Surface design of orthopaedic drug delivery implants: X-ray photoelectron spectroscopy of bone-derived apatites

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Incorporation of foreign ions onto a biomineral surface as well as removal from it of native (physiological) ions during deproteination procedures, are discussed in view of surface design of orthopaedic implants serving as drug delivery carriers. Surface properties of bone-derived apatites, purified at low temperature in non-aqueous medium, and of several commercial samples are examined via XPS technique. It has been demonstrated that, in spite of the fact that the initial bone demineralized matrix is derived from the same bovine bone, different deproteination procedures lead to different surface properties. Correlation between carbon 1s deconvoluted peak and the percentage of the residual organic fraction on the bone-derived apatites' surface at different stages of purification has been established.

1. Introduction

Bone-derived apatites (demineralized bone matrix) have a considerable potential as remodeling implants, prosthetic bone replacement and drug delivery devices [1–4]. They can be used in orthopaedic surgery for replacement of bone loss, which is a result of trauma, for hip revisions, for remodeling in cranio-maxillofacial surgery, as well as for filling periodontal defects and tooth extraction sockets. The therapeutic potential of such applications could be greatly enhanced when combined with simultaneous delivery of active therapeutic agents.

One of the key parameters for drug delivery applications is the surface functionality of the implant since cells and proteins are very sensitive to surface properties. For example, surface functional groups affect endothelial and bone cells' attachment and proliferation [5]. The incorporation of ions onto an implant surface may change the orientation of binding proteins and, consequently, change the binding mode of cells to the implant [6, 7]. Lack of physiological ions at the implant surface may also cause an alteration of membrane potential of cells closest to the surface [8,9].

All these factors are very important to take into account when planning to design or use biomaterial surfaces as carriers for drug delivery. For orthopaedic applications, an important protein family to be properly delivered is Bone Morpho-genetic Proteins (BMPs) which are bone-derived growth factors able to promote the differentiation of non-committed mesenchymal cells onto chondro – and osteogenic – pathways *in vivo*. BMPs play a significant role in fracture repair and bone remodeling but their therapeutic applications critically depend on a carrier system and carrier surface properties [10, 11].

To prevent undesirable immunological response during and after implantation, the organic fraction associated with biominerals should be completely extracted via deproteination procedures. In biological mineralization processes, as well as in attempts to mimic these processes synthetically [12–14], an organic matrix, usually anionic in character, serves as a template for nucleation and growth of biominerals. This organic fraction is composed mostly from collagenous proteins, phosphoproteins and lipids [15].

Numerous purification procedures were used to completely eliminate the organic component from inorganic material [16–19]. For example, the treatments with following reagents were applied: hydrazine, sodium hypochlorite, chloroform/ methanol, enzymatic degradation, concentrated KOH, ethylene diamine, as well as radio frequency discharge in oxygen at low pressure. All these deproteination procedures lead to various degrees of bone mineral properties distortion. As a result, compositional and structural identification of bone inorganic phase is ambiguous and has remained controversial for the past century [20].

We examine the surface properties of bone-apatite crystals extracted by non-aqueous method at low temperature [21] and those of commercial bone-derived materials [Osteograf/N 300 (CeraMed Corporation, Lakewood, CO) and Bio-Oss (Ed. Geistlich Sohne AG, Switzerland)] using X-ray photoelectron spectroscopy

(XPS). In this paper, we show how the surface properties of bone-apatite crystals are affected by various deproteination procedures.

2. Materials and methods

2.1. Analysis of surface composition

X-ray photoelectron spectroscopy (XPS) gives a semiquantative elemental surface composition with about $\pm$ 10% accuracy, limited functional group analysis and estimation of the distribution of elements of functional groups [22]. XPS analysis was performed on a Surface Science SSX-100, using a hemispherical analyzer and Mg K$\alpha_{1,2}$ X-rays (300 w, 15 kV and 20 mA). To prevent surface charging, a flood gun (5 eV) was used. All spectra were charge-corrected to carbon 1s at 284.8 eV for the C_xH_y-type hydrocarbons. High resolution scans were performed for carbon and phosphorus. To identify the various components of their peaks which reflect the different chemical functions in which these elements are present, peak decomposition was performed based on a least-squares curve fitting.

