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Study on short-term implants of a fluorinated glass in bone

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The behaviour of a proposed fluorinated glass implanted in femurs of rabbits for a short time was studied on contact microradiographs by electron microscopy and X-ray microprobe. The glass appeared to be surrounded by several bony trabeculae starting from the endosteal surface; some trabeculae were in contact with the outer part of the glass. X-ray analysis showed that degradation occurs in the outer layer only, although without uniformity. Si-rich zones were randomly located in the deeper part of the layer that externally formed zones with P and Ca content higher than that in the non-implanted glass.

1. Introduction

Biological glasses are studied as materials that might give rise to direct bonding with living bone tissues. As is well known, 45S5 Bioglass was the first controlled-surface reactive glass [1], free of toxicity [2], to show an evident tendency towards this required capability. Such bonding is physiological or biological in nature, even though a chemical rearrangement by ionic diffusion is involved which led initially to the improper term of chemical bonding for it [3]. After this first biological glass of high alkaline content, others containing also F were proposed by the same researchers. The addition of fluorides to the Bioglass resulted in compositions with a slower biochemical reactivity. The F ion was thought to act as a corrosion inhibitor promoting the formation of a thin gel layer with a high surface silica concentration [4–6]. Certainly it stabilizes the apatitic compounds inside the glass [7] and those that can form in its neighbouring areas by interaction with tissues. Some researchers proposed other kinds of glasses with low alkaline content [8, 9]. All proposed glasses were and are carefully tested [10–14], particularly *in vivo* to check their capability to be utilized for different roles and in different sites as prosthetic materials. A great number of new glasses were later proposed for use in the field of bone replacements, and systematic studies were carried out to explain the interaction between the host tissue and biological glass [4, 15].

Unfortunately, the mechanical strength of glasses is inadequate for most of the proposed uses in the fields of orthopaedics or dental implantation (joint replacements, dental roots, substitution of segments of long bones or jaw, etc.). Since in these fields of application the compressive and flexural strength are very high, the biological glasses are considered by us mainly as materials for coating of metallic prostheses or granules for the repair of bone defects. Particularly in the granules, for which the surface/volume ratio is high, the degree of surface reactivity of these glasses plays a decisive role; if, in fact, the reactivity is high, most of the granules may disappear [16], in many cases with no possibility of occurrence of repair of the bone defects. It goes without saying that the degree of reactivity in the biological environment depends on the chemical composition of the glass. A moderately low reactivity and a suitable chemical composition are obviously needed also in glass coatings to avoid their fast break-up. However, different glass-making operations and different thermal treatments can also produce, in a glass having the same chemical composition, different arrangements of its molecular network and even a precipitation of microcrystals inside. Consequently, it will have different physicochemical properties [17], including obviously those concerning the interaction with living bone tissue [18].

The formation of microcrystals can improve certain properties, and the ratio between network formers and modifiers is important in order to obtain proper characteristics from a glass system [19]. On the basis of these considerations, a controlled-surface reactive glass has been made whose chemical composition was adjusted to obtain good stability, osteo-inductive capability, low reactivity and improved stabilization of the apatitic layer eventually formed. To obtain all of these characteristic properties, β-TCP, K^+, Mg^{2+} and F^- ions were considered for the formulation of the base composition of the glass. The glassy system obtained and studied was thermodynamically stabilized by shifting its softening temperature upwards,

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and it is typically glass–ceramic for the high presence of microcrystals in it. In this paper results are reported for this type of glass implanted for a short time in the long bones of rabbit, with the aim of verifying the role played by the microcrystallinity dispersed inside the glass.

2. Materials and methods

The glass used in this study had the following composition (wt %): 44.3 SiO_2, 24.5 β-TCP, 18.6 CaO, 4.6 Na_2O, 0.2 K_2O, 2.8 MgO and 5.0 CaF_2. This composition looks like an apatite–wollastonite glassy system, although it differs from that.

