Early Interaction of Biomaterials with Dynamic Simulated Body Environment

J. Strnad[1], Z. Strnad[1], J. Protivínský[1], A. Helebrant[2]

in Proc. 5th Asian Symposium on Biomedical Materials (Yasuhiko Tabata ed.), Hong Kong University of Science & Technology, Hong Kong 2001, pp. 51-55

Introduction

Bioactive materials like bioglass, certain glass-ceramics or alkali-treated titanium bond to living bone through an apatite layer, which is formed on their surfaces after implantation[1]. The process of apatite nucleation and crystal growth is initiated by an ion interaction between bioactive material and surrounding blood and it can be reproduced in simulated body fluid (SBF)[2]. The exposure in SBF is usually carried out as a static experiment, where a sample is placed in the SBF solution of a constant volume, or the solution is periodically renewed[3]. If the solution volume is sufficiently high to keep approximately constant solution composition it is difficult to detect compositional changes in the solution caused by the material-SBF interaction. If the volume is small, concentration changes are measurable but in case of apatite formation the driving force for nucleation and crystallization decreases as calcium and phosphate ions are consumed from the solution. Static exposure also does not resemble the conditions at implantation, where blood flow always occurs. Therefore a dynamic exposure is suggested to keep constant composition during the experiment and to detect compositional changes in SBF during the initial stages of interaction.

Materials and Methods

In the experiment we used samples of bioactive chemically treated titanium (Ti-bio), sintered hydroxyapatite (HA1100) and dried hydroxyapatite (HA120) granulates. As reference bioinert materials we used c.p.titanium (grade 4) and silica glass granules. Ti-bio samples were prepared from titanium turnings, washed in isopropanol, dried at 120°C, acid etched in 2%HF and then treated in alkaline solution. C.p. titanium reference samples were pure Ti turnings without further treatment. Samples of hydroxyapatite were prepared as precipitates in aqueous solution, dried at 120°C (HA120) and subsequently sintered at 1100°C (HA1100). The grain size was in the range of 0,6-1 mm. Surface area of samples was measured by krypton adsorption using B.E.T. isotherm, porosity was determined by mercury porosimetry (Tab. 2).

Tab. 1: Surface area and porosity of used samples.

surface area m^2/g			
Ti-bio	c.p. Ti	HA120	HA1100
turnings		granulate	
0,4392	0,0013	93,69	0,002
	porosity %		
-	-	65	7

Samples were exposed in SBF having ion concentrations given in tab 2.

Tab. 2: Composition of SBF used in experiments

SBF	mmol/l
Na^+	142
K^+	5
Ca^{2+}	2,5?
Mg^{2+}	1
Cl^-	131
HCO_{3-}	5
SO_4^{2-}	1
HPO_4^{2-}	1

The solution was prepared using following reagents: KCl, NaCl, $NaHCO_3$, $MgSO_4*7H_2O$, $CaCl_2$, TRIS, NaN_3, KH_2PO_4. TRIS buffer was used to adjust the pH to 7.55-7.60 at 25°C. Sodium azide was added to inhibit bacterial growth. Samples were exposed in a single pass flow through reaction cell with SBF preheated at 37°C (Fig.1). The flow rate of SBF solution through the cell was 0.042ml/min. Samples of the output solution were collected for calcium and phosphate ion concentration measurement. Ca concentration was determined by atomic absorption spectroscopy, phosphates were determined spectrophotometrically. After exposure surface of titanium samples was analyzed using energy dispersive spectroscopy (SEM-EDS).

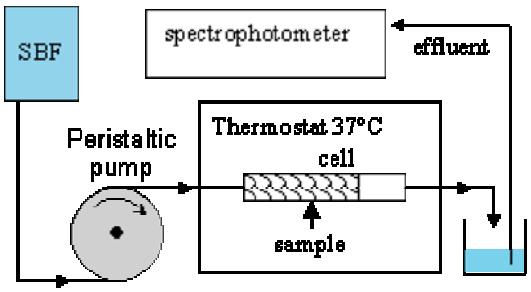

Fig. 1: Experimental arrangement of the flow-through SBF test

In the series of preliminary experiments we studied the effect of the ion exchange reactions (measured in flow through test) on a living cell culture. The culture medium consisted of Hank's balanced salt solution (HBSS, Eagle MEM) and bovine serum. Bone marrow cells extracted from chicken long bones were seeded in the medium. After addition of samples, morphology and population of living cells was observed.

