Energy dispersive analysis (EDX) of a degradable bioactive-glass coating on Ti6Al4V *in-vivo*

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The bioactive-glass coating of metallic substrates provides a gradually degrading interface which can be used to favor the bony integration of the implant by the physiologic processes of bone turn-over and remodeling. Twelve New Zealand White rabbits, about 2700 g of weight, were operated by the sagittal insertion of a bioactive-glass coated plate of Ti6Al4V. Retrievals were performed at 4, 8 and 12 weeks. Undecalcified specimens were embedded in methyl-metacrylate and sectioned at 100 microns of thickness. Blocks were grinded and had an electroconductive coating to be examined by scanning electron microscopy (SEM), back scattering electron microscopy (BSEM) and X-ray energy dispersive spectroscopy microanalysis (EDX). EDX allows to evaluate quantitatively the gradual process of coating degradation. Areas of 200 microns in square were analyzed at the interface between bone and coating to determine their elemental composition. Silicon was the key marker for the presence of the glass. Morphological analysis confirms that a tight apposition with bone can be obtained by utilizing the bioactive glass coating of metal. Results of energy dispersive analysis support the mechanism of a gradual degradation of the bioactive glass coating and its integration with bone, since the presence of silicon can be documented within the newly formed bone after the coating has disappeared.

1. Introduction

Bioactive glasses have been studied for many years [1, 2] because of their high compatibility both as intramuscular and intraosseus implants [3].

Degradable glasses are able to elicit an interface with bone which is characterized by a tight apposition between the two components. This characteristic has been used to improve the Ti6A14V-bone interface in the intramedullary prosthesis like hip prostheses [4, 5], after a suitable coating methodology, using the plasma-spray technique, was developed [6].

The bioactive glass coating of metallic substrates provides a gradually degrading interface. This interface can be used to favor the bony integration of the implant by the physiologic processes of bone turn-over and remodeling.

Degradable bioactive glass coatings are needed since the rationale of the coating is to lead the bone gradually towards the Ti6A14V. This process should happen without the production, at its end, of bulky non-degradable particles as those observed with the fragmentation of the crystalline phase of hydroxyapatite coatings [7].

2. Materials and methods

Twelve young adult New Zealand White rabbits about 2700 g of weight were operated by the sagittal insertion, in the proximal tibia, of a bioactive glass coated plate of Ti6A14V. Control rabbits were implanted an uncoated plate.

The bioactive glass, coded for the *in-vivo* experiments as B01A, is a glass designed to be degradable and resorbable and has a percentual molar composition, before the coating process, of SiO_2 47.1%; P_2O_5 6.9%; CaO 20.6%; MgO 1.0%; Na_2O 18.5%; K_2O 4.9%; Al_2O_3 1.0%. A suitable plasma-spray methodology was developed and described in literature [6]; the percentual molar composition of the glass, after the coating process, was measured on scraped-off material and resulted to be: SiO_2 50.5%; P_2O_5 5.3%; CaO 23.1%; MgO 1.1%; Na_2O 14.9%; K_2O 3.8%; Al_2O_3 1.3%. Bioactive glass was prepared at Stazione Sperimentale Vetro in Murano (I) and plasma-spray was performed by Flametal, Fornovo (I).

Plates were sterilized by ethylene oxide and single-packaged in sterile envelopes. Anesthesia was obtained by administration of intramuscular Valium (5 mg/kg),

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intramuscular Ketalar (50 mg/kg) and subcutaneous Xilocaine. Antibiotic prophylaxis was based on administration of intramuscular Rifocin (250 mg) daily. Bone growth was marked by giving a tetracycline injection one week before sacrifice.

Retrievals took place at 4, 8 and 12 weeks. To retrieve and process the samples, the rabbit is placed in a special sealed chamber where the atmosphere is quickly saturated with CO_2 and left there for 3 min. The retrieved sample is, then, placed in 70% ethyl alcohol, dehydrated in serial passages in alcohol and embedded in polymethyl-meta-acrylate (PMMA).

