Characterization and histological analyses of a coral–collagen composite used for bone-replacement graft material: a report of clinical cases

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Several studies, devoted to the osteogenic potentialities of natural $CaCO_3$ have already been reported. However, it seems questionable if the data obtained from natural calcium carbonates can be extrapolated to a composite biomaterial incorporating coralline material. For these reasons, in the present investigations the structural and crystallographic features of the biomaterial (Biocoral® gel) were thoroughly analyzed prior to implantation, with the aid of X-ray diffraction and electron microscopy. Then, biopsied samples, taken from Biocoral® gel-filled sites, respectively after 7, 8, 9, 12 and 29 mon implantation, were studied with optical and electron microscopy. It could be concluded from the histological analyses of the biopsies, that mineral still remained after long implantation periods. This composite biomaterial may thus be considered for uses in clinical situations where neither incorporation nor dissolution of the implanted biomaterial are essential, i.e. maintenance of edentulous ridge volume.

1. Introduction

Numerous biomaterials have been developed to be used as bone substitutes for the filling of osseous defects. Among them, natural coral skeletons have been proposed as bone grafts. Clinical results have shown a maintenance of the edentulous ridge volume after grafting of coral particles [1]. In other studies, the resorption of the coral after implantation and its replacement by new tissue [2, 3] was found. Some authors found it preferable to use osseous transplants which offer a matrix with better osteoblastic regeneration potentialities [4]. Others are of the opinion that without long-term studies, it is preferable to use autogenous bone as graft material [5]. The knowledge of the evolution, after implantation, of the employed coral particles is fundamentally important, especially if the biomaterial is used in a site where endosseous implants will be inserted. In fact, little is known about the osseointegration properties of endosseous implants located in a site preliminary treated with bone-replacement graft materials. The association of coral particles with collagen has also been proposed. This seems, in particular, more convenient for the clinician, as it can be easily adjusted to the size of the lesion to be filled. Such biomaterials are thought to avoid migration of coralline particles before connective tissue ingrowth or substitution by newly formed bone tissue. However, to our knowledge, no electron microscopical investigations have been realized on the tissue responses for this composite biomaterial type (Biocoral® gel). Moreover, it remains highly questionable whether the behavior of a coral–collagen composite material can be extrapolated from the reports related to the use of coralline calcium carbonate alone. The purpose of this work was to characterize the grafting material (Biocoral® gel) prior to implantation and to study the tissue responses of this composite biomaterial used for the filling of extraction sites in humans after different implantation times.

2. Materials and methods

2.1. Implant material

A coral–collagen composite biomaterial (Biocoral® gel, Inoteb, 56920 Saint-Gonnery, France) was used for the filling of freshly extracted alveoli.

Biocoral® gel is a bone-replacement graft material mainly composed of coralline calcium carbonate, under the form of aragonite (50%), of collagen (3%) and of glycerol and lactic acid (47%) as excipient. Magnesium and trace elements such as fluoride, strontium, zinc, iron and copper, are present in concentrations similar to those found in embryonic mammalian bone. Other mineral and organic components vary in nature and levels according to the coral species and harvesting site.

2.2. Clinical procedures

The samples were taken from five different patients, aged between 40 and 60 y (two women and three men), during the surgical operation immediately preceding the introduction of endosseous implants. For all reported cases, Biocoral® gel was placed in the alveolar socket immediately after tooth extraction. The socket was carefully cleaned and thoroughly rinsed with a sterile serum solution. Biocoral® gel was introduced in the socket and packed using a fuller or small damp sterile compresses. The flap was then pulled over the entire wound and stitched using an interrupted suture. An antimicrobial therapy with penicilline (Amoxicilline and clavulanic acid) was supplied for 7 d (1 g d^{-1}). For postsurgical care, the patients rinsed their mouth with a 0.1% chlorhexidine solution for 2 wk.

Biopsies were all taken during surgical intervention, just before introducing the endosseous implants. Biopsy samples were taken from Biocoral® gel-filled sites, respectively after 7, 8, 9, 12 and 29 mon implantation.

2.3. Tissue preparation and investigation methods

2.3.1. Light microscopy

The biopsies were fixed in a 10% neutral formaldehyde solution adjusted at pH 7.4, decalcified in a 15% sodium formate/85% formic acid mixture and stained with a haematoxylin/eosin solution.

2.3.2. Electron microscopy

The specimens were fixed in a 2% paraformaldehyde–glutaraldehyde solution buffered at pH 7.4 with 0.1M sodium cacodylate and post-fixed in a 1% osmium tetroxide solution in the same buffer. Non-decalcified ultrathin sections from the samples embedded in Epon® 812, were prepared with a microtome (Sorvall®, MT1, Porter-Blum, Norwalk, USA) equipped with a diamond knife. After uranyl acetate and lead citrate staining, the sections were observed in transmission electron microscopy (TEM) (Jeol 100B, Tokyo, Japan). The Biocoral® gel composite material was also investigated prior to implantation. Scanning electron microscopical (SEM) (Jeol 35C, Tokyo, Japan) observations were made after sputter-coating (Hummer-Junior, Siemens, Karlsruhe, Germany) of the biomaterial with a gold–palladium alloy.