[1] LASAK, Ltd, Papírenská 25, Prague 6, Czech Republic

[2] Institute of Chemical Technology, Technická 5, Prague 6, Czech Republic

Results

The time dependence of Ca^{2+} and PO_4^{3-} ion concentration in the output solution, measured after exposure of Ti-bio samples indicated three main phases of interaction (Fig 2.). First, a rapid but temporary decrease of calcium and phosphate concentration in the effluent was detected. This decrease indicated a significant adsorption of Ca^{2+} and PO_4^{3-} ions by the material. During the second phase concentration returned almost to its initial value (100 mg/l Ca^{2+}, 96 mg/l PO_4^{3-}). After approx. 25hours another concentration decrease was detected indicating the starting crystal growth. The output concentration stabilized at the level of 60-63mg/l of PO_4^{3-}, indicating crystal growth at the constant rate.

During exposure of bioinert samples (c.p.Ti and silica glass) no changes in the effluent concentration were detected (fig.2,3).

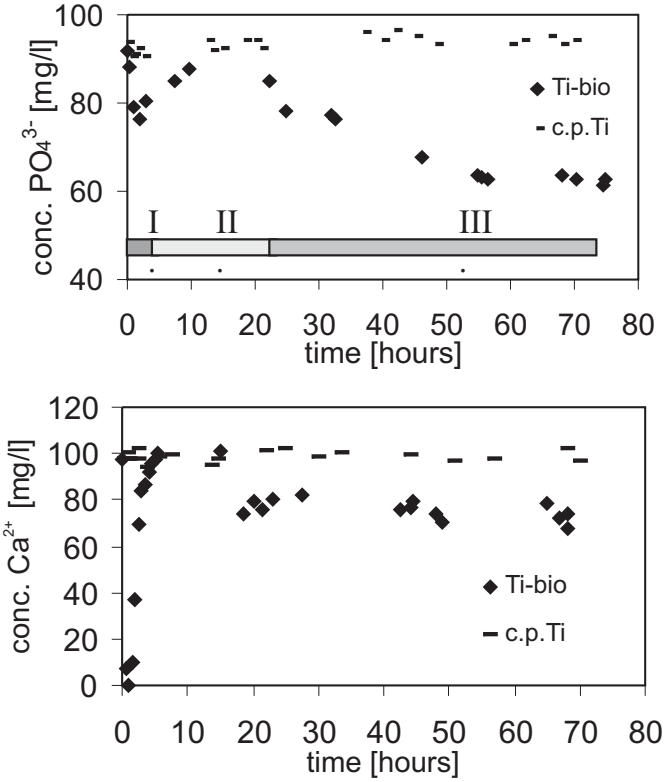

Fig. 2: Time dependence of the PO_4^{3-} and Ca^{2+} concentration in the output SBF solution during exposure of Ti-bio and c.p.Ti sample, indicating three phases of interaction

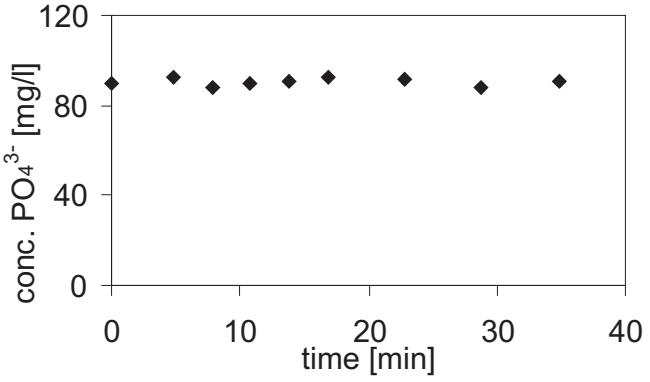

Fig. 3: Time dependence of the PO_4^{3-} concentration in the output SBF solution during exposure of silica glass granulate

The time dependences plotted after exposure of hydroxyapatite samples had similar shape as those of Ti-bio samples but apatite precipitation on hydroxyapatite samples exhibited apparently shorter induction periods (Fig 4).