Undecalcified specimens were sectioned at 100 microns of thickness using a toroidal rotating diamond-saw (Leitz Wetzlar, D). Polarized light microscopy and ultra-violet (UV) fluorescence microscopy was performed on the sections. PMMA blocks were grinded and had and electroconductive coating to be examined by scanning electron microscopy (SEM), back scattering electron microscopy (BSEM) and X-ray energy dispersive spectroscopy microanalysis (EDX). These techniques give information about morphology and elemental composition at the interface with bone.

Scanning electron microscopy, back scattered electron microscopy and X-ray energy dispersive spectroscopy were performed at the facilities of the Center for Materials Engineering (CEMUP) and the National Institute of Biomedical Engineering in Porto, Portugal (see Acknowledgments). Samples were sputter-coated with gold and analyzed in a Jeol JSM 6301F scanning electron microscope equipped with X-ray energy dispersive spectroscopy microanalysis capability (Voyager XRMA System, Noran Instruments).

3. Results

Polarized light microscopic analysis shows how bone achieves a tight apposition with the coating (Fig. 1) which was designed to be degradable *in vivo* in a period of about 3 months. Sections which show, in polarized light microscopy, the apposition of bone towards the bioactive glass coating (Fig. 2) were analyzed by UV fluorescence microscopy and it was possible to demonstrate newly formed bone tissue apposed to the coating (Fig. 3). X-ray energy dispersive spectroscopy microanalysis allows to evaluate quantitatively the gradual process of degradation of the coating. Areas of 200 microns in square were analyzed at the interface between bone and coating to determine the elemental composition (Fig. 4). Silicon was the key marker for the presence of the glass.

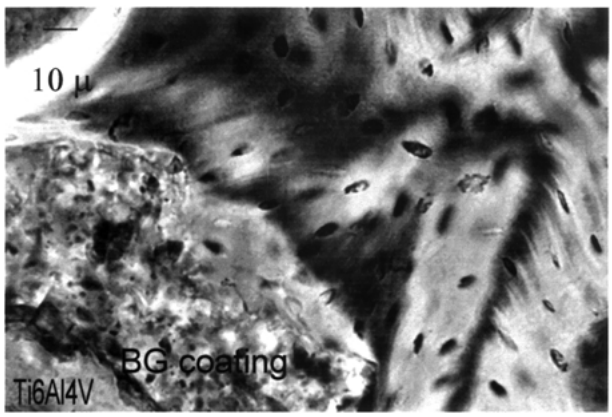

Figure 1 Polarized light microscopic analysis shows how bone achieves a tight apposition with the coating.

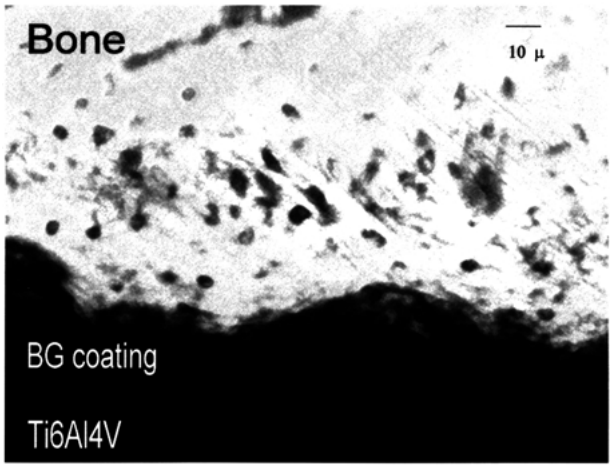

Figure 2 A section which shows, in polarized light microscopy, the apposition of bone towards the coating; on this same section an UV fluorescence microscopy will follow.

