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Granules of osteoapatite and glass-reinforced hydroxyapatite implanted in rabbit tibiae

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Granules of a modified hydroxyapatite, glass-reinforced hydroxyapatite composite and commercial hydroxyapatite were implanted in rabbit tibiae. Histological studies were carried out after 1 and 2 months implantation periods using light and fluorescence microscopy. A much higher percentage of bone contact was developed for both the glass-reinforced hydroxyapatite composite and the modified hydroxyapatite when compared to commercial hydroxyapatite (89–91% versus 66%) after 2 months implantation. The mechanism of bone formation and growth around implants is discussed in terms of the influence of elements incorporated into these novel materials which are commonly found in bone tissues, such as Na, K and Mg, and the presence of a soluble β-tricalcium phosphate phase in the microstructure of the composite.

1. Introduction

Hydroxyapatite is a calcium phosphate which has been used as a bone substitute for many biomedical applications [1], both as granules and block materials [2]. Granules allow for bone reconstruction and bone apposition on the material surface. Several chemical modifications have been tried to increase the bioactivity of hydroxyapatite [3]. Bioactive materials with higher solubility than HA, such as β and α tricalcium phosphate structures, for example, have demonstrated much faster osseointegration [4]. However, a very high solubility rate may produce rapid material biodegradation before the formation of new bone on the material surface. Therefore, there is a need to prepare implant materials which can promote high bioactivity without great resorption. The aim of this work is to study two novel materials which may fulfil this condition.

2. Materials and methods

Granules of three different bioactive materials were prepared and implanted in rabbit tibiae: a commercial hydroxyapatite powder (HA) supplied by Merck, an osteoapatite and a glass-reinforced HA composite. The osteoapatite is a modified HA which incorporates the following oxides, in wt%, Na_2O-3.1, K_2O-1.0, Fe_2O_3-0.34 and MgO-0.7 [5]. The glass-reinforced HA composite is prepared by a liquid phase sintering process [6], with the addition of 2.5 wt% of 45.0-P_2O_5, 28.0-CaO, 27.0-Na_2O glass to HA powder. The powder of each material was uniaxially pressed at 200 MPa and sintered at 1250 °C for 1 h. Samples were then milled to produce granules of 0.15–0.85 mm size.

Phase content of each material was analysed using X-ray diffraction analysis (XRD), and only hydroxyapatite phase was detected for both commercial HA and osteoapatite. The microstructure of the glass-reinforced HA composite was composed of a mixture of β-tricalcium phosphate and HA [6]. In order to determine changes of pH, 0.5 g of each material was immersed in 50 ml deionized water at room temperature for 4 h, with an initial pH of 6.1. The pH was continuously monitored throughout the test.

The *in vivo* response of these ceramic materials was assessed by implanting granules in the right posterior tibiae of adult rabbits. All rabbits were operated on by a standard procedure in aseptic conditions. They were given intramuscular anesthesia (Ketalar and Metazolam) complemented with local anesthesia using 1.8 ml of 2% lidocaine/adrenaline solution. After a 10 cm skin incision and periosteal flap to expose the anteromedial face of the tibial proximal metaphysis, the implant site was prepared with a spherical burr with continuous cooling. The hole was then well

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packed with the granules of the sample materials and the wound was closed in two layers. Ten days before sacrifice, the animals received 20 mg/kg body weight of tetracycline (Oxitetracicline) subcutaneously as a marker of new bone formation.

Rabbits used in this assays were sacrified 4 and 8 weeks after the surgical procedure with an overdose of intravenous Pentobarbital. The bone blocks were immersed in 10% neutral buffered formalin for 24 h. Subsequently, specimens were dehydrated in a series of alcohols and embedded in a mixture of methylmethacrylate, plastoid N and Perkadox. After polymerization, specimens were sectioned with a diamond saw to a thickness of about 250–350 μm and ground down to about 40 μm with a polish superfine disc, ref. 3M, SF 737. Slices were then stained with hematoxylin and eosin. Histological characterization was performed under light and fluorescence microscopy to detect newly formed bone. Percentage of bone contact was measured on 50 implanted granules using a curvimeter device.

3. Results

Fig. 1 shows the XDR results for the three types of ceramic granules after sintering.

No phase transformation was detected for both commercial hydroxyapatite and osteoapatite. The glass-reinforced hydroxyapatite composite showed a biphasic structure composed of HA and β-tricalcium phosphate. A phase proportion in the microstructure of HA-65% and β-tricalcium phosphate-35% was determined using relative intensities of the three main peaks of each phase [6].

The changes of pH in deionized water versus immersion time is presented in Fig. 2.

Both the osteoapatite and the glass-reinforced hydroxyapatite composite provoked an increase in pH towards alkaline values. This pH increase was detected a short time after immersion, i.e. after the first 5 min. No significant change was monitored for the commercial hydroxyapatite.

