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Guided bone regeneration using osteopatite® granules and polytetrafluoroethylene membranes

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Granules of a modified hydroxyapatite, Osteopatite®, were implanted in the right posterior tibiae of adult rabbits. We studied the extent of bone regeneration in bone holes. In the right tibiae, that were filled with granules of this biomaterial covered with a polytetrafluoroethylene (PTFE) membrane using, as a control, uncovered granules. In the left tibia, an empty hole was covered with PTFE membrane and a second hole was left empty to be used as a control. A histomorphometric study was carried out using light microscopy, four and eight weeks after the surgery. The covered granules presented a higher percentage of bone contact than the uncovered ones, and it was also possible to observe a better bone tissue organization, mainly produced by the immobilization action of the PTFE membrane. Empty bone defects covered with PTFE membranes, two months after implantation, presented large areas of Haversian bone and direct bone contact to the PTFE membrane.

1. Introduction

In previous work [1, 2], the mixing of granules of hydroxyapatite with calcium sulfate (plaster of Paris) with a solution of K_2SO_4 (3 wt %) produced a paste, that could be spread on bone surfaces thereby ensuring that the granules were immobilized during initial bone healing.

The use of this paste to fill large bone defects, appears to be inefficient because the plaster is reabsorbed in two to three weeks, and bone requires more time to regenerate and heal in these conditions. The use of a membrane could be an alternative to the use of the plaster of Paris. The use of a membrane is a widespread method for guided tissue regeneration in implant surgery [3–5] which can be extended for use with bone filling biomaterials, such as hydroxyapatite.

The aim of this work is to study bone regeneration using modified hydroxyapatite granules, covered with a polytetrafluoroethylene (PTFE) membrane and compare these results with those obtained with uncovered particles under the same conditions. In previous work [2, 6] this biomaterial has been shown to promote faster bone healing and we are interested in finding out whether any further improvement can be obtained.

This *in vivo* study was performed on rabbits. The results showed a higher percentage of bone contact when the granules were covered with a PTFE membrane as will be discussed in detail in this paper.

2. Materials and methods

Osteopatite® is a modified hydroxyapatite with several ions added to the basic apatite formula. The composition of Osteopatite® matches that of bone hydroxyapatite: Na_2O–3.1, K_2O–1.0, Fe_2O_3–0.34 and MgO–0.7 wt%. This material is also microporous thereby allowing the possibility of ionic release from within the granules.

The membranes, with a thickness of 0.2 mm, were prepared by sintering very thin strips of commercial PTFE. This treatment increases the thickness of the material thereby providing a stiffness level suitable for surgical applications.

A careful control of the sintering temperature was necessary to ensure the adhesion of the strips without melting the material. The membranes prepared by this technique are fully continuous. Osteopatite® granules with sizes in the range of 0.15–0.85 mm, were implanted in bone holes that had been drilled in the posterior tibiae of 10 adult rabbits. All the rabbits were operated on using a standard procedure in aseptic conditions. They were sedated with an intramuscular anaesthesia (Ketalar and Metazolam), complemented with a local anaesthesia, a 2% lidocaine/adrenaline solution. After a 10 cm skin incision a periostal flap was raised thereby exposing the anteromedial face of the tibial proximal metaphysis, two holes were drilled with a continuously cooled spherical burr in each posterior tibiae. All the holes had a diameter of 5 mm.

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In the right bone, one hole was densely packed with granules and covered with a PTFE membrane. As a control, the other hole was only packed with granules but no membrane was used. In the left bone, two holes were drilled under the same surgical conditions. One, was covered with a PTFE membrane but did not contain any filling materials and, the other was left empty and uncovered to be used as a control.

The rabbits were sacrificed either four or eight weeks later with an overdose of intravenous Pentobarbital. The bone blocks were immersed in a 4 wt% neutral buffered formalin solution for 24 h. Subsequently the specimens were dehydrated using a graded series of ethanol washes and embedded in a methylmethacrylate resin. After the polymerization, specimens were sectioned with a diamond saw to a thickness of about 250–350 μm and were subsequently ground to a size of about 30 μm with a polish superfine disc. Slices were then stained with hematoxylin and eosin. The histological characterization was performed using light microscopy to detect newly formed bone. Samples were analysed one and two months after implantation and the percentage of bone contact was measured on 40 implanted granules using a curvimeter device.

3. Results and discussion

The presence of some interstitial or substitution ions in the original Osteopatite® crystalline structure highlights the possibility of ionic release without dissolution of the hydroxyapatite crystal. As was previously reported the release of alkali ions from the granules, via ion exchange with H_3O^+, results in an increase of the pH around the particles [1]. This mechanism induces the precipitation of Ca^{2+} from the medium resulting in the formation of a natural hydroxyapatite film on the surface of the granules. This behaviour results in a material with an increased bioactivity but which is not absorbable as is usual with other bioactive ceramics.

As a result of its composition and the presence of microporosities, Osteopatite® is an excellent bone filling material. The consequence of this great bioactivity is that even in the worse conditions, the uncovered hole, the regeneration of bone was significant.