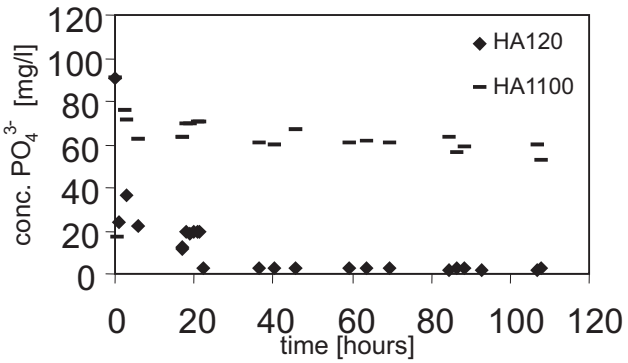

Fig. 4: Time dependence of the PO_4^{3-} and Ca concentration in the output SBF solution during exposure of HA120 and HA1100 samples

Titanium samples exposed in SBF were analyzed using SEM-EDS. Apatite crystals formed on the surface of Ti-bio sample are shown in fig. 5. Fig. 6 shows the EDS semi quantitative analysis determining the Ca/P molar ratio as 1,74. Table 3 shows results of EDS analysis of Ti-Bio and c.p.Ti samples after exposure in SBF.

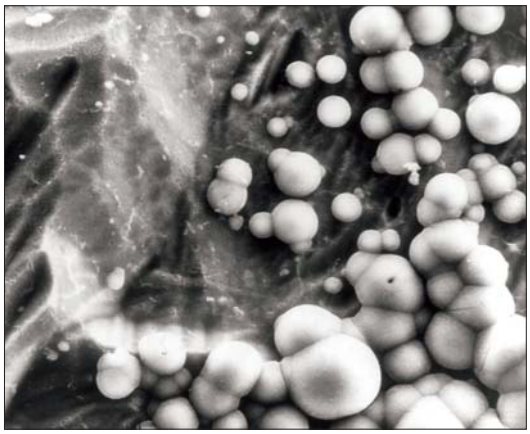

Fig. 5: SEM image of apatite crystals formed on the surface of Ti-bio sample after 74 hours of dynamic exposure in SBF (magn: 1000x)

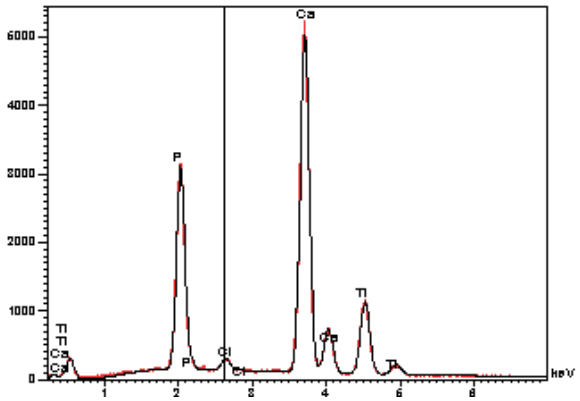

Fig. 6: EDS analysis of apatite crystals formed on the surface of Ti-Bio sample after 74 hours of dynamic exposure in SBF

Tab. 3: Amount of calcium and phosphorus present in the surface of of Ti-bio and c.p. Ti sample after 2 minutes, 20 and 74 hours of dynamic exposure in SBF (Analyzed by EDS, the residual elements are Ti and O)

at. %	Ca	P	Ca	P
2min.	1,4	ND	ND	ND
20h	6,7	0,7	ND	ND
74h	23,0	13,2	-	-
				ND - not detected

Discussion

In the case of Ti-bio samples, measured curves of the output solution concentration vs. time showed a significant Ca^{2+} and PO_4^{3-} ion retention by the sample within first minutes of interaction (Fig.2). This can be caused by ion adsorption onto the hydrated bioactive surface of sodium titanate gel layer formed on the surface of titanium after alkali etching. This finding is in agreement with previous result of Kokubo et.al.[4], who detected calcium by TEM-EDX on the surface of alkali-treated titanium after 0.5h of exposure in SBF. The presence of calcium and traces of phosphorus in the surface before apatite crystal growth starts was proved even by relatively surface insensitive EDS analysis (Tab.3). The effect is definitely supported by approx. 2 orders of magnitude higher surface area of Ti-bio samples compared to c.p.Ti (tab.1). On the other hand no calcium was found on the surface of c.p. Ti exposed in SBF for 2 minutes and 20hours (tab.3) and also no apatite crystal growth was detected after 74 hours. Similar inert behavior exhibited silica glass granulate (fig.3) exposed in SBF.