High-purity powders of these compounds were carefully mixed and milled together to obtain a homogeneous mixture that was melted at 1520 °C in a Pt crucible for 1 h. The molten glass was then poured into initially cold graphite dies to obtain glass rods (the use of graphite prevented chemical pollution of foreign cations into the glass, allowed its slow cooling and allowed its contraction free from adhesion problems). The rods were sawn into rectangular blocks (2 mm × 5 mm × 10 mm), surface polished and sterilized by autoclaving. Checks by scanning electron microscopy (SEM)-microscope (SEM 500; Philips, The Netherlands) equipped with an energy-dispersive X-ray analyser (EDAX 9900) on some samples were carried out to ensure that no surface changes had occurred after sterilization. Ten glass blocks were implanted as a press-fit in a lateral fissure, previously made in the proximal end of the diaphysis, in the femurs of five white adult male rabbits kept under general anaesthesia. Each glass block was inserted intracortically in the fissure with its longest axis parallel to the longitudinal axis of the femur. After 20 days from the implant intervention the femurs were carefully dissected, fixed with paraformaldehyde, dehydrated and embedded in methyl methacrylate resin. One after the other, thin (150 μm thick) and thick (500 μm thick) cross-sections of the femurs were taken from the levels of the implants by means of a diamond saw microtome. After polishing with emery paper and alumina, low-resolution microradiographs of thin sections were made under an X-ray generator (Italstructures, Italy) at 8 kV and 4 mA on EM-Ilford film. SEM observations and EDAX microanalyses at 25 kV were performed on such polished thick sections after sputtering their surface with a thin conductive layer of carbon. The ZAF correction (Z = atomic number, A = absorption, F = fluorescence) routine was always called and applied on the detected intensities, but highly reliable final values were achieved by comparison with those obtained for a series of glasses of known composition.

3. Results

The SEM analyses of sections of non-implanted glass show that the surface of the glass obtained was not homogeneous: some needle-shaped structures, randomly arranged and with dimensions up to 10 μm × 150 μm (diameter × length), were visible in the glassy matrix (Fig. 1). X-ray analysis of some of these needle-shaped structures showed an Si content about 5% higher than that of the homogeneous part of the glass. Their chemical composition and shape were reminiscent of wollastonitic ($CaSiO_3$) crystals. The majority of the needle-crystals nevertheless appeared to be calcium-phosphatic in nature; the Ca/P ratio detected by microprobe on these microcrystals was practically equal to that of the utilized powders of β-TCP, although their crystalline habitus and shape were completely different.

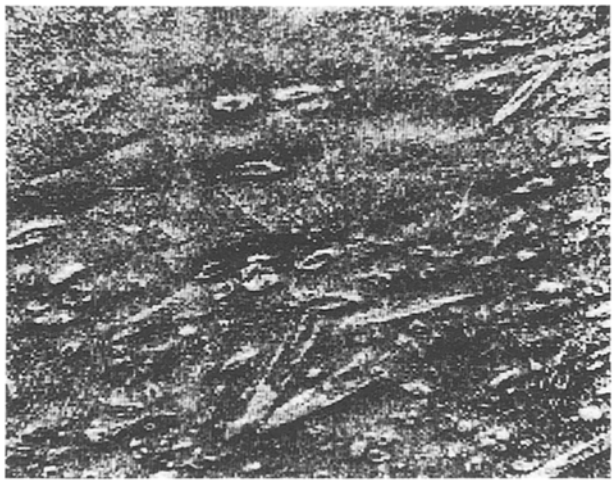

Figure 1 Digital SEM micrograph of a cross-section of the non-implanted glass (field width 0.35 mm).

The microradiographs of thin sections showed that in all of the implants the rectangular blocks of glass were not in contact with the compact bone; furthermore, a number of implants showed the rectangular block obliquely oriented with respect to the longitudinal axis of the femur (Fig. 2a). It also appeared that there was a conspicuous remodelling of the whole compact bone of femurs; the new bone, less than 20 days of age, showed a degree of radiopacity similar to that of the old bone. It should be pointed out that in many implants, starting from the endosteal surface, a conspicuous network of bony trabeculae surrounded the intramedullary portion of the glass (Figs 2a and 3se). These trabeculae had a degree of radiopacity lower than that of the remodelled bone; some trabeculae came into direct contact with the external surface of the implanted glass (Figs 2b, 3se, 4se and 5se). At the periphery of the implant a layer (with thickness ranging between 100 and 150 μm) showed an increased radiopacity and a different morphology compared with the core.