Macroscopic observations showed new bone formation around implanted granules without any infection or inflammatory response. However, percentage of bone contact measurements were much higher for both the osteoapatite and glass-reinforced hydroxyapatite than commercial apatite, as shown in Table I.

Fig. 3a–3c shows typical undecalcified sections of the three materials after 2 months implantation.

New bone (NB) can be clearly distinguished from old bone (OB) as it appears not too well organised and with a light red colouration, as presented in Fig. 3b. Newly formed bone was also revealed by the yellow-coloured tetracycline fluorescence (see Fig. 4)

While for commercial hydroxyapatite, gaps among granules are clearly seen, particularly when pronounced angles are present, osteoapatite granules are almost completely surrounded by newly formed bone with cuboidal-shaped cells, probably active osteoblasts. The glass-reinforced HA granules seem to have suffered some surface dissolution since their tips

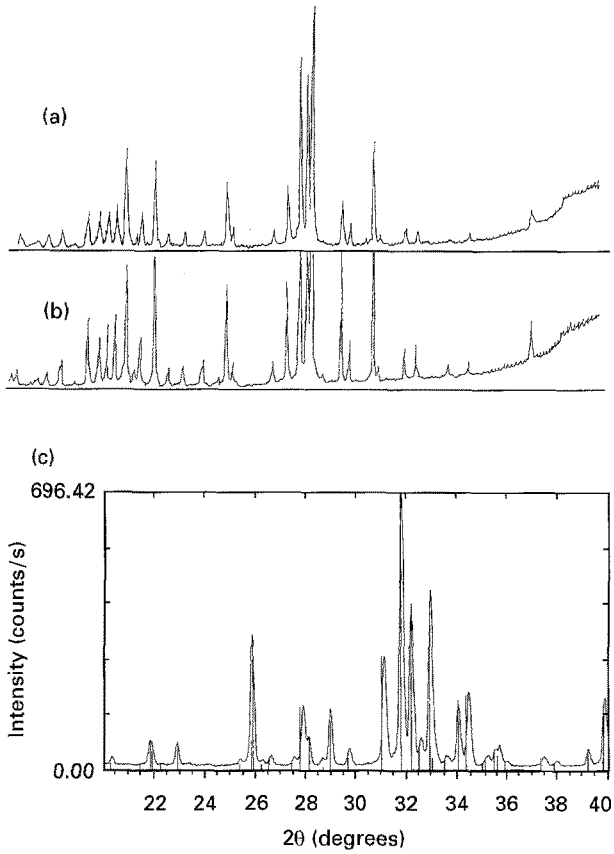

Figure 1 X-ray diffraction analysis of the ceramic granules after sintering at 1250 °C: (a) hydroxyapatite; (b) osteoapatite; (c) glass-reinforced hydroxyapatite.

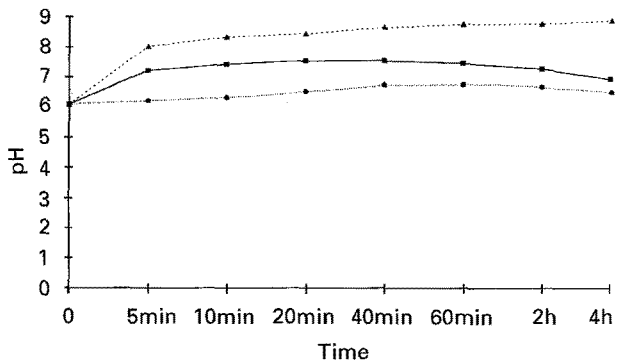

Figure 2 pH variation versus immersion time for each ceramic material: ● HA; ▲ osteoapatite; ■ glass-reinforced HA.

TABLE I Percentage of bone contact with implant materials after 1 and 2 months implantation

	Bone contact after	
	1 month (%)	2 months (%)
Commercial HA	64 ± 7	66 ± 9
Glass reinf. HA composite	90 ± 9	89 ± 8
Osteoapatite	82 ± 12	91 ± 9

appear rounded off after implantation and the darker zone around particles is possibly due to some resorption.

4. Discussion

Both the glass-reinforced HA composite and osteoapatite granules promoted much higher bone