2.2. Amino acid analysis

The various degrees of bone-derived apatite purification were estimated based on amino acid analysis (AAA). For amino acid analysis of a bovine, powdered apatite crystals were dissolved in hydrochloric acid. The solution was then hydrolyzed for 24 h at 110 °C to decompose any remaining proteins in corresponding amino acids. The amino acid solution was then placed into the analyzer to determine the percentage and weight of each amino acid.

2.3. Bone-derived apatites

Sample 1 was only ground at liquid nitrogen temperature without applying deproteination procedure. It contains maximum percentage of organic fraction ($\sim$ 22%) composed from collagenous proteins, phosphoproteins, lipids and cells. Samples 2-4 represent different stages of deproteination with corresponding decreasing percentage of organic fraction on their surfaces. Comparative studies were done on similar (bone-derived) commercial samples-Osteograf/n-300 (CeraMed Corporation, Lakewood, CO) and Bio-Oss (Ed. Geistlich Sohne AG, Switzerland). These commercial samples due to their corresponding processing procedures were transformed mainly to hydroxylapatite as opposed to our carbonate apatites. Our samples were identified as carbonate apatite since no hydroxyl groups were detected by resolution-enhanced Fourier transform infrared spectroscopy (FTIR) and magic angle spinning proton nuclear magnetic resonance spectroscopy (MAS NMR) characterization [23]. This identification of bone apatite as a carbonate apatite is in agreement with previous observations [24-27].

3. Results and discussion

3.1. Surface chemistry of bone-derived apatites

All the results obtained by XPS technique are summarized in Table I.

Fluorine

We detected the presence of fluorine atoms on the surfaces of the Samples 2-4, (Fig. 1). Significant percentage of organic fraction on Sample 1 ($\sim$ 22.0%) prevented fluorine detection by XPS. For Samples 2-4, we observed direct correlation between various degrees of purification and the percentage of fluorine on the crystal surfaces (see Table I):

$$y = 11.67 - 0.86\,x; \quad R = 0.998$$

where x percentage of fluorine determined by XPS; y, estimated percentage of organic fraction remaining on the apatite surface at various degrees of deproteination; R, correlation coefficient.

Thus, the percentage of fluorine in this purification procedure could serve as a criterion for estimation of purification degree. Presence of fluorine could also serve as indirect proof of hydroxyl groups absence. Fluorine exchanges for hydroxyl groups, decreasing the affinity of the apatites for water, and thus, increasing the strength of the adsorption bond [28, 29].

Fluorine ions were not detected on the surface of

TABLE I Surface composition of bone–derived apatites determined by XPS

	1–3 weeks bovine cortical bone				Osteograf	Bio–Oss
	Sample 1	Sample 2	Sample 3	Sample 4	(Natural HA)	(Natural HA)
Organic fraction	$\sim$ 22.0	$\sim$ 10.0	$\sim$ 6.0	$\sim$ 0.22	$\sim$ 0.0	$\sim$ 0.0
PO_4^{3-} (132.8 $\pm$ 0.2 eV)	$\sim$ 5.03	—	—	—	$\sim$ 10.0	—
HPO_4^{2-} (133.8 $\pm$ 0.2 eV)	—	$\sim$ 12.38	$\sim$ 10.84	$\sim$ 11.02	—	$\sim$ 12.09
F^-	—	$\sim$ 2.17	$\sim$ 6.37	$\sim$ 12.68	—	—
K^+	—	$\sim$ 1.0	$\sim$ 1.0	$\sim$ 1.0	$\sim$ 4.0	$\sim$ 1.0
Na^+	—	—	—	—	$\sim$ 4.0	—
C total	$\sim$ 45.07	$\sim$ 17.50	$\sim$ 16.27	$\sim$ 12.19	$\sim$ 20.09	$\sim$ 16.28
C—C & C—H (284.8 eV)	$\sim$ 27.42	$\sim$ 10.55	$\sim$ 11.55	$\sim$ 8.56	$\sim$ 11.19	$\sim$ 6.96
C—CH_2—N (285.4 eV)	—	$\sim$ 5.06	$\sim$ 3.67	—	—	—
C—OH (286.0 eV)	—	—	—	—	$\sim$ 6.17	$\sim$ 5.75
C—O or C—N (286.3 $\pm$ 0.1 eV)	$\sim$ 10.15	—	—	—	—	—
C—C—O or C(288.0 $\pm$ 0.1 eV)	$\sim$ 7.50	—	—	—	—	—
C—C (289.0 eV)	—	$\sim$ 1.89	$\sim$ 1.05	$\sim$ 0.68	—	—
CO_3^{2-} (289.7 eV)	—	—	—	$\sim$ 2.95	$\sim$ 2.73	$\sim$ 3.57