2.3.3. X-ray diffraction

X-ray diffraction (Kristalloflex D5000, Siemens, Karlsruhe, Germany) was performed using CuK_α radiation ($\lambda_{CuK_\alpha} = 0.154$ nm). The diagrams were compared to the JCPDS (Joint Committee of Powder Diffraction Standards) files 41-1475 corresponding to aragonite ($CaCO_3$), 5-586 corresponding to calcite ($CaCO_3$) and to 33-268 corresponding to vaterite ($CaCO_3$).

3. Results

As observed by SEM and before implantation (Fig. 1), the composite biomaterial shows irregularly shaped coralline particles, together with fibrous material. The mean size of the particles determined from about 50 representative grains seen on scanning electron micrographs was 456 μm with a standard deviation of 114 μm. Similarly, about 25 round-shaped holes were measured within the particles. Their mean diameter was about 84.5 μm and had a standard deviation of 28.5 μm. These holes crossing the composite biomaterial can be considered as pores. Using transmission electron microscopy (Fig. 2), the particles appear as large crystals with smooth surfaces. X-ray diffraction revealed that the crystalline material of Biocoral® gel corresponds to the crystalline aragonite form (JCPDS file 41-1475) of $CaCO_3$ (Fig. 3) having a density of 2.95 g cm^{-3}. Radiological evaluation (Fig. 4) of an implanted site showed a satisfactory filling of the extraction socket. A biopsy realized 8 mon after implantation of Biocoral® gel and observed under light microscopy showed the presence of bone trabeculae in the implantation area (Fig. 5). The bone tissue, rich in

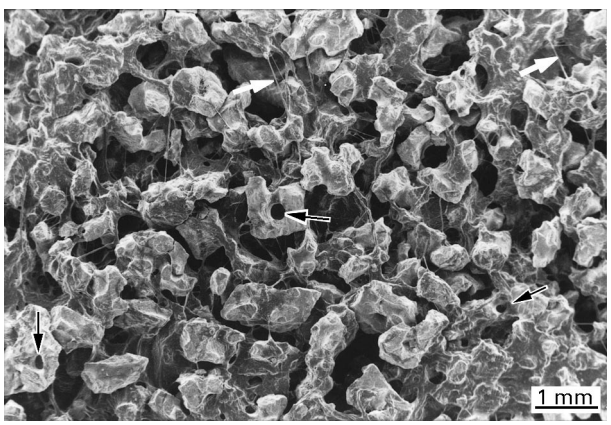

Figure 1 Scanning electron micrograph showing the structure of the coral–collagen composite biomaterial before implantation. The coral particles are interconnected by fibrillar material (white arrows). Round holes (black arrows) can be noticed within the mineralized compound of the biomaterial.

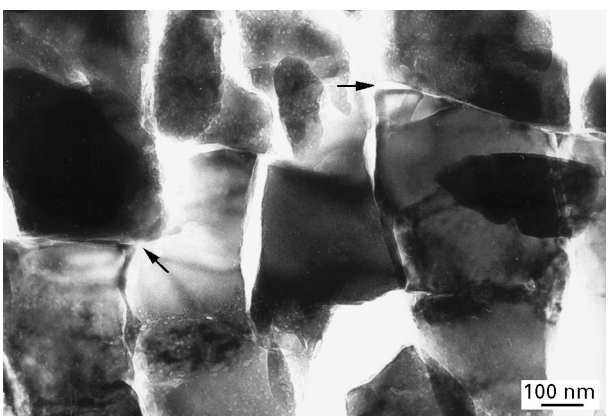

Figure 2 Transmission electron micrograph of the mineralized part of the composite biomaterial prior to implantation. The cracks (dark arrows) are probably due to the sectioning process.

normal osteocytic lacunae, exhibited regular osteoblast linings. By TEM and after 9 mon implantation, calcified islands were seen in the osteoid tissue (Fig. 6). However, 8 mon after implantation, histological observations also revealed the presence of granulated tissue within the connective material located in the apical area of the filled extraction socket (Fig. 7). Several mastocytes are noticed by TEM in the implantation site of a 29 mon biopsy (Fig. 8). In Fig. 9 the coralline material implanted for 7 mon shows similar

