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Evaluation of tricalciumphosphate/hydroxyapatite cement for tooth replacement: an experimental animal study

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A calciumphosphate cement, consisting mainly of tricalciumphosphate (85% α-TCP and 15% β-TCP), was inserted in 16 surgical defects created in the tibia of goats. X-ray diffraction (XRD) and energy dispersive spectroscopy (EDS) showed that after 3 months of implantation the α-TCP was transformed to hydroxyapatite (HA). Histological evaluation revealed that the presence of cement stimulated the ingrowth of bone compared with unfilled cavities. Active resorption and remodelling of cement particles was observed. The cement did not evoke an inflammatory reaction. At 6 months after implantation no further changes in the composition of the cement occurred. All remaining material was surrounded by mature bone.

1. Introduction

Retention of complete dentures can be provided only when sufficient alveolar jaw bone is maintained after the loss of the natural teeth. During the last few decades several procedures have been developed to delay alveolar bone resorption. The main principle behind these methods is to keep the alveolar tooth sockets filled with a synthetic material to prevent collapsing of the buccal and lingual socket walls. In most studies, solid prefabricated hydroxyapatite cones are used [1–4]. This method, however, suffers from some serious disadvantages concerning the initial fit and proper fixation of the implants. An alternative method is the use of mouldable calciumphosphate cements as a space maintainer between the socket walls. These cements set in *in situ*, are highly compatible with bone and are supposed to resorb slowly, while being replaced by bone [5–10]. However, for practical applications, the properties of the currently available calciumphosphate cements are still not adequate. Problems are encountered with the mechanical strength, setting time, delivery and maintenance in the bone defect, and the final biological behaviour. Recently, Driessens *et al.* [11, 12] developed a calciumphosphate cement in which some of these problems are overcome. For example, the setting time is reduced (7–13 min) and the compressive strength is improved (41 ± 4 MPa).

The purpose of the present study was to obtain data on both physicochemical and biological properties of this cement implanted in artificial bone cavities in goat tibia.

2. Materials and methods

2.1. Tricalciumphosphate/hydroxyapatite cement

The solid component of the calciumphosphate cement consisted of a powder which contained tricalciumphosphate and hydroxyapatite. The cement was mixed with disodium phosphate solution to a paste which could be applied and moulded with a spatula into the surgical defect. The initial setting time was 7 min and the final setting time was 13 min. The final compressive strength, reached after 15 h, was 45 MPa.

The chemical composition of the cement, directly after mixing and after storage for 24 h in Ringers solution, was characterized by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and energy dispersive spectroscopy (EDS).

2.2. Animals and surgical methods

For the animal study four adult goats were used, equally divided over two experimental periods: 3 and 6 months. Under general anaesthesia, a longitudinal incision was made on the medial side of the left and right tibia. After exposure of the cortical bone, five pilot holes were drilled into the right and four into the left tibia. These holes were gradually widened to a final diameter of 1.5 cm. The bone preparation was performed with a very gentle surgical technique and continuous cooling. After preparation the holes were irrigated and then packed with sterile cotton gauze to stop bleeding. Subsequently four holes in the right and all holes in the left tibia were filled with cement. One

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hole in the right tibia was left unfilled to serve as control. Following the setting of the cement, the soft tissues were closed in separate layers. Finally, radiographs were taken. To reduce the perioperative infection risk, prophylactic antibiotic Albipen® was administered for 3 days starting 1 h postoperatively.

2.3. Histological procedures

At the end of the experimental periods, the animals were sacrificed and the tibias excised. Following fixation in 10% buffered formalin solution, blocks of tissue containing the filled and empty holes were sawn out. Subsequently, these specimens were embedded in methylmethacrylate. After polymerization, non-decalcified serial sections were made using a modified circular saw microtome technique [13]. Sections were made in a transversal plane perpendicular to the surgical defects and proceeding from the periosteal to endosteal side. The sections were stained with methylene blue and basic fuchsin. Histological evaluation of the sections was done by light microscopy.