The membranes generally used in surgery are made of expanded polytetrafluoroethylene (PTFE) [4–6]. The use of these membranes is influenced by four factors [7]: (a) the barrier material should be of sufficient stiffness and pore size to ensure the desired volume of the compartment and to enhance blood clot stabilization, (b) healthy vascularized bone, (c) immobilization of the membrane and submerged healing, and (d) an appropriate healing time.

The membranes used in this work are also made of PTFE, and have all the characteristics referred to above, except for the high porosity which does not appear to be necessary for the healing process. In fact when used in oral surgery, since the oral cavity is a bacterial rich medium, micro-organisms may colonize and extensively invade the open microstructure of the PTFE membranes [8, 9]. All the animals investigated in this study healed well, with no infection being observed during the two-month period, and in addition no signs of inflammatory cell infiltration were observed in the area surrounding the implanted materials. One month after implantation differences between the filled defects and the control samples could be observed. However after two months these differences were less evident. The granules of Osteopatite® covered with the PTFE membranes showed 100% bone contact, one month after implantation. The granules that were not covered had, one month after implantation, 84 ± 7.1% of bone contact, and after two months this figure rose to 92 ± 6.7% (Table I).

TABLE I Percentage of bone contact, one and two months after implantation

	One month	Two months
Osteopatite® granules covered with PTFE membrane	100%	100%
Uncovered Osteopatite® granules	84 ± 7.1%	92 ± 6.2%

The samples in which the granules were covered with a membrane had more particles per volume, compared with the uncovered samples. Consequently, the space between particles was smaller allowing these small gaps to be filled with new bone (Fig. 1 (a and b)). The membrane seems to be useful to ensure the immobilization of the granules avoiding their dispersion around the defect.

When the empty bone defects were covered with PTFE membranes it was possible to observe, one month after implantation, bone regeneration under the membrane with large areas of osteoid bone (Fig. 1c) that filled approximate 22% of the cavity.

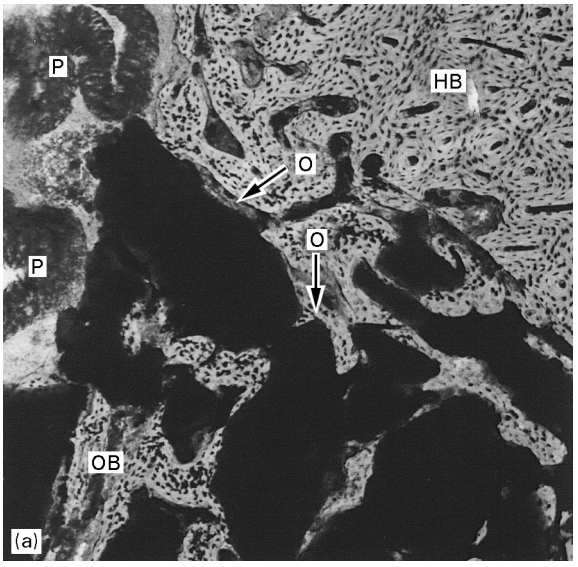
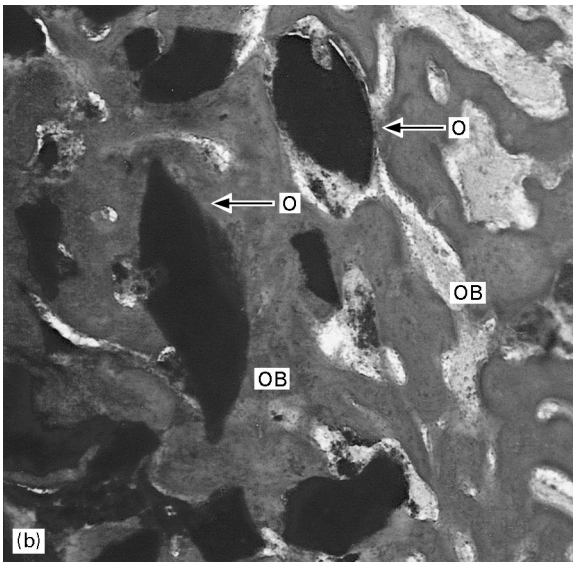


Figure 1 Microscopic view of undecalcified section one month after implantation (X40). (a) The granules (O) are completely surrounded by osteoid bone (OB) and the close proximity and the large number of granules present is evident. (b) Uncovered granules surrounded by osteoid bone. It is possible to observe fewer granules per volume of bone and some gaps between the bone and the granules. (c) Bone regeneration under the PTFE membrane. (*O – Osteopatite® granule, OB – Osteoid bone, IIB – Haversian bone, P – PTFE membrane; distance bar 100 μm*).