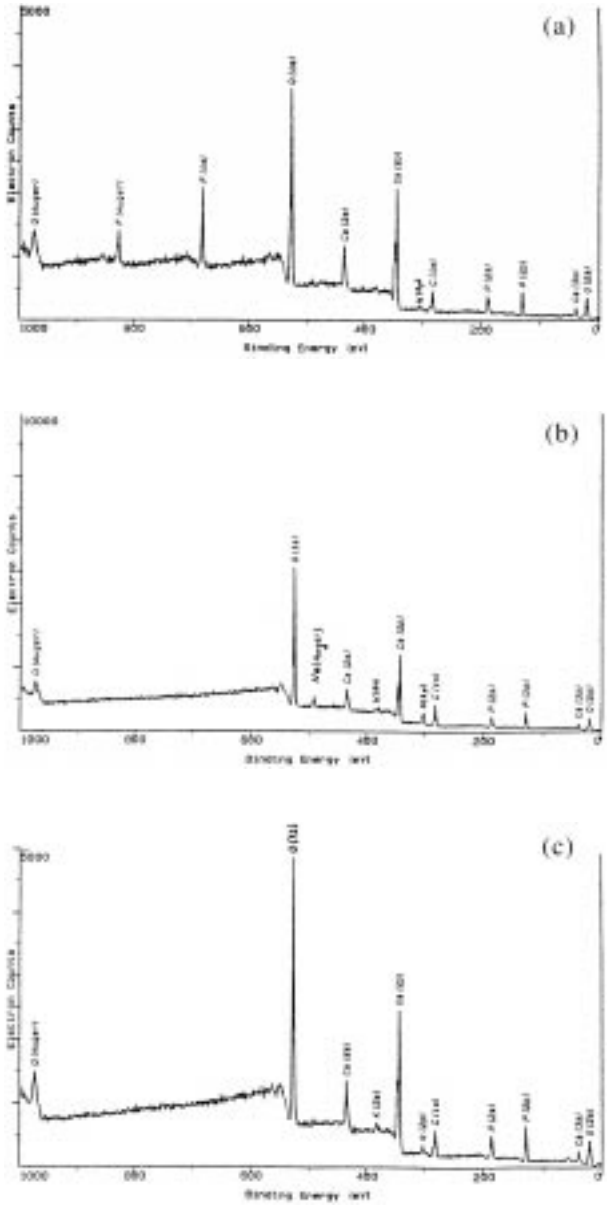

Figure 1 XPS analysis of bone–derived apatites surface composition: (a) Sample 4 ($\sim$ 0.22% of organic fraction; (b) Osteograf; (c) Bio–Oss.

OsteoGraf/N-300 and Bio-Oss, Fig. 1. Apparently, they were removed from the surfaces due to corresponding purification procedures. The possibility of fluorine removal was demonstrated previously via treatment of biominerals with KOH solution [30]. The presence of fluorine on the surfaces of apatite processed in non-aqueous medium could be due to the fact that the fluorine was adsorbed onto the crystal surfaces as a result of the blood/bone equilibrium-inequilibrium processes and the *in vivo* accumulation of ions in bone. Fluorine is not irreversibly deposited in the bone, but can be mobilized during the osteoclastic-osteoblastic cycles of the Haversian systems.

The mechanism of therapeutic agents adsorption (bone growth factors) and their release profile are affected by the presence of fluorine on the apatite surfaces. Fluorine is contributing to the increase in the number of adsorption sites and to the strength of the adsorption bond [6]. The presence of fluorine on the surface of apatite results in a surface with a lower surface energy, hence its interaction with water should be weaker [31].

Potassium

In Samples 2-4 and in commercial samples, we have detected the presence of K^+ (see Table I, Fig. 1). This might be related to osteocytes activity. It is known that the osteocytes continuously pump potassium into Bone Extracellular Body Fluids (BECF) [32]. The efficiency of the potassium pump of living cells depends on the magnesium concentration [33]. Chronic magnesium deficiency induces a pathological mineralization of calcium phosphate salts. Potassium depletion is often observed secondary to magnesium deficiency [34]. During periods of slight magnesium deficiency, when the potassium pump of the osteocytes does not work sufficiently, the pH of BECF may become lower than that of the other body fluids. This can lead to hypercalcemia, hyperphosphatemia and pathological calcifications. [20]. We suggest that within the same deproteination procedure, it is possible to estimate the level of activity of osteocyte potassium pump. This may lead to potentially distinguishing a healthy bone from pathological cases.