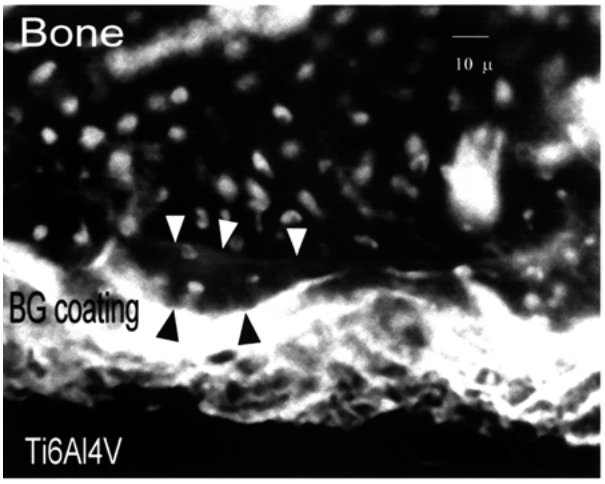

Figure 3 The section presented in Fig. 2 was analyzed by UV fluorescence microscopy and, by the changing in color of bone tissue (medium gray in picture) due to the florescence of tetracycline labels, it was possible to demonstrate newly formed bone tissue apposed to the coating (arrows).

Figure 4 To perform X-ray energy dispersive spectroscopy microanalysis, areas of about 200 microns in square (A) were analyzed at the interface between bone and coating to determine the elemental composition.