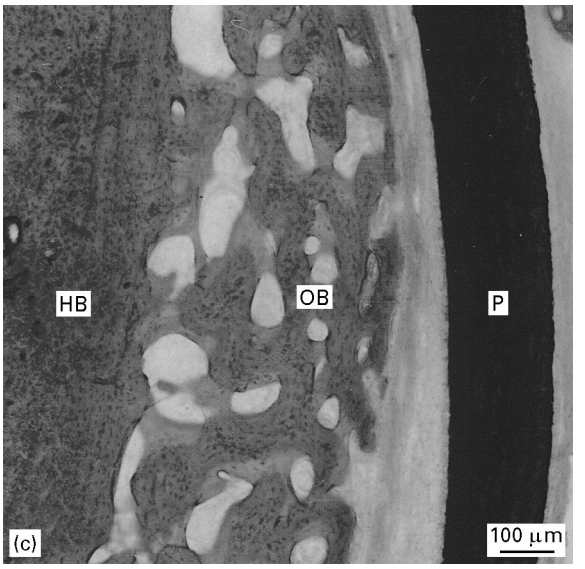
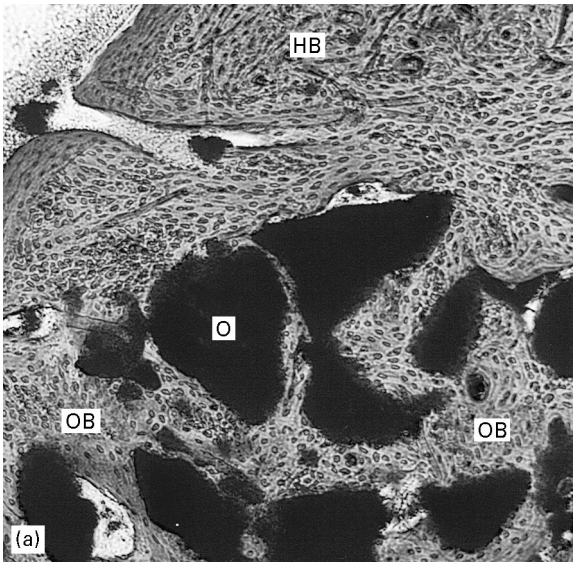


Figure 1 (continued)



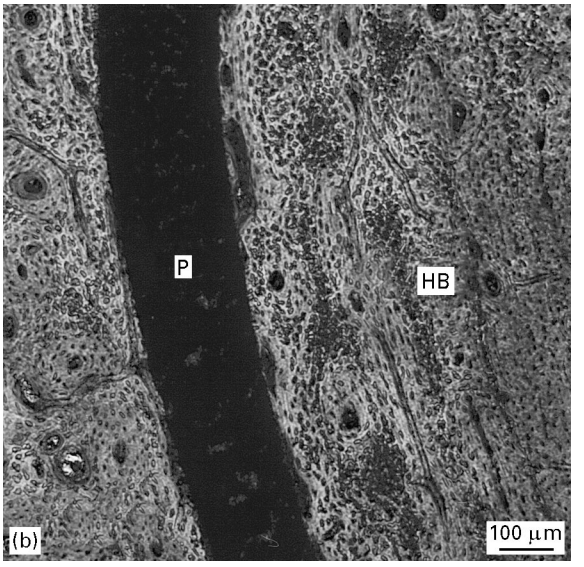


Figure 2 Microscopic view of undecalcified section two months after implantation (X40). (a) Uncovered granules surrounded by osteoid bone and some Haversian bone. Notice the presence of large areas of Haversian bone (HB). However we can observe fewer Osteopatite® granules (O) compared to when a PTFE membrane is used. (b) The PTFE membrane (P) is completely surrounded by new Haversian bone (HB) without fibrous tissue encapsulation. (*O – Osteopatite® granule*, *OB – Osteoid bone*, *IIB – Haversian bone*, *P – PTFE membrane*; *distance bar 100 μm*).

This should be contrasted to the behaviour of the empty and uncovered holes, that showed no signs of bone growth one month after implantation.

At the end of two months, it was possible to observe haversian bone formation in the case where the granules were covered by the PTFE membrane (Fig. 2a). However, the surrounding bone tissue of the uncovered granules, showed areas of osteoid tissue and less Haversian bone. These differences are more noticeable one month after implantation (Fig. 1(a and b)).

Two months after implantation, the PTFE membrane was surrounded by new bone without fibrous tissue encapsulation and it was possible to observe a significant amount of Haversian bone (Fig. 2b). In the control bone hole, which was not covered by a PTFE membrane, it was possible to observe a delay in the bone healing process with the presence of more areas of woven bone, and fewer areas of lamellar bone being observed. If the study of bone regeneration in rabbits exceeds eight weeks, the test sites will heal at the same time as the control sites [10]. This is the reason why this study was terminated eight weeks after the implantation.

4. Conclusions

It was possible to demonstrate that commercial PTFE could be used to prepare a membrane adapted to surgical needs. The porosity of the membranes used by other researchers appears to be unnecessary to obtain a good material for guided tissue regeneration.

The use of PTFE membranes produces a barrier effect, avoiding soft tissue proliferation into the bone defects and in addition immobilizing the granules prevents their movement, improving significantly, the percentage of bone contact. Even in the case of empty

bone defects covered with PTFE membranes, some areas of woven bone were present, one month after implantation and at two months, it was possible to observe large areas of lamellar bone.

This study indicates that the combined use of granules of Osteopatite® and PTFE membranes enhances bone regeneration and its organization.

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