Low-magnification SEM observations confirmed the microradiographic findings: a layer, 100–150 μm thick, less compact than the core, was present at the periphery of the implanted glassy sample (Fig. 3se). Here the X-ray analysis showed a lack of continuous Si-rich layer and the presence of a P-rich layer (Fig. 3).

At higher magnification the SEM observations revealed in the external layer of the implanted glass easily distinguishable needle-shaped structures (corresponding to those shown in Fig. 1) surrounded by an apparently homogeneous material (Fig. 4se).

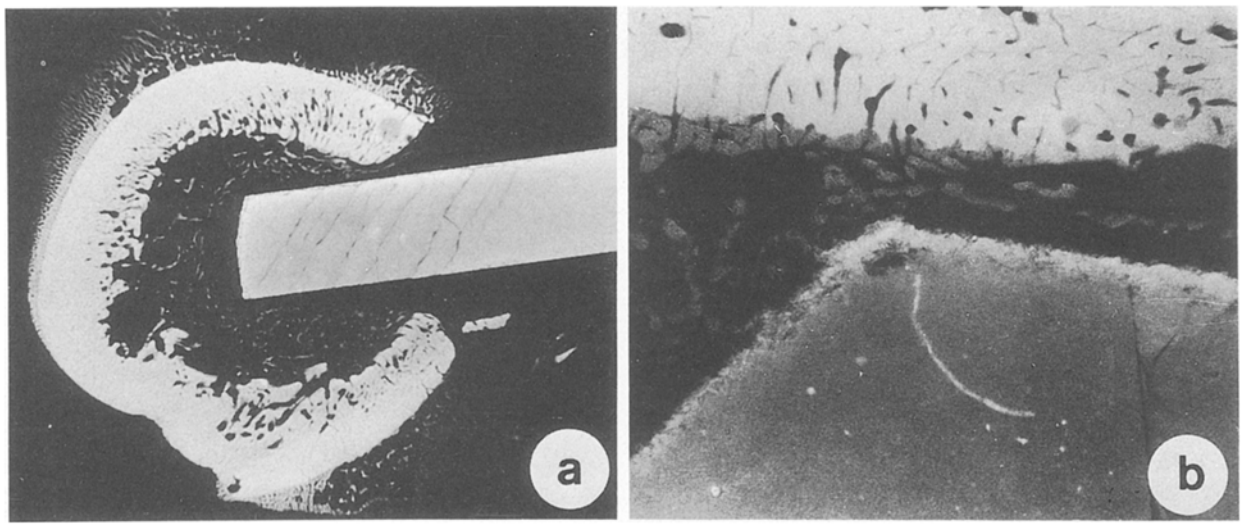

Figure 2 Microradiographs of cross-sections of the rabbit femurs containing an implanted sample of this glass for 20 days (field width. a, 12 mm; b, 2.9 mm)