In addition, on six of the retrieved samples XRD and EDS measurements were carried out to obtain information about structural changes during the implantation period.

3. Results

3.1. Physicochemical structure

Fig. 1 shows the XRD pattern of the cement powder and of the cement after setting and storage for 24 h in Ringers solution. It can be seen that the cement powder was composed of about 85% α- and 15% β-TCP with some hydroxyapatite. When incubated in Ringers solution, the α-TCP transformed into hydroxyapatite. Fig. 2 shows XRD patterns of the cement 3 and 6 months after implantation. In the 3-month specimens, the peaks were broadened. The α-TCP had almost completely disappeared. The rest of the material consisted of about 85% hydroxyapatite and 15% β-TCP. At 6 months, no further changes of the crystal structure were observed.

FTIR-spectroscopy revealed, for the powder as well as in the Ringers soaked cement, two clusters of P–O peaks from 900–1150 and 550–600 cm^{-1}. Furthermore, at 3750 cm^{-1} a small OH-peak was found (Fig. 3).

EDS measurements indicated that, before implantation, the Ca/P ratio of the set cement was 1.67. At

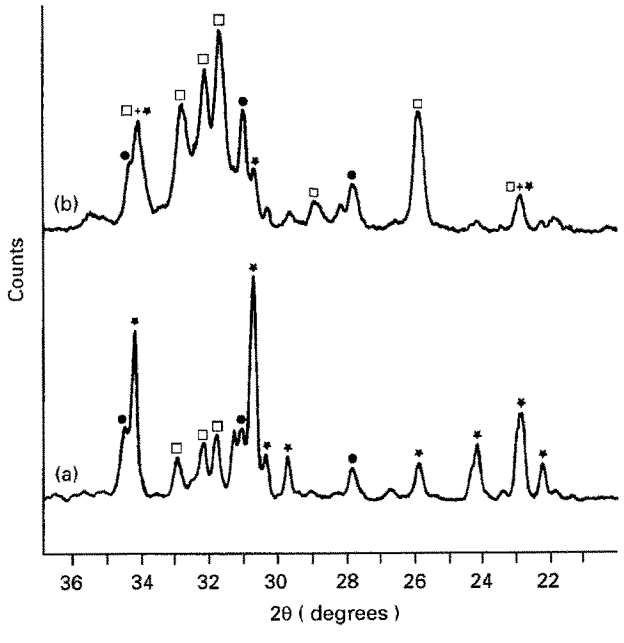


Figure 1 X-ray diffractograms of (a) the cement powder and (b) cement after storage for 24 h in Ringers solution: * = α-TCP; ● = β-TCP; □ = HA.

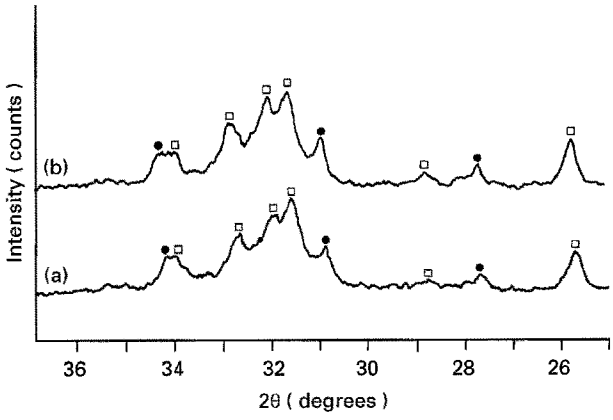


Figure 2 X-ray diffractograms of the cement (a) 3 and (b) 6 months after implantation: ● = β-TCP; □ = HA.