The fast adsorption was also detected during exposure of hydroxyapatite samples. In this case, the adsorption was immediately followed by apatite crystal growth and the induction time was much shorter than in the case of Ti-bio samples (tab.4). It can be assumed that the rapid adsorption of Ca^{2+} and also PO_4^{3-} ions from SBF is the first step of bioactive behavior. The induction time after which crystal growth starts depends on the interfacial energy between the precipitating phase and the substrate [5,6,7] and it can be used to quantify bioactivity of the studied substrate. The extremely long induction period for apatite formation on c.p.titanium determined by Li and Ducheyne[8] may indicate a material, which stands on the border of bioactive and bioinert materials and under certain conditions exhibits so called osseointegration.

Tab. 4: Induction time values for samples Ti-Bio, HA120 and HA1100

	HA120	HA1100	Ti-Bio
Induction time (h)	3,8	2,5	24,5

The dynamic experimental arrangement enabled simple determination of the induction times using a plot of precipitated PO_4^{3-} weight vs. time and by extrapolation of its linear part to the zero weight (Fig 7,8). The value of induction

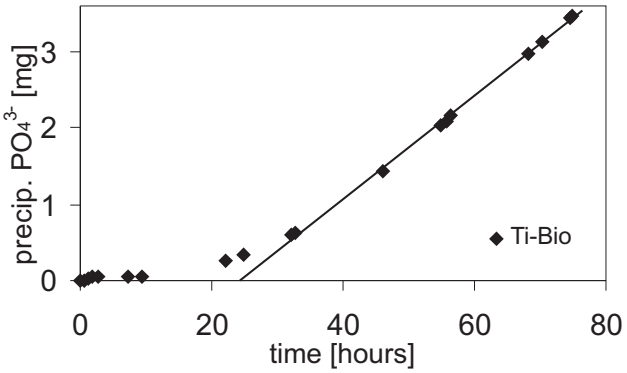

Fig. 7: Time dependence of the precipitated PO_4^{3-} weight plotted for Ti-Bio sample

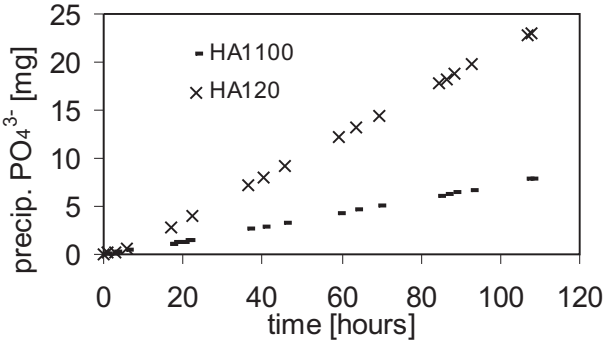

Fig. 8: Time dependence of the precipitated PO_4^{3-} weight plotted for HA120 and HA1100

time is in good correlation to the results obtained from static exposure and reported earlier[7]. The induction times for hydroxyapatite samples were much shorter (tab.4) than those of Ti-Bio probably as a result of much higher structural similarity in the case of apatite precipitating on the hydroxyapatite substrate.

The rate of PO_4^{3-} ions consumption by HA120 sample was higher compared to HA1100 probably due to higher surface area available for reaction (Fig. 8).

In preliminary experiments studying the effect of materials' surface reactions on the culture of chicken bone marrow cells, HA120 sample exhibited behavior-resembling cytotoxicity, when it was added to the cell culture in higher amounts (fig. 9). This phenomenon was not observed in sample HA1100 and it was minimized by pre-incubation of the HA120 sample in culture medium. It is possible that high surface area, inducing massive ion consumption by the adsorption and the crystal growth, causes the cytopatological effect as a result of the concentration decrease in the culture medium.

