In vitro surface reactions underlying bone bioactivity of calcium-alkali-orthophosphate bone grafting materials

C. Knabe*,**, J. Kim, C. Chen, V. Meausoone, S. Radin, P. Ducheyne
Center for Bioactive Materials and Tissue Engineering, Dept. of Bioengineering, University of Pennsylvania, Philadelphia, PA,
* Division of Orthopaedic Research, Thomas Jefferson University, Philadelphia, PA, ** Dept. of Experimental Dentistry,
Charité University Medical Center Berlin, FRG

Introduction: The ability to bond to bone tissue and to stimulate bone formation is a unique property of bioactive calcium phosphate ceramics (1, 2). This has led to their wide clinical use in both orthopaedics as well as dentistry. A key element of bone bioactive behavior of these ceramics is the development of a carbonated apatite surface in biological fluids and its influence on fibronectin (Fn) adsorption. Recently, calcium alkali orthophosphate materials were developed to address the significant need for greater resorption rates without jeopardizing bioactive behavior. In this study, we report on 1) the kinetics of formation of bioactive surface reaction layers; 2) Fn adsorption on theses ceramic surfaces.

Methods: The test materials were two calcium alkali orthophosphates with a crystalline phase $Ca_2KNa(PO_4)_2$ and with a small amorphous portion containing either magnesium potassium phosphate (GB14) or silica phosphate (GB9). These materials were compared to β-tricalcium phosphate (β–TCP). Three types of solutions were used for the *in vitro* evaluation, including 0.05M tris buffer (T), T supplemented with physiological electrolytes (TE), and TE supplemented with 10% fetal calf serum (TES). All discs were immersed at a surface area-to-solution volume ratio of 0.1 cm^{-1}. Post-immersion solutions were analyzed for changes in Ca concentrations using atomic absorption spectrophotometry (AAS). After 21 days of incubation the structure and composition of the surface reaction layers were determined using Fourier transform infrared spectroscopy (FTIR).
Fn adsorption to these ceramics after immersion in the 10% fetal bovine serum-containing tissue culture medium was determined using Western blot and was quantified using Fuji Image Reader.

Results/Discussion: When immersed in T, all materials tested showed a time-dependent dissolution behavior. The rate of Ca release from GB9 and GB14 was comparative to that of β–TCP. However, when immersed in TE, the behavior of samples changed dramatically. Decrease in cumulative ion concentration indicates uptake of Ca ions. β–TCP and GB9 showed their time-dependent uptake of Ca ions. GB14 displayed only a minor uptake. In contrast, after immersion in TES, β-TCP and GB14 had a minor uptake whereas GB9 did not show release or uptake.
FTIR spectra of GB14 before and after immersion in T and TE (Fig. 1) showed that bioactive surface transformations of this material occurred in both solutions. GB9 also showed surface transformations in both solutions.
The relative amount of Fn adsorbed onto the surface of each ceramic is shown in Fig. 2. The greatest adsorption was observed on β–TCP (1.8 folds of GB14) followed by GB9.

Conclusion: Surface characteristics, dissolution and precipitation reactions of calcium alkali orthophosphate materials were tested. Both GB9 and GB14 displayed the desirable dissolution properties and the bioactive surface transformations. In addition, amount of Fn adsorbed onto the surfaces of GB9 and GB14 was comparative to that of β–TCP. These findings are in accordance with the results of previous studies regarding the effect of GB9 and GB14 on osteoblast differentiation *in vitro* and *in vivo*, in which GB9 exhibited the greatest stimulatory effect on osteoblast differentiation and bone formation (3-5)

Figure 1. FTIR spectra of GB14 before and after immersion in T and TE for 21 days

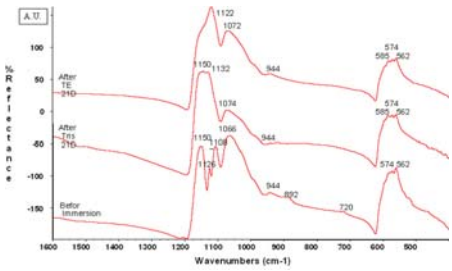
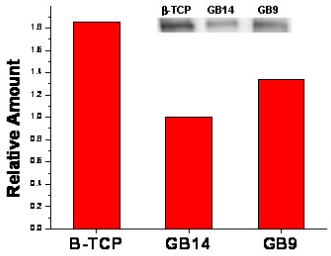


Figure 2. Level of Fn adsorbed after 24h of immersion in serum containing tissue culture medium



Acknowledgements: This work was funded by the German Research Foundation (DFG Grant KN 377/5-1), the Osteology Foundation and the NIH (5-RO1-AR051303).

References

[1] Ducheyne P. Biomaterials. 1990;20:2287-303.
[2] Hench LL. None-Implant Interface. 1994;9:181-90.
[3] Knabe C. JBMR A. 2008;85:856-68.
[4] Knabe C. Trans SFB. 2007;32:262.
[5] Knabe C. Trans 8th WBC. 2006;p.035.