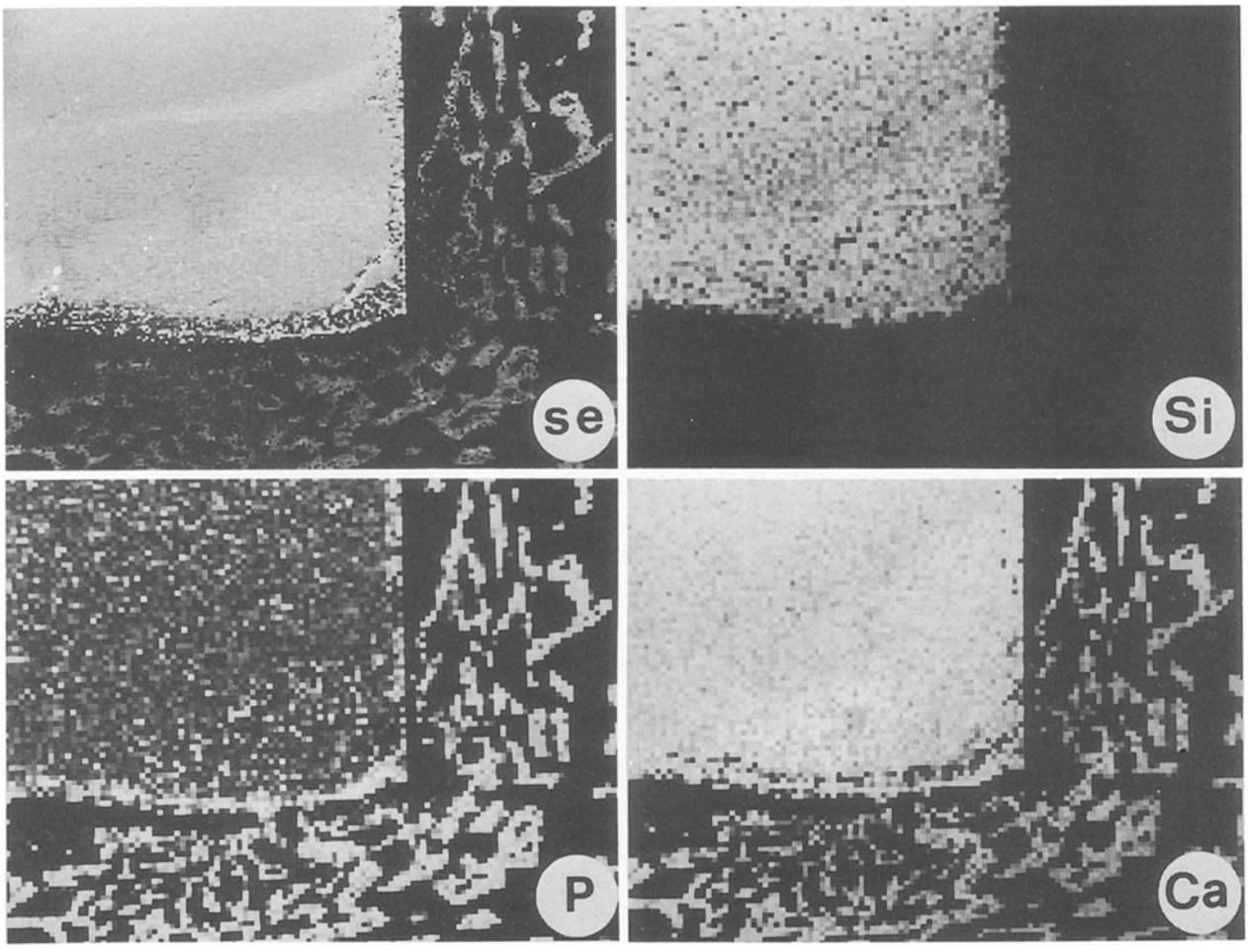

Figure 3 Digital SEM micrograph (se) and digital X-ray dot maps for Si (Si), P (P) and Ca (Ca) of a sample of this glass implanted in the rabbit femur (field width 2.8 mm). Note the irregular P-rich layer at the periphery of the glass.

Sometimes inside the homogeneous part of the external layer there were some zones that could reflect the electrons less than the surrounding ones did, so the former are darker in the SEM images (Fig. 5se). These zones were randomly located in the layer and had a high content of Si (Fig. 5Si). The Si-rich zones (Fig. 6b) were connected with the non-degraded glass inside (a in Figs 5se and 6) and both with non-degraded glass (Fig. 5Si), with Si-poor zones (c in Figs 5Si and 6) and sometimes with P- and Ca-rich zones externally (Fig. 5). Conversely, the external needle-shaped structures had a very low Si content (Figs 4, 5 and 6d) but P and Ca contents (with an atomic Ca/P ratio of 1.61) higher than those of the