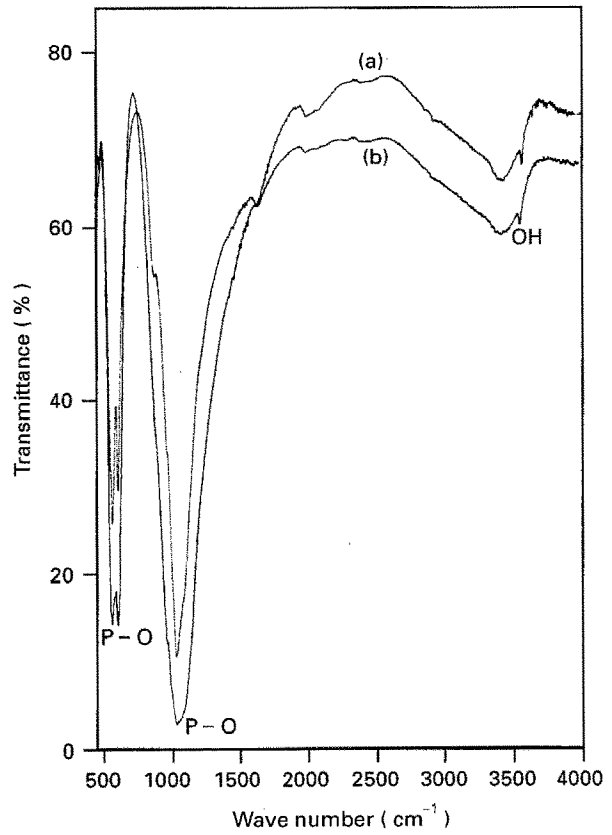


Figure 3 FTIR spectra for (a) the powder and (b) cement after storage in Ringers solution.

3 and 6 months after implantation, the Ca/P ratio was increased to 1.78 ± 0.1. No other elements than Ca and P were found. The Ca/P ratio of the surrounding bone was 1.85 ± 0.1.

3.2. Light microscopic evaluation

Microscopic evaluation of the 3-month specimens revealed incomplete filling of the created defects (Fig. 4). The sections of the periosteal and endosteal bone side showed that the cement had extensively disappeared. In these areas, the cement was found only at the outer margins of the hole. In the midsections, the cement did not always match exactly the circular shape of the drilled hole. Nevertheless, in all specimens a considerable amount of the material was retained. The missing material was completely replaced by new bone. The structure of this bone did not yet correspond to the surrounding bone. The newly formed bone was found to be in intimate contact with the ceramic particles of the cement surface. Apparently, the cement formed part of the bone remodelling process. In some areas, active remodelling was characterized by the presence of osteoclasts and osteoblasts, resorption of the cement, and deposition of osteoid (Fig. 5). The cement never elicited an inflammatory reaction.

By 3 months, the empty control holes were incompletely filled by new bone. In the sections, bone deposition was observed only on the surrounding walls of the drilled hole, leaving the centre free (Fig. 6).

At 6 months after cement installation, Haversian remodelling of the new bone had occurred. In all sections well-organized bone, characterized by the presence of mature secondary osteons, were observed (Fig. 7). Although, similar to the 3-month specimens, in some of the holes cement loss was observed, substantial amounts of the cement were present. Formation of a fibrous capsule around the cement material

