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# FTIR monitoring of the growth of the carbonate containing apatite layers from simulated and natural body fluids

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## Abstract

The aim of this work is to perform such a chemical modification of the implant that in vivo conditions on its surface, heterogeneous nucleation of apatite from the body fluid could be easily induced and then its growth successfully performed. The laboratory experiments were carried out with carbon–carbon biocomposites and carbon needled clothes. The surface of carbon was coated with the sol–gel silica or calcium silicate layer and then, under physiological conditions, thermostatically soaked in the synthetic or natural body fluid. Successive steps of the apatite growth were monitored by infrared spectroscopy. It was found that the nucleation and growth of carbonate containing apatite took place at the surface and was more effective on silica–calcium than on silica substrate. The natural body fluid, compared with synthetic body fluid much enhanced the apatite precipitation. This observation supports suggestion that also proteins can act as nucleation centres. © 1999 Elsevier Science B.V. All rights reserved.

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# 1. Introduction

Bioactive materials like hydroxyapatite, phosphate ceramics, bioglass and glass ceramics possesses important property to form a strong chemical bond with natural bone [1,2]. Their surface region can be transformed to the biological type of apatite (carbonate apatite) through a series of surface reactions including dissolution-precipitation and ion exchange [3]. However, high brittleness and low tensile strength of all these bioactive materials inhibit their direct use in the reconstruction of the hip and knee joints.

The formation of the carbonate apatite on the surface of biomaterials is thought to be one of the

events leading to form a strong chemical bond being conditio sine qua non of the strong implant—bone joint [1,2]. For this reason hydroxyapatite (HAP), having most similar chemical composition and crystallographic structure towards the bone apatite is considered as the joining material suitable to achieve strong early fixation of uncemented prostheses.

Biocompatible carbon composites are very promising materials for medical applications [4]. Since the biological activity of carbon composites is low, the coating of their surface with a hydroxyapatite layer can enhance it. This task is not easy for serious differences in chemical nature of the interacting substances, though numerous methods for the coatings preparation were suggested, including: electrophoretic deposition [5,6], biomimetic processes [7,8], plasma spraying [9], sputtering [10], pulsed

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Fig. 1. FTIR transmission spectra of biological bone: (a) heated at 1100°C; (b) heated at 600°C.

laser deposition [11], polymeric route [12] and solgel deposition [13]. Actually, the plasma spraying is the most favourable one.

In this work we coated the biocompatible carbon with the biologically active carbonate apatite by growing from the natural body fluid (NBF) or synthetic body fluid (SBF) during the soaking procedure. It has already been observed [14,15] that in SBF the reactions that in vivo would take place near the implant surface are in vitro well reproduced. In such conditions a bone like apatite is deposited, similar to that which forms in vivo on bioactive materials.

# 2. Experimental

In our experiments we used carbon–carbon composites and a carbon biomaterial based on carbon fibres in the form of needled cloth. Prior to soaking the samples were coated with the sol–gel silica or  $SiO_2$ –CaO sol–gel layer [16]. The soaking procedure was performed in SBF or NBF [7,16].

Carbon biomaterials used in this study were made

in the Department of Special Ceramics of AGH. The SBF solution was prepared according to Kokubo [17] with the ion concentrations almost equal to those of human blood plasma. The fluid was buffered at pH 7.2 with the mixture of 50 mM tris(hydroxymethyl) aminomethane and 45 mM HCl.

The carbon samples were initially coated with the silica sol or with silica–calcium sol–gel solutions using dipping–withdrawing technique [18] and after this process were baked for 30 min at 600°C in an argon atmosphere. Then, the samples were soaked thermostatically under physiological conditions in SBF or in NBF for up to 30 days, at various daily intervals. During the soaking SBF was renewed every 5 days.

FTIR spectroscopy was used for the phase analysis of the phosphate precipitates produced during the soaking. The FTIR spectra were recorded in the transmission spectral mood in the  $400-4000 \text{ cm}^{-1}$  range using Bio-Rad FTS-60V spectrometer.

XRD analysis used as a complementary technique was performed on Seifert FPM XRD 7 instrument (CuK $\alpha$ ). The morphology of deposits was monitored using scanning electron microscope (SEM) Phillips



Fig. 2. FTIR transmission spectra of phosphate precipitates after soaking (5 days) in SBF: (a) carbon with no precoating; (b) carbon with silica coating; (c) carbon with silica-calcium coating.

XL 30, equipped with an energy dispersive X-ray analyser (Link ISIS-EDX).