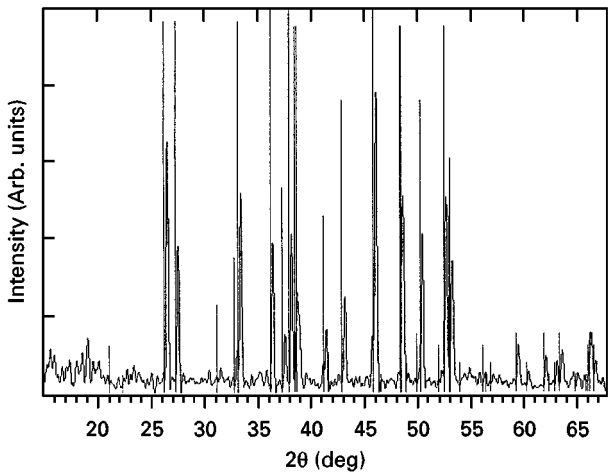

Figure 3 X-ray diffraction pattern ($2\theta = 15°–65°$) of Biocoral® gel before implantation.

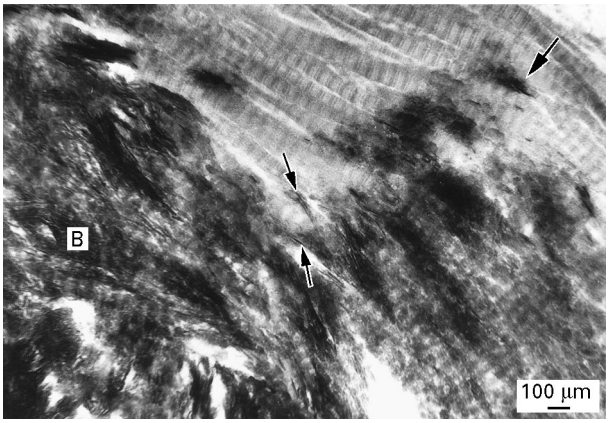

Figure 6 TEM observation of mineralizing collagen fibrils (large arrow), 9 mon after implantation of the composite biomaterial in a human alveolar socket. B, mineralized bone substance. Small arrows, edge-on view of bone mineral crystals.

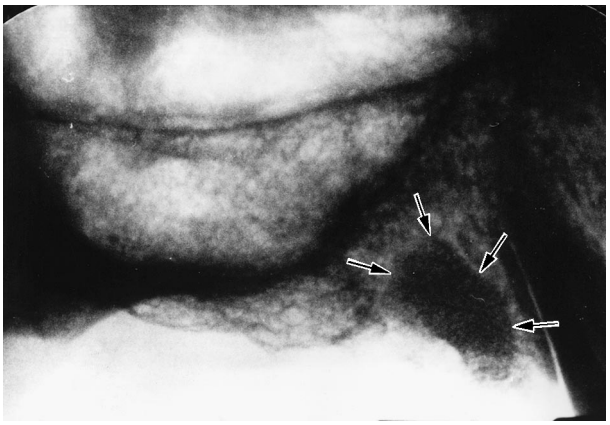

Figure 4 Radiography realized just after filling of the alveolar socket with Biocoral® gel. Arrows delimit the implantation site (upper first right premolar).

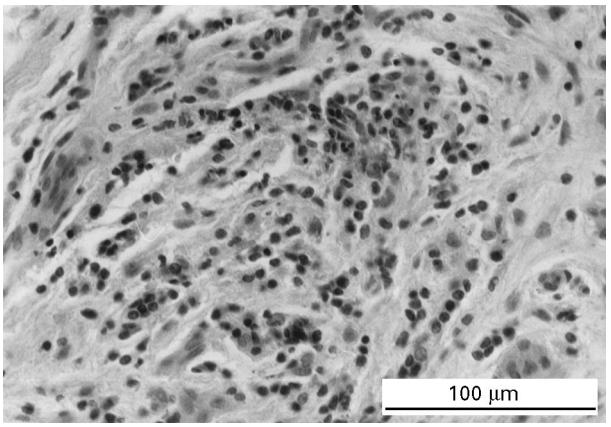

Figure 7 Optical micrograph showing connective tissue with inflammatory round cell infiltration in the apical area of an implantation site, after 8 mon implantation. Note the absence of polymorphonuclear neutrophil.

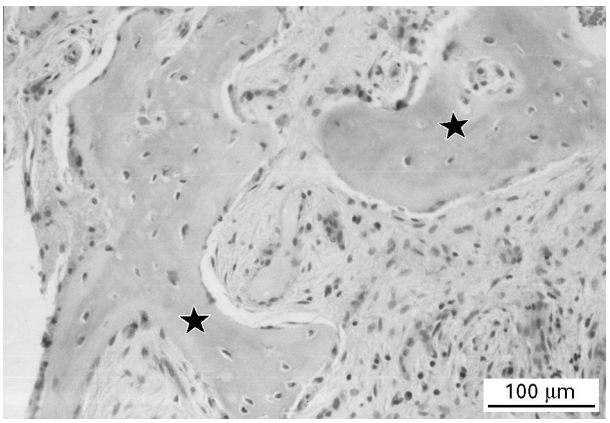

Figure 5 Presence of bone trabeculae (stars) with numerous lacunae containing osteocytes in the implantation area, 8 mon implantation of Biocoral® gel in an extraction socket, under light microscopy.