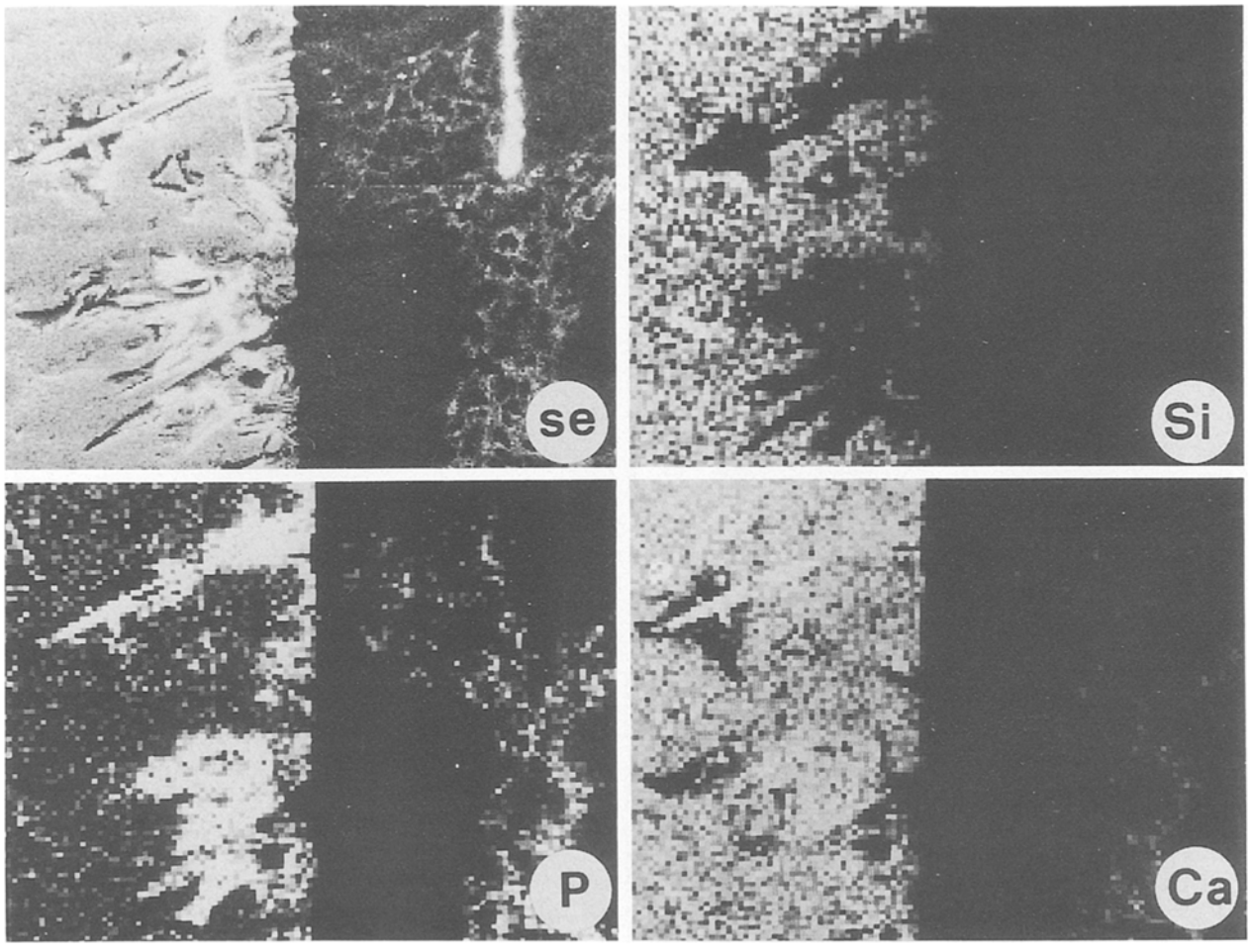

Figure 4 Digital SEM micrograph (se) and digital X-ray dot maps for Si (Si), P (P) and Ca (Ca) of a sample of this glass implanted in the rabbit femur (field width 0.35 mm). Note the needle-shaped structure rich in P and Ca

peripheral layer of the glass (with an atomic Ca/P ratio of 1.74) close to the bone (Fig. 6e). On the basis of their Ca/P ratio these external needle-shaped structures proved to be constituted typically of hydroxyapatite. No Si was detectable by this method in the bone and in the glass in contact with it (Fig. 6e and f); moreover, in the glass close to the bone, the P and Ca contents were higher than both in the non-implanted glass (atomic Ca/P ratio of 4.06; Figs 5 and 6a) and in the bone (atomic Ca/P ratio of 1.65; Figs 5 and 6f).

4. Discussion and conclusions

The non-specific host response, due to the surgical procedures to create space for the insertion of materials in bone, is normally mainly responsible for results in short-time implants. Even if bone healing and regeneration of bone segments are not related to the chemical and physical properties of the implanted material, the harmful influence caused by these processes may lead to failure of the implant. The displacement of the rectangular glass blocks observed in the experiments was probably due to an intermediate period of bone resorption, initially pressing the glass. The failure of implant press-fitting leads to implant movability and, around the glass, normal osteogenetic processes may be inferred by implant movements as long as they are stopped again by the fibrous tissue growth. Perhaps these are the reasons for the heterogeneity in the degree of bony trabeculae growth around some of these implants. On the other hand, this is a question that concerns the methods of insertion of the samples into the bone. Comparisons of the behaviour *in vivo* of different glassy systems can be made only in the presence of equal surgical modalities and techniques for their implantation: equal hole or cut, same bone site and same kind of animal. This matter should be looked into for the formulation of a standard.