#### 3. Results and discussion

Hydroxyapatite, the main mineral component of a biological bone, with its formula  $Ca_{10}(PO_4)_6(OH)_2$  shows absorption due to vibrational modes from phosphate and hydroxyl groups. In biological apatites, some of  $PO_4^{-3}$  ions are substituted by  $CO_3^{-2}$  ions and IR spectrum is very sensitive to these carbonate substitutions, so even a very small amount of carbonate can be detected. The phosphate group has a  $T_d$  symmetry resulting in four internal modes being IR active:  $\nu_3$  asymmetric stretching mode of vibration characterised by a strong, complex band in the

1000–1150 cm<sup>-1</sup> range (degeneracy = 3) and a medium intensity band at about 960 cm<sup>-1</sup> due to  $\nu_1$ symmetric stretching vibration (degeneracy = 0). The  $\nu_4$  bending vibration of PO<sub>4</sub> is characterised by bands located at 560–610 cm<sup>-1</sup>. Weak band at 430– 460 cm<sup>-1</sup> correspond to  $\nu_2$  bending vibration (degeneracy = 2). Carbonated hydroxyapatite yields the  $\nu_3$  vibrations of C–O in the high-energy region between 1410 and 1470 cm<sup>-1</sup> and  $\nu_2$  vibrations in the low-energy region between 850 and 890 cm<sup>-1</sup>. The peak position of the carbonate  $\nu_2$  mode depends upon whether the CO<sub>3</sub><sup>-2</sup> ion substitutes for OH<sup>-</sup> or PO<sub>4</sub><sup>3-</sup> in the HAP lattice.

Crystalline hydroxyapatite generates two characteristic OH bands at about 3570 and  $630 \text{ cm}^{-1}$ . Sometimes they are absent in IR spectra (Fig. 1(b)) and some authors attributed this missing OH modes to a



Fig. 3. FTIR transmission spectra of phosphate precipitates after 30 days of soaking in SBF: (a) carbon with silica coating; (b) carbon with silica–calcium coating.

perturbation of hydroxyl stretching and bending modes on the apatite surface by the hydrogen bonding of water molecules to the surface OH ions. It was noticed (see also Fig. 1(a)) that after water removing from the apatite surface the OH bands in the infrared patterns became sharp [19]. The OH stretching vibration is unique for crystalline hydroxyapatite and its intensity is considerably weaker compared to the strong P–O stretching vibration because of the hydroxyapatite stoichiometry.

Fig. 1 shows the FTIR spectra of a biological bone. The material heated at 600°C (b) is represented by the whole set of bands characteristic of the biological apatite. After heating at 1100°C the carbonate and water bands quenched while bands due to crystalline OH appeared. These changes indicate that during the heating of the bone at 1100°C it was transformed into a highly crystalline form of hydroxyapatite. Fig. 2 shows the FTIR transmission spectra of the phosphate precipitates at the carbon surface subjected to different modifications. After 5 days of soaking in SBF the FTIR spectrum of uncovered carbon (Fig. 2(a)) does contain no phosphate and carbonate bands, indicating that the surface is inactive towards the apatite nucleation.

In the case of the silica overlayer after 5 days of soaking (Fig. 2(b)) the PO<sub>4</sub> bands in the 550–650 cm<sup>-1</sup> region are very weak and Si–O vibrations in SiO<sub>4</sub> tetrahedra dominated the FTIR spectrum. It does mean that the apatite nucleation and precipitation already proceeded at a moderate rate.

It is seen that the calcium–silica precoating (Fig. 2(c)) most profitably influences the calcium phosphate precipitation from SBF. The PO<sub>4</sub> bending vibrations with maximum at  $562 \text{ cm}^{-1}$  and at  $603 \text{ cm}^{-1}$  are distinct but the FTIR spectrum is dominated by the



Fig. 4. SEM micrograph of phosphate precipitates after soaking in NBF during 1 day for carbon with silica-calcium coating.

stretching PO<sub>4</sub> vibration in the 1000–1150 cm<sup>-1</sup> range. The strong band with a pronounced peak with maximum at 1425 cm<sup>-1</sup> appeared along with a well-defined band at 876 cm<sup>-1</sup> known to be specific for a carbonated apatite. The intensities of both bands significantly decreased with the immersion time. The OH bands at about 630 cm<sup>-1</sup> and at about  $3570 \text{ cm}^{-1}$  are absent, pointing to a poor crystallised apatite structure. The presence of the distinct phosphate bands in the FTIR spectra shows that the calcium–silica precoating is the most favourable substrate for the apatite nucleation and deposition.