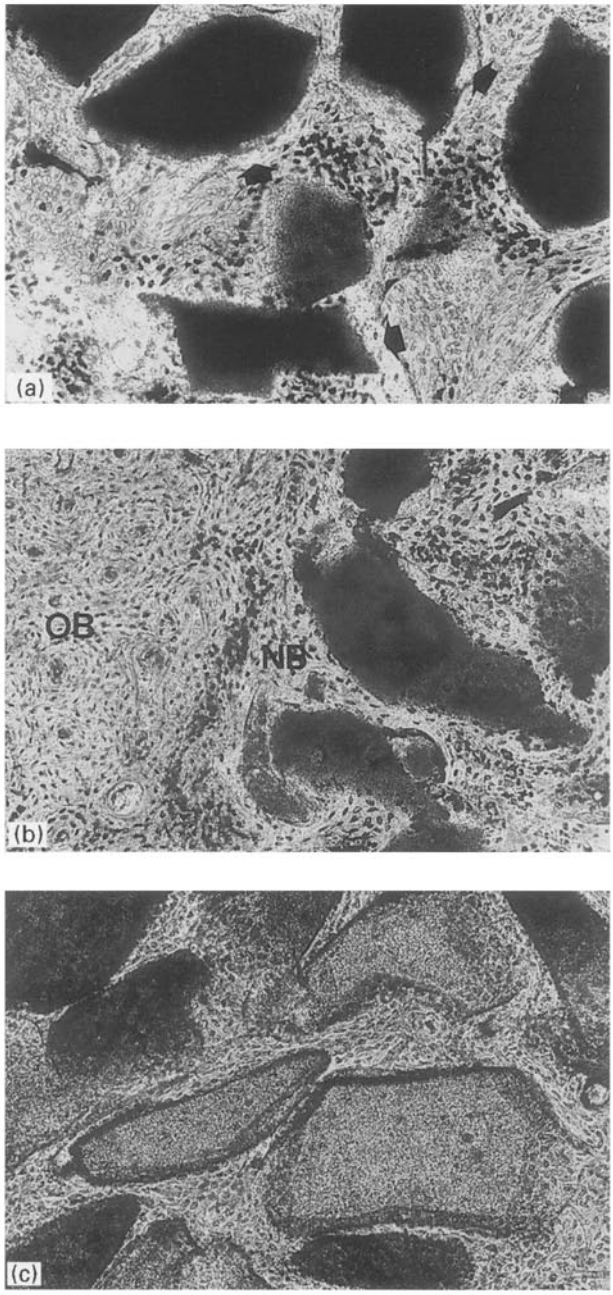

Figure 3 Undecalcified sections after a 2 month implantation period. Some gaps are clearly seen among hydroxyapatite granules (a) (see arrows). Higher bone apposition was detected for both osteoapatite (b) and glass-reinforced hydroxyapatite composite granules (c). (×100)

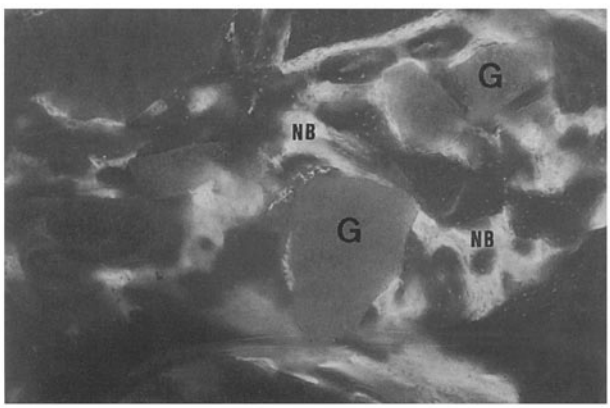

Figure 4 Fluorescence microscopy showing new bone formation (NB) yellow-coloured around glass-reinforced HA granules (G). (×100)

formation than unmodified commercial HA around implants. Because of the difference in the composition of the materials, chemically pure HA does not change the pH level, particularly in the first few hours, while osteoapatite provokes an immediate increase of pH level and the release of Na^+ and K^+ ions. The glass-reinforced HA sample does not significantly change pH values. Although the mechanism of bone formation and growth is not yet clearly understood for these novel materials, two different approaches may be applied which may explain the biological behaviour observed. Recent work has demonstrated [7–9] that composites with a biphasic structure, based on HA and β-tricalcium phosphate phases, exhibit higher biological response than sintered HA. It is believed that, the highly soluble β-tricalcium phosphate phase causes local Ca and P enrichment, which then reprecipitates with proteins from the biological fluid enhancing the formation of new bone [9]. A similar mechanism is apparent for glass-reinforced HA composite granules as some surface dissolution occurred after implantation, while for HA granules no dissolution was observed. This dissolution can be proved by the fact that most granules became smaller and rounder after implantation. Similar findings were detected by LeGeros *et al.* [9] for biphasic HA/β-tricalcium phosphate structures, where not only the size of the granules decreased after implantation but also some of the smaller granules disappeared, particularly for higher β-tricalcium phosphate/HA ratios.

The better biological performance of osteoapatite when compared to commercial HA may be explained in terms of its chemical similarity to the mineral part of bone. In fact, osteoapatite contains a great variety of trace elements such as Na, K and Mg which enhances ion exchange and bone formation at the bone–implant interface. On the other hand, osteoapatite promotes a change in the pH of the medium towards alkaline values, inducing early calcium phosphate precipitation on the material surface. This effect on the pH of the medium and the ion leaching have been shown in a previous *in vitro* study [5].

5. Conclusions

Glass-reinforced HA composite and osteoapatite granules exhibit greater biological activities than commercial HA. This enhancement of bone growth was achieved without any detectable degradation of the osteoapatite granules and with only a slight solubility of the glass-reinforced HA material.

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