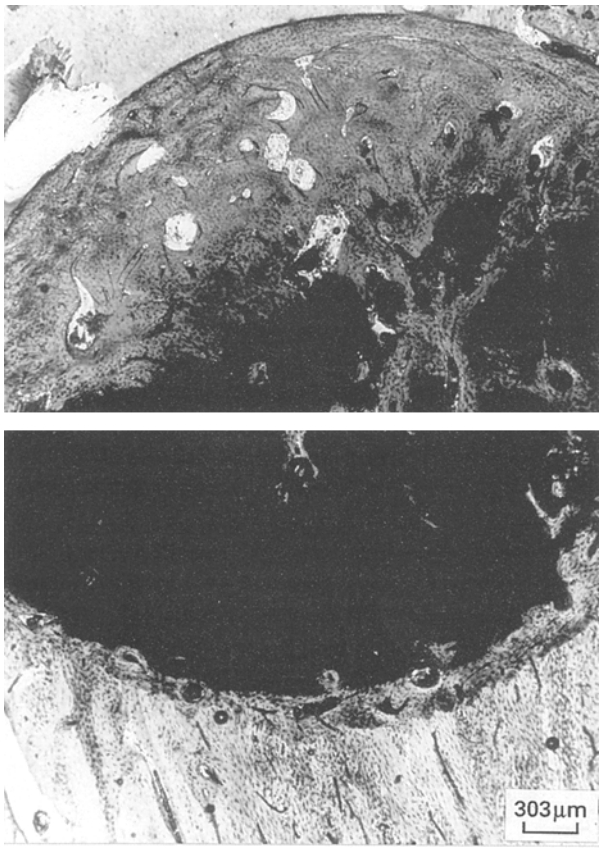
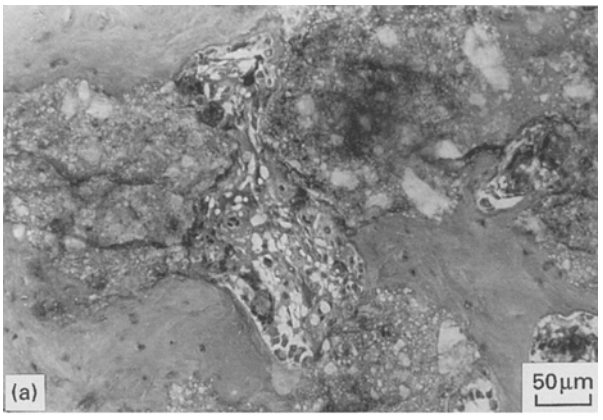


Figure 4 Light micrographs of a 3-month specimen. At one side of the defect no cement is present, while the other side is still completely filled.



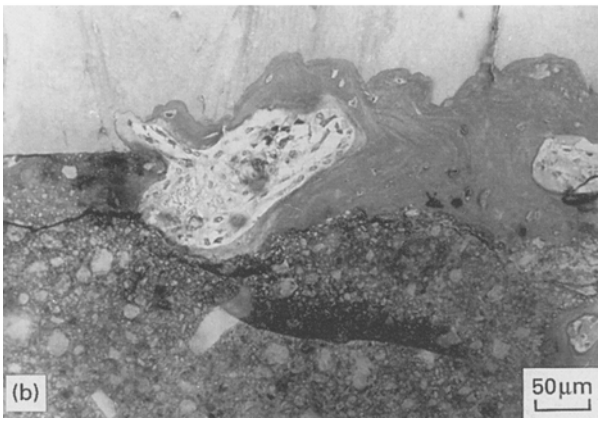


Figure 5 Photomicrographs showing active remodelling in the 3-month specimens. This remodelling occurred in the central area (a) as well as peripheral areas (b) of the defects.

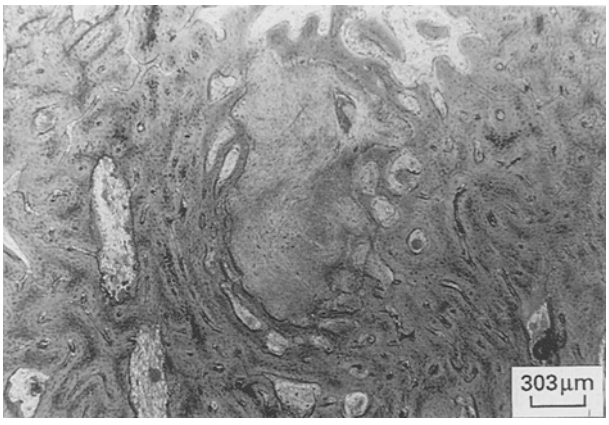


Figure 6 At 3 months, the control holes were incompletely filled. No bone was present in the centre of the defect.