The rich network of trabeculae and the direct contact of some of them on the glass may be indicative of the degree of osteoconductive capability of this tested material. The absence of a continuous Si-rich layer, the so-called "silica barrier" of 45S5 Bioglass [1, 3, 4], does not seem to affect the capability of the glass. The lack of Si-rich zones can be ascribed to the composition of the glass: the low Na content in the glass, an element having a high diffusion rate, and the use of β-TCP in its preparation are probably the causes of the presence of very few zones having low Na, low Ca and high Si contents [20]. It is likely that the needle-shaped structures in the glass are sites around which glass degradation and calcium phosphate precipitation quickly occur; this leads to a rapid formation of sites suitable to bind the bone. All around each microcrystal of wollastonite, the glass

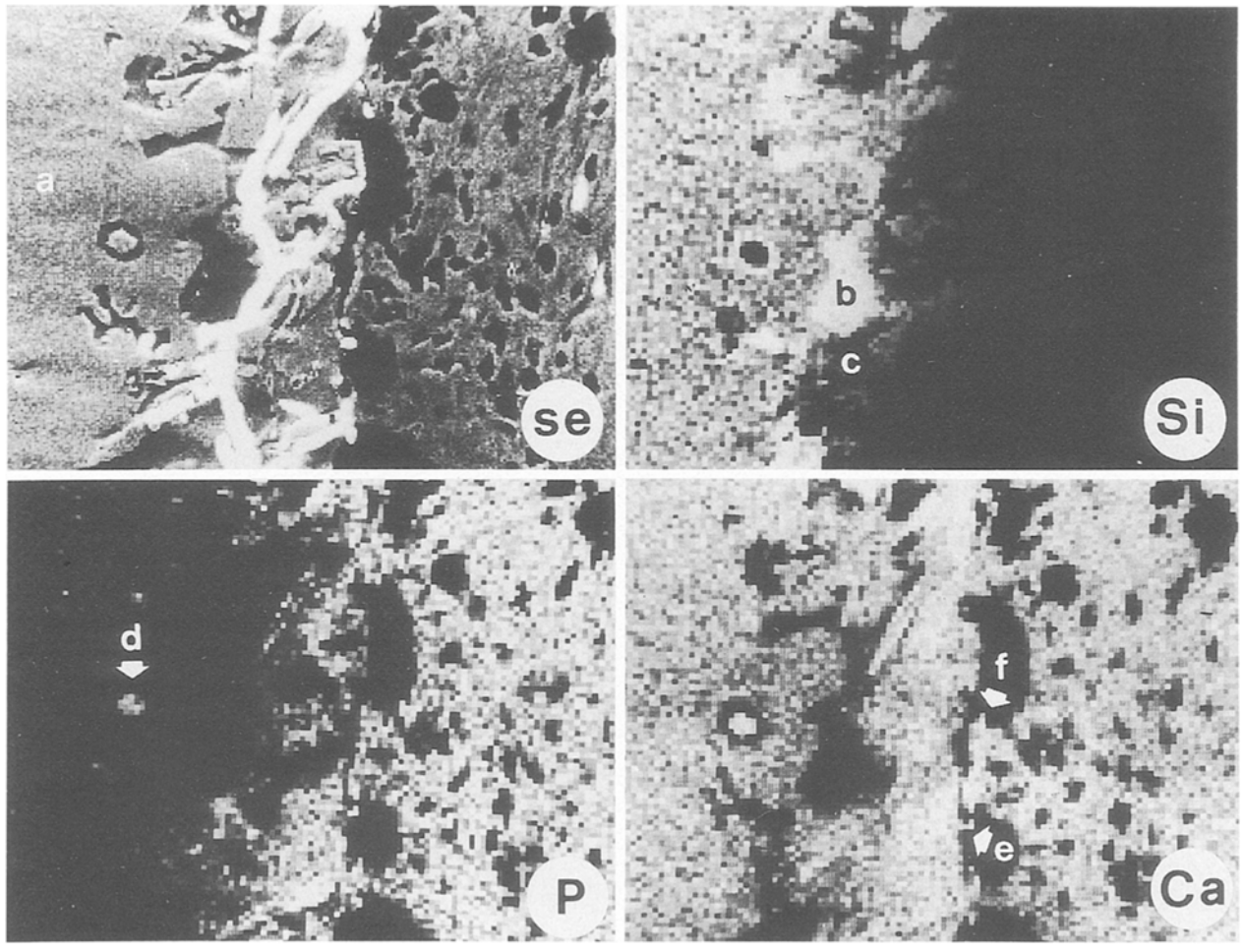

Figure 5 Digital SEM micrograph (se) and digital X-ray dot maps for Si (Si), P (P) and Ca (Ca) of a sample of this glass implanted in the rabbit femur (field width 0.35 mm). The letters indicate the sites in which X-ray spot analyses were carried out. In the P image the arrow points to a needle-shaped structure transversally sectioned.

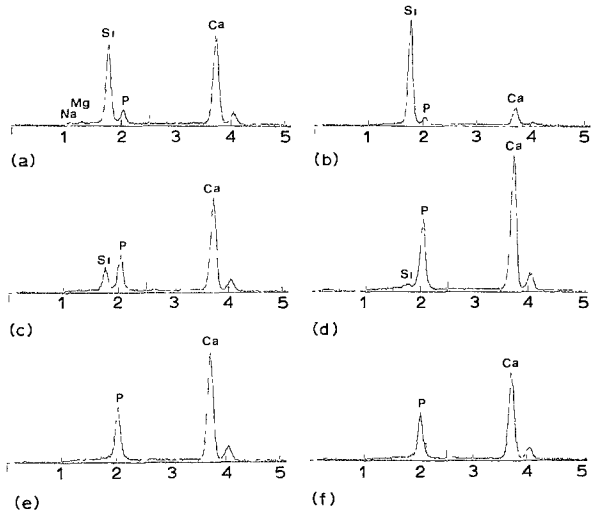

Figure 6 X-ray spot microanalyses carried out in the sites indicated in Fig. 5, recorded with the same preset time and plotted at the same full scale: (a) non-implanted glass, (b) Si-rich point, (c) Si-poor point, (d) needle-shaped structure, (e) glass close to the bone and (f) bone in contact with the glass.

close to it was surely impoverished of Ca^{2+} and silica. As a consequence it enriched itself with the other components (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} and F^{-}) that are easily solubilized and involved in the metabolic biochemical chains of the neighbouring cells; this may promote a local bioactivity that leads to the formation of calcium-phosphatic precipitates. However, the precipitation of calcium-phosphatic salts from the outer physiological fluids was shown to