Carbonate ions

Carbonate ions were detected in Sample 4 and on both commercial samples – osteoGraf/N-300 and Bio-Oss – but not on Samples 1-3 (see Table I). Absence of CO_3^{2-} in Samples 1-3 might be caused by a high percentage of organic fraction on the surfaces of the crystals. Such a high percentage of organic fraction may interfere with the detection of CO_3^{2-} ions, similarly to the case of fluorine. In spite of the fact that 30% of the carbonate is lost due to heating at 400 °C [20] as in the case of OsteoGraf/N-300 and Bio-Oss, we were still able to detect CO_3^{2-} on their surfaces. The partial loss of these ions during heating might be compensated by conversion of citrate into carbonate at this temperature.

Phosphate and protonated phosphate ions

We were able to detect the surface hydrolysis of PO_4^{3-} into HPO_4^{2-}, that could not be directly detected by any other methods (see Table I). The presence of phosphate ions in Sample 1 ($\sim$ 22% of organic fraction) might be attributed to contribution of acidic phosphoproteins and phospholipids from bone matrix [35]. We also detected PO_4^{3-} ions in OsteoGraf/N-300 samples and HPO_4^2 ions in Bio-Oss ones.

3.2. Evaluation of biomineral surface protein content at various degrees of purification

XPS was used previously to detect the presence of adsorbed immobilized proteins and to estimate the amount of proteins present. It was previously observed that the amount of adsorbed proteins is proprtional to N (adsorption on the surface of hydrophilic polymers) [36]

or N/C (adsorption on the surface of hydrogel contact lenses) [37]. These correlations cannot be applied in our case since N was detected ($\sim 7.8\%$) only for Sample 1 ($\sim 22\%$ of organic fraction) and not on Samples 2-4 in spite of the fact that these samples contain some percentage of organic fraction based on AA analysis. Thus, we propose a different correlation for our case based on analysis of C 1s decomposed peaks for Samples 1-4. We have found that the following components –C—CH_2—n at 285.4 eV [22], C—O or C—N at 286.3 ± 0.1 eV [38], O—C–O or C=O at 288.0 ± 0.1 eV [38] and O=C—O—H at 289.0 eV [38] are directly related to the percentage of organic fraction present on these samples during different stages of purification. The following correlation equation has been established:

$$y = 0.05 + 1.27\,x; \quad R = 0.998$$

where x, percentage of organic fraction determined by XPS based on carbon 1s deconvoluted peaks. Thus, this equation might be used for evaluation of the degree of purification during deproteination process or for estimation of protein percentage adsorbed on a surface of deproteinized bone crystal in the process of designing drug delivery carriers.

In summary, we have demonstrated that, in spite of the fact that the initial bone demineralized matrix is derived from the same bovine bone, different deproteination procedures lead to different surface properties. This, in turn, provides important knowledge for drug delivery design.

The presence or absence of physiological ions on the surfaces, as well as the difference in their content on the implant surfaces due to the difference in the deproteination procedures, lead to different surface energies, since the latter are defined by charge density and net polarity. Incorporation of ions onto a surface can change the orientation of binding proteins and consequently the binding of cells to the apatite surfaces. Lack of physiological ions at the implant surface may cause alteration of membrane potential of cells closest to this surface.

Future studies will be devoted to quantitative predictions and criteria which could be formulated regarding our findings above. In view of using the orthopaedic implants for drug delivery applications, the following parameters need to be evaluated, taking into account presence or absence of the corresponding ions, or the ratio of their content on the surfaces: bone growth factors (BGFs) adsorption strength; BGFs release profile; extent of BGFs inactivation due to their incorporation into the matrices, and extent of their native conformation preservation upon their release.

XPS data compliments other experimental techniques (e.g., neutron activation analysis, XRD, FTIR, FT-Raman and NMR spectroscopy) to study the bulk chemical composition of the bone inorganic phase. It can also yield valuable information about composition of the inorganic phase and the location of ions and atoms in bone-derived apatites.

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