on the half of the samples directly ("direct" coating) and for the other half on samples already pre-coated with SBF ("with pre-coating").
BSA/Alexa Fluor TM 488 conjugates were applied to visualize the incorporated proteins into the surface. To evaluate a role of sedimentation of protein in the solution, the coating produced on horizontal and vertical samples were compared. Samples were imaged by using fluorescence microscope. To determine the morphological changes on the substrate surfaces after soaking in SBF, scanning electron microscopy was applied. Carbon-content of the HAp-coating dependent on concentration of BSA in the solution were established by using EDX measurements. Moreover, the thickness of HAp-coatings could be measured by imaging of cross-section of ZrO_2-substrates.

Results and Discussion

The control samples (0 gL^{-1} BSA) as well as samples coated in SBF-solution containing 0.01 gL^{-1} BSA exhibit typical coral-like crystal structures [2] with app. 100 nm long crystal-plates. In contrast, with BSA-concentrations >0.1 gL^{-1} the crystal structure appears to be altered or protein-overlayed (FIG. 1).
The incorporation of protein within the HAp-coatings was visualized by using fluorescence microscopy to detect BSA/Alexa-FluorTM-488 conjugates, which gives a green signal. The intensity of green signal is stronger with increasing protein concentration in the solution. Additionally, the content of carbon was measured by EDX. The results show a logarithmic growth of carbon content in the HAp-coating with increasing BSA concentration in SBF solution by the precipitation process (FIG. 2).
The influence of the sedimentation process on the intensity of fluorescence signals proportional to the amount of proteins in the coating could also be observed. The results were in correlation with the chemical analysis of the coated surfaces (EDX).
Analysis of the cross-section of the obtained coating on CaP-pre-coated samples showed the apatite growth for all tested samples in comparison to the pre-coated control sample. The thickness of the coating decreases with the increase of protein concentration in the solution, which is in correlation with the SEM images.

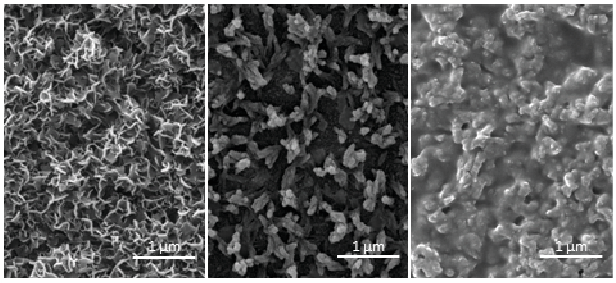

FIG. 1. Morphology changes through different BSA concentrations.

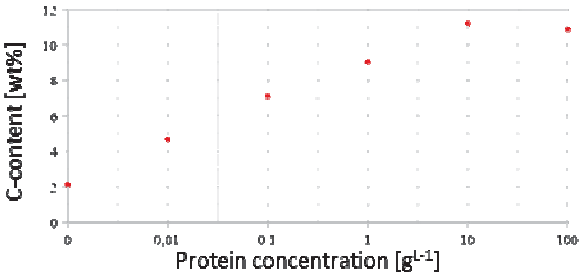

FIG. 2. Measurement of Carbon content by EDX.

Conclusions

It could be shown, that it is possible to co-precipitate an organic/non-organic coating based on HAp and biological agents such as BSA. This method could create a new biomaterial group, which surfaces could be tailored designed according to its desires and requirements. Based on these results with a standard protein, BSA has been replaced by specific proteins like Bone Morphogenetic Protein 2 (BMP-2) as a potential osteoinductive factor and Hepatocyte Growth Factor (HGF) as a growth factor. These proteins have already evidenced a strong influence on the crystal growth and the HAp-coating morphology as well. Further systematic analyses and cell culture tests are still on going in order to better understand biologically efficacy or bone growth factor response of the protein incorporated into the CaP-coating.

Acknowledgments

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