Fig. 3 shows the FTIR spectra of the phosphate precipitation course during 30 days of soaking in SBF. Comparing the spectra it may be noticed that for both silica (Fig. 3(a)) and calcium–silica (Fig. 3(b)) precoatings the spectral features of apatite strongly point to the presence of the well-formed

phase of the carbonate apatite. The morphology of precipitates (Fig. 4) and broadening of the XRD peaks at  $2\Theta$  of about 26 and  $32^{\circ}$  (Fig. 5) indicate that a crystal size of this apatite is very small. It is poorly crystalline, forming very close-packed globules (Fig. 4). Each globule is composed of flakes united together. However the carbonates content and crystallinity of the deposits on calcium–silica are higher than that on silica. Comparing all considered cases it is seen that the calcium–silica precoating yields the most positive influence on the carbonate phosphate precipitation.

The FTIR spectra of the results obtained on carbon samples covered with the calcium–silica layer after 1, 3 or 5 days of soaking in NBF is shown in Fig. 6. It is seen that even after 1 day of soaking the phosphate precipitation is quite effective. The strong  $PO_4$  bands in the 550–650 cm<sup>-1</sup> region are present,

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Fig. 5. XRD pattern of phosphate precipitates after 30 jdays of soaking in SBF (carbon with silica-calcium layer).



Fig. 6. FTIR transmission spectra of phosphate precipitates after soaking in NBF: (a) carbon with silica-calcium, 1 day; (b) carbon with silica-calcium, 3 days; (c) carbon with silica-calcium, 5 days.



Fig. 7. SEM micrographs of phosphate precipitates after 30 days of soaking in SBF: (a), (b) carbon with silica coating; (c), (d) carbon with silica–calcium coating.

while the Si–O and C–O band in SiO<sub>4</sub> and CO<sub>3</sub> groups dominated the spectra. The adsorbed water yields a broad band in  $3000-3500 \text{ cm}^{-1}$  range and at about  $1650 \text{ cm}^{-1}$ . An additional band at about

 $1530 \text{ cm}^{-1}$  is the evidence that the phosphate precipitates formed in NBF contain also proteins. More intense and developed FTIR spectra of phosphate precipitates formed in NBF compared to that in SBF indicate that an environment of NBF containing proteins facilitates formation of the phosphate deposits. It supports the suggestion that also proteins act as a nucleation centres of apatite [7,19]. The process is so easy that even after 1 day of soaking the deposits are already present (Fig. 7), well revealed by Kevex microanalysis (Fig. 8).

Since precoatings accelerate the deposition, it can be concluded that during the soaking SBF reacts with silica forming silanol groups that induce the apatite nucleation [7,14]. On the other hand the calcium ions that are released from the silica–calcium precoating by overcoming the ionic product with respect to apatite, also accelerate the apatite nucleation [16].

The same processes make the very essence of the bone remodelling and the mineralisation at the boneimplant interface and so they are fundamental elements of good implant-bone fixation. Therefore



Fig. 8. X-ray microanalysis of phosphate precipitates after soaking in NBF during 1 day for carbon with silica-calcium coating.

the success of the prosthesis incorporation depends on the presence and activity of free calcium and phosphate ions in the interface, that make the implant to be able to interact with the surrounding bone. We can also conclude that the precoated carbon biocomposite can provide a solid material for the subsequent attachment of a broad spectrum of biochemical active molecules.

### 4. Conclusions

Vibrational spectroscopy is useful in the investigation of chemical reactions on the surface of biomaterials. The FTIR spectra can control the transformations in surface layers on the carbon precoated with silica or silica–calcium phases and also they can detect formation of the poorly crystallised carbonated apatite.

Both silica and silica–calcium precoatings applied on the biocarbon surface act as an effective interface with higher ability to precipitate the carbonate apatite in vivo. It can be also concluded that the precoatings improve the mineralisation process on the biocarbon surface.

The formation of the carbonate apatite (biologically equivalent) on the surface of carbon biomaterials in SBF may be an important step leading to direct bonding with bone. It is believed that the formation of the biologically active bone—like apatite (containing carbonate) on the implant surface in the body—is the most important condition for obtaining the direct, strong chemical bonding between the implant to living bone.

The NBF, compared with SBF, much enhances the growth of apatites on carbon biocomposites.

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