be greatly increased on those specific sites of the glass surface where there come out parts of needle crystals already having a calcium-phosphatic composition. Such precipitation tends to involve all of the free surface of these crystals, and the precipitation seems to be favoured and the precipitated material stabilized by its partially direct incorporation in the growing crystals. The glass matrix also interacts with the neighbouring tissue, but at a biochemical kinetic rate slower than that of other glasses with higher Na content.

In the glass studied, with a high annealing temperature similar to the apatite–wollastonite glasses [13], the refractoriness corresponds to their higher thermodynamical stability, which in turn gives rise to a general lower activity of ionic exchange with the physiological fluids of the environment. An important question is whether the expected lower ionic release permits better bone growth around the glass with respect to less-refractory glasses. In any case, the amount of external needle-shaped structures (probably crystals of hydroxyapatite) observed on the surface allows a precipitate deposited from biological

fluids surrounding the implanted sample to be thought of as the unique logical source.

In conclusion, this fluorinated glass seems to be promising for clinical applications. At present it may be used as granules for bone defect repair only; for use as coating, the glass must be suitably doped with elements conferring to it a capability of developing good adhesion to any specific substrate, obviously without loosing its biological characteristics. The study of its behaviour in implantation evidences the importance of the ingrowth of the crystalline phases within the matrix of the glass. On the basis of what has been observed, the bioactivity of this kind of glass–ceramic system can be controlled by intervening on the formulation of the composition to induce the formation of a suitable amount of the observed micro-crystals and of a suitable ratio between wollastonitic and calcium-phosphatic ones.

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