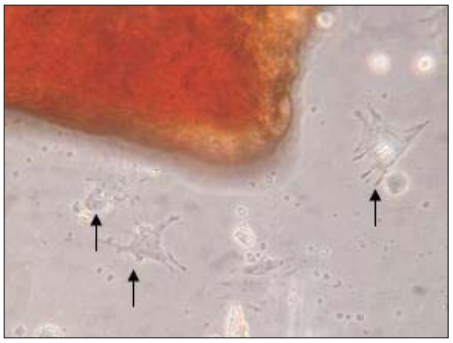

Fig. 8: Bone marrow cells (arrows) surviving near HA120 sample 5 hr after addition of small number of sample particles

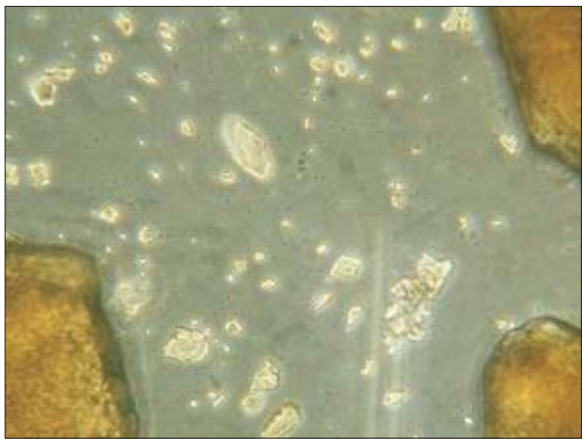

Fig. 9: No living cells present 5 hours after addition of higher number of HA120 sample particles

Conclusion

The dynamic SBF test proved to be a simple tool for detection of early ion exchange reactions between biomaterial and SBF. The ability of bioactive materials to adsorb calcium and phosphate ions immediately from surrounding fluid can have a significant effect on later adsorption of larger molecules (such as amino acids or proteins) and thus affect or even predetermine biocompatibility and bioactivity before apatite crystal growth starts.

The dynamic arrangement also enables the evaluation of precipitation parameters (induction time, growth rate), which can be used for bioactivity quantification.

Acknowledgements

Study was supported by the Department of Industry of the Czech republic under the project No.: FB-CV/64/98.

Literature

1. T.Kokubo, F. Miyaji, H.M. Kim and T. Nakamura: Spontaneous formation of bonelike apatite layer on chemically treated titanium metals, J. Am. Ceram.Soc., 79[4],1127-1129 (1996)
2. T. Kokubo, H. Kushitani, S. Sakka, T. Kitsugi and T. Yamamuro: Solutions able to reproduce in vivo surface-structure changes in bioactive glass-ceramic A-W3, J. Biomed. Mat. Res., 24, 721 (1990)
3. K. Hata, T. Kokubo, T. Nakamura, T. Yamamuro: Growth of a bonelike apatite layer on a substrate by a biomimetic process, J. Am. Ceram. Soc., 78 [4] 1049 (1995)
4. H. Takadama, H.M. Kim, T. Kokubo and T. Nakamura: TEM-EDX study of process of apatite formation on bioactive metal in simulated body fluid, Proceeding of the 13th Int. Symp. On Ceramics in Medicine, Bologna, Italy, Bioceramics 13, 22-26.11.2000, p.51
5. Wu W.; Nancollas G.H., Bioceramics 11, Proceedings of the 11th int. symposium on ceramics in medicine, New York, USA (1998) 469
6. Song.L.; Campbell A.A.; Bunker B.C.: Hydroxyapatite and related materials, Brown P.W., Constanz B. (eds), CRC Press (1994) 197
7. J. Strnad, A. Helebrant, Hamackova J.: Bone-like apatite formation on titanium and silica glass, Glastech. Ber. Glass Sci. Technol. 73 C1 (2000)
8. P. Li, P. Ducheyne: Quasi-biological apatite film induced by titanium in a simulated body fluid, J. of Biomed. Mater. Res. 41 (1998) 341