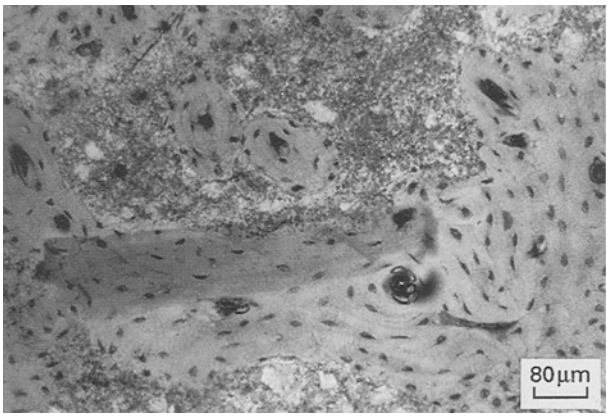


Figure 7 At 6 months, bone remodelling had proceeded. Secondary osteons and a close bone/cement contact were observed.

was not observed in any section. The interface was composed of bone, no osteoclasts being present in the sections examined.

Evaluation of the 6-month control specimens revealed that these unfilled holes were difficult to localize. The holes were completely covered with matured bone.

4. Discussion and conclusions

The α-TCP component of the cement powder used in this study was observed to transform into HA both under *in vitro* and *in vivo* conditions. This phenomenon has already been described earlier [9]. In our experiments, we found that, apparently *in vivo*, this process is completed within 3 months after application of the cement. The histological evaluation showed that during these first 3 months, the cement was actively resorbed by osteoclasts. Resorption of Ca–P ceramic is determined by the physico-chemical nature of the ceramic and the environment in which the cement is placed. For example, it is known that TCP is much more resorbable than HA [14, 15]. Further, a decreased pH during the initial stages of the wound healing process can influence the degradation process of Ca–P ceramics. The biological examination at 6 months showed that the resorption and bony apposition to the ceramic cement particles was almost completed. In these sections no signs of active remodelling with involvement of osteoclasts and osteoblasts were observed. This observation is especially important for the final application of Ca–P-based bone cement. After all, in situations where the cement is used as a gap filler around orthopaedic or dental implants, a delay in replacement of the cement by natural bone can result in a weakening of the mechanical properties of the bone resulting in decreased loading conditions. In addition, when used as a bone filler for tooth extraction sockets, a lag in the course of cement resorption will complicate the installation of dental implants at a later moment.

The tested cement showed very biocompatible bone behaviour. At no time were inflammatory or foreign-body reactions present, and the ceramic particles had become covered with bone. This observation corresponds with the earlier findings of Munting [7] and Koshino [16]. Similar to our experiments, in both studies powders made of TCP were used. On the other hand, Yoshimine [8] and Kurashina [9] found that cements based on TCP powder were surrounded by fibrous tissue. As already suggested by Kurashina [9], probably the hardening of the cement by mixture with acid, as done in these last mentioned studies, is responsible for this fibrous tissue formation. The final prepared cement becomes too acidic resulting in an incompatible behaviour. For this reason, mixing with sodiumphosphate or water is to be preferred.

The EDS data obtained from the various samples were slightly increased compared to the expected values. This discrepancy is in line with earlier publications [17, 18] discussing the accuracy and difference of this analysis technique compared with other methods.

Finally, in the prepared histological sections, incomplete filling of the created defects with cement was observed. Probably this problem occurs because the cement paste comes in contact with blood before it is set. Evidently, the bone response was not influenced by this loss of cement. In all holes, sufficient material was left to form a scaffold for complete coverage of the holes by ingrowing bone.

In summary, we conclude that the TCP/HA cement is very biocompatible material which stimulates new bone formation in experimental bone cavities. Therefore, although some important questions have to be answered, this cement can be regarded as a promising material for future applications in alveolar bone maintenance or bone defect filling.

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