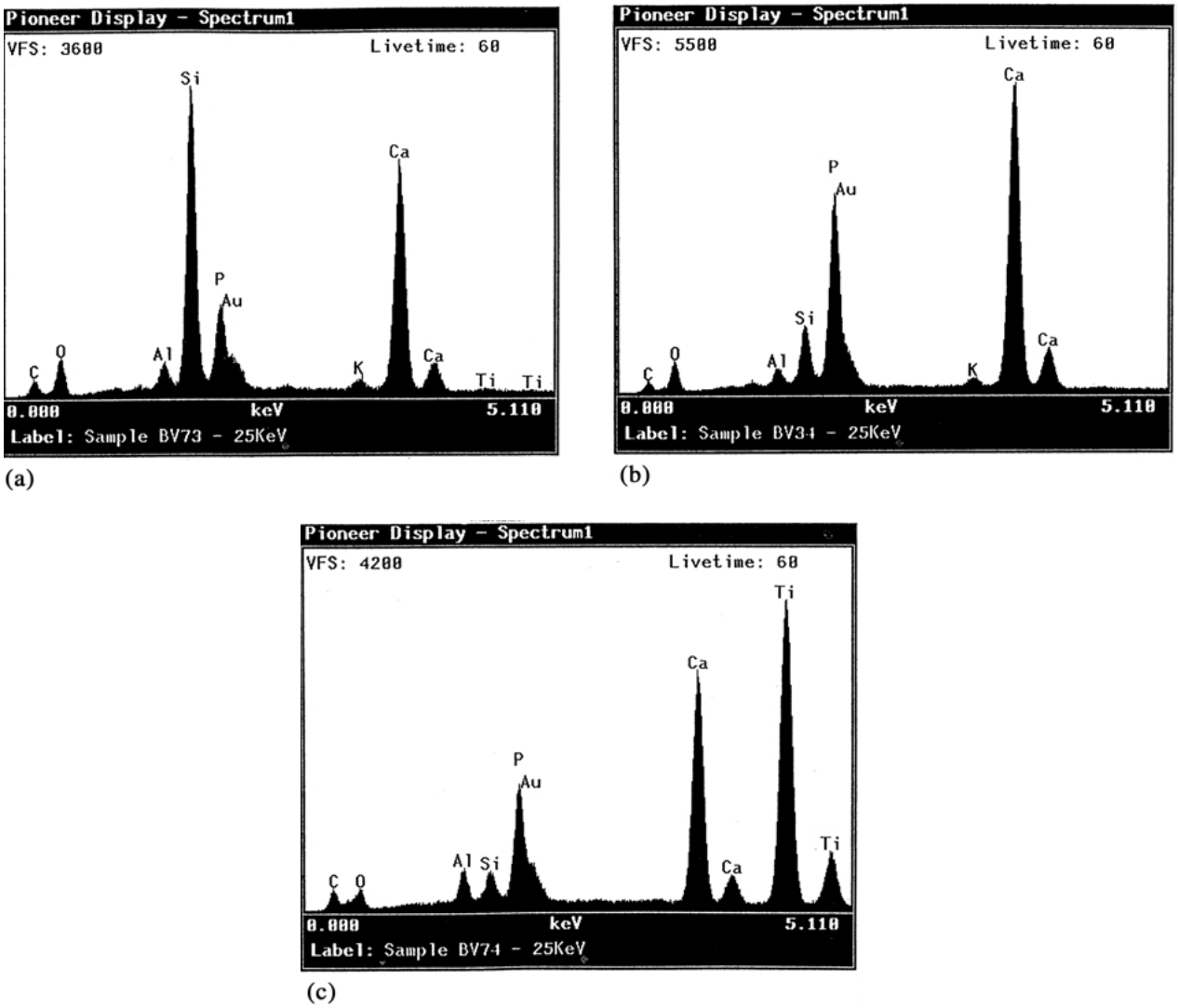

Figure 5 In spectrum (a), silicon has a maximum peak in a sample retrieved after 4 weeks. Spectrum (b) shows that after 8 weeks of implantation there is a significant reduction of the signal for silicon. Spectrum (c) shows a very little but stable presence of the signal for silicon despite the morphological absence of any coating and the direct apposition of bone to the metallic substrate.

In spectrum a (Fig. 5), silicon has a maximum peak in a sample retrieved after 4 weeks. Spectrum b (Fig. 5) shows that after 8 weeks of implantation there is a significant reduction of the signal for silicon.

Newly formed bone gradually replaces the coating and, after 12 weeks, it is apposed to an uncoated metallic substrate (Fig. 6). Spectrum c (Fig. 5) shows a very little but stable presence of the signal for silicon despite the morphological absence of any coating and the direct apposition of bone to the metallic substrate, as shown in Fig. 6, from which the spectrum was taken, and also documented by the appearance of the peaks for titanium.

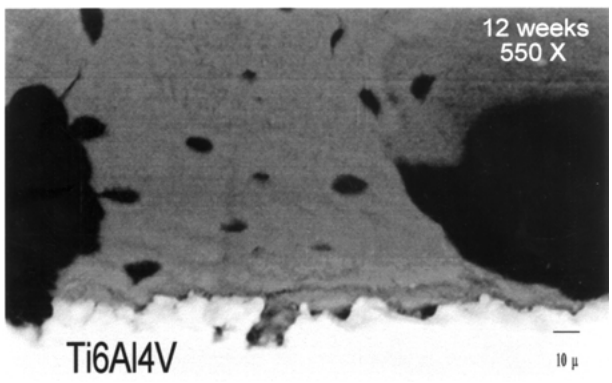

Figure 6 BSEM analysis shows the direct apposition of bone to the metallic substrate once the coating has become morphologically invisible because of its degradation.

In the three spectrums we can notice the distribution of K, mostly from the glass, and Al, which comes scarcely from the glass and mostly from the Ti6A14V alloy; they both provide little help to evaluate the coating degradation. Au comes, obviously, from the electro-conductive coating required for SEM and BSEM analysis.

4. Discussion

This study has documented the morphological and chemical aspects, in an *in-vivo* experimental model, of the degradation of a bioactive glass coating and of the eventual integration of the metallic implant with bone.

The early work of Larry Hench appreciated that a peculiar composition of glass may be ‘‘bioactive’’ and,

eventually, promote the growth of newly formed bone in tight apposition with the glass itself [1]; in this study the authors show that this characteristic is retained when a bioactive glass is used as a plasma-sprayed coating.

After the newly formed bone has gradually replaced the coating, apposing to the Ti6A14V substrate, silicon can still be traced by EDX. The Silicon signal which is present despite the morphological absence of coating, is likely to be originated from glass particles, or components, which are now incorporated inside the newly formed bone. Then, the rationale of coating Ti6A14V by a degradable bioactive glass may be defined as: providing a mean by which the bone tissue has time to repair from the surgical procedure and then to start remodeling in contact with a surface which is more acceptable, biologically, than the bare metal. The extremely gradual coming-into-contact with the metallic substrate probably favors a more physiological apposition of the bone to the metallic implant.

In conclusions, morphological analysis confirms that a tight apposition with bone can be obtained by a bioactive glass coating while elemental analysis supports the hypothesis of a mechanism of gradual degradation of the bioactive glass coating that leads, at the end, newly formed bone into direct apposition with the Ti6A14V metallic substrate.

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