Cellular and Bacterial response to Bioactive and Antibacterial Chemically-modified Titanium and Bioactive glass surfaces

92 **Novel biomimetic anti-adhesive implant coatings based on tetraether lipids** (#218)

A. Scholte[1], M. Frant[1], R. Schade[1], G. Hildebrand[1], K. Liefeith[1]

93 **Electrophoretic deposition of a metal-ceramic coating containing MCrAlY (M:Ni,Fe) bond coat for cardiovascular implants.** (#250)

N. Grigorev[1], S. Neuss-Stein[2, 1], N. Labude[2], R. Telle[1], K. Schickle[1, 2]

94 **Intracellular calcium-ion mobilization on charged titanium surface modifications** (#291)

M. Grüning[1], J. Lehnfeld[2], T. Distler[3], K. Fricke[4], M. Dubs[5], C. Völkner[6], R. Müller[2], M. Schnabelrauch[5], A. R. Boccaccini[3], S. Speller[6, 7], B. Nebe[1, 7]

95 **Surface modification of zein-based composite coatings** (#338)

L. Ramos Rivera[1], A. R. Boccaccini[1]

96 **Cellular and Bacterial response to Bioactive and Antibacterial Chemically-modified Titanium and Bioactive glass surfaces** (#341)

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Introduction

Bone repair is a complex process whose success is based on a continuous and definite ions exchange at the interface between the tissue itself and the implantable substitute. Accordingly, there is nowadays a large research aimed to improve the bioactivity of the most employed bone substitutes such as bioactive glass and titanium (Ti) alloys [1,2]. However, an external factor that can hinder the healing process is represented by bacterial infection. In fact, infections are currently the most problematic reason of prosthetic failure due to the high bacteria antibiotic resistance [3]. So, it is evident that the design of bone repair dedicated biomaterials must include both bioactive and antibacterial properties. Based on these premises, here different surface chemical treatments were applied onto Ti alloys and bioactive glasses in order to improve and faster apatite formation by stimulating the microenvironment chemistry or to enhancing ions exchange. Moreover, silver (Ag) was introduced in the above-mentioned treatment in order to provide a strong and broad-range antibacterial activity [4].

Experimental Methods

Ti6Al4V alloys and a SiO_2-Na_2O-CaO-P_2O_5-B_2O_3-Al_2O_3 bioactive glass were applied as bare materials for further modifications. Ti alloys (named Ti64(Sr-Ag)) were first soaked in a 5M NaOH solution and then in a 50 mM $CaCl_2$ and 50 mM $SrCl_2$ mix solving. Afterwards, specimens were heated at 600°C (1 hour) and soaked once more in a 1M $Sr(NO_3)_2$ solution doped with 1 mM $AgNO_3$ aimed to introduce silver. The bioactive glass samples (named SBA2-Ag) were soaked in a 30 mM $AgNO_3$ solution to incorporate silver ions too. Specimens' physical-chemical characterization was performed by means of FESEM and XPS, while apatite formation was evaluated by soaking in body simulated fluid (SBF) [5]. Specimens' cytocompatibility was evaluated by means of metabolic activity in direct contact with human osteoblasts progenitors (hFOB 1.19) that were selected as representative for cells deputed for bone self-healing. Then, antibacterial activity was tested against a multi-drug resistant *Staphylococcus aureus* strain biofilm by applying both a well-established protocol from

literature [6] and the ISO 22196 standard to compare results. Finally, specimens' ability to protect cells from infection was evaluated by 3 co-culture systems: (i) cells were pre-seed onto specimens' surface and then infected, (ii) bacteria were pre-applied to infect specimens' surface and then cells were plated, and (iii) bacteria and cells were applied together onto specimens' surface to simulate a "road to the surface" competition.

Results and Discussion

Surface morphological analysis done by FESEM showed a nano-textured surface for Ti64(Sr+AG) specimens and a smoother one for SBA2-Ag. XPS analysis confirmed that Ag was successfully introduced onto both Ti64(Sr+AG) and SBA2-Ag surfaces as ions. Apatite formation was correctly observed for both Ti alloys and bioactive glass; in particular, the latter resulted as faster showing apatite after 1 day. Biological evaluations are summarized in Figure 1; ISO 22196 standard and methods 1 and 2 were used as representative examples for antibacterial and co-cultures results. Ag-doping did not cause any toxic effect as cells metabolism was comparable between treated and control (cnt) specimens (Fig. 1a, $p>0.05$). On the opposite, Ti64(Sr-Ag) and SBA2-Ag showed a marked antibacterial activity as the *S. aureus* biofilm viability was significantly decreased by comparing Ag-doped Ti alloys and SBA2-Ag with their bare counterparts (Fig. 1b, $p<0.05$ indicated by §). Moreover, this strong antibacterial effect was effective in cells viability preservation in co-culture models. In fact, both that infection was applied after (method 1) or prior cells seeding (method 2), the number of viable cells onto Ag-doped surfaces was significantly higher then what observed in the controls (Fig. 1c, $p<0.05$, indicated by § and #, respectively).

Conclusion

Both Ag-doped Ti alloys and bioactive glass obtained by the here described surface chemical treatments can be considered as very promising for bone tissue engineering due to their strong bioactivity and antibacterial properties.

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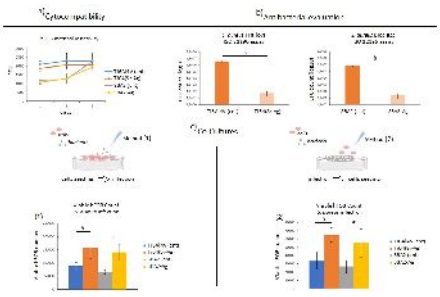

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Ag-doping did not turned specimens to toxicity as no differences were noticed between bare (cnt) and treated specimens in terms of cells metabolism (a). On the opposite, both Ag-doped Ti alloys and SBA2-Ag determined a significant reduction of bacteria viability (b, $p<0.05$, indicated by §). The antibacterial activity was confirmed by co-culture systems where the number of viable cells in presence of bacteria was significantly higher for Ag-doped materials (c, $p<0.05$, indicated by § and #, respectively).