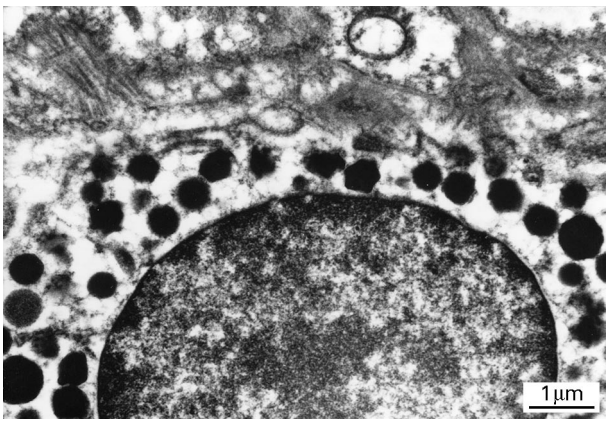

Figure 8 Transmission electron micrograph of a mastocyte observed from a biopsy realized in an alveolar socket being filled with Biocoral® gel for 29 mon.

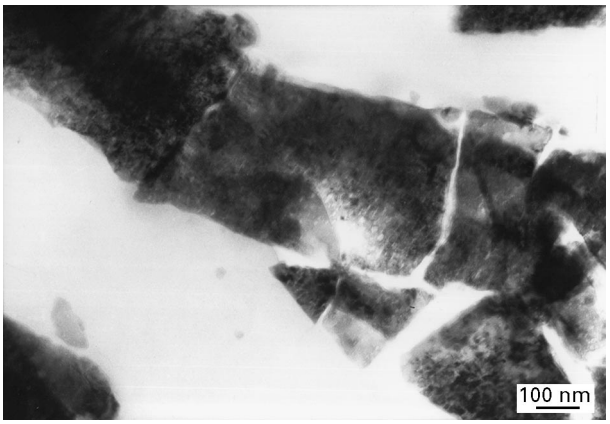

Figure 9 Transmission electron micrograph showing the aspect of the coralline material having been implanted for 7 mon in an extraction socket.

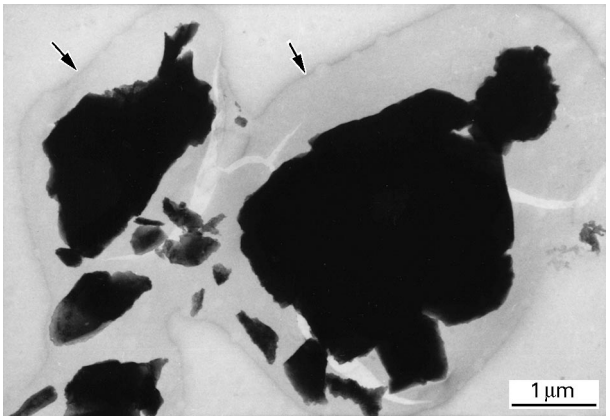

Figure 10 TEM image visualizing electron-dense material seemingly encapsulated in an amorphous substance, 12 mon after implantation of Biocoral® gel.

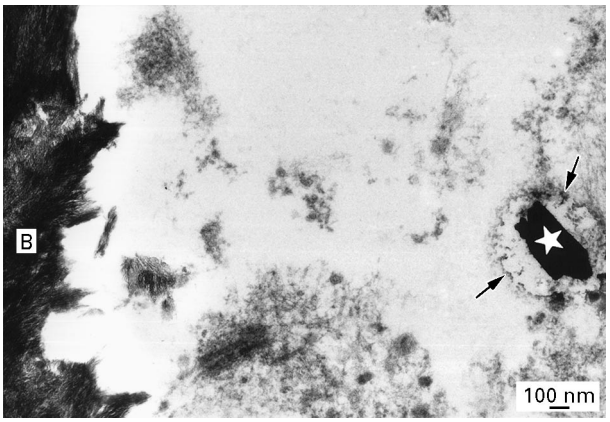

Figure 11 Transmission electron micrograph showing the presence of possibly encapsulated (arrows) implanted material (star) at distance from mineralized bone tissue (B), after 12 mon implantation.

aspects to the mineral particles observed before implantation (Fig. 2). In some areas and after 12 mon implantation, the remaining coralline material seems to be circumscribed by an amorphous envelope (Fig. 10). The mineral (possibly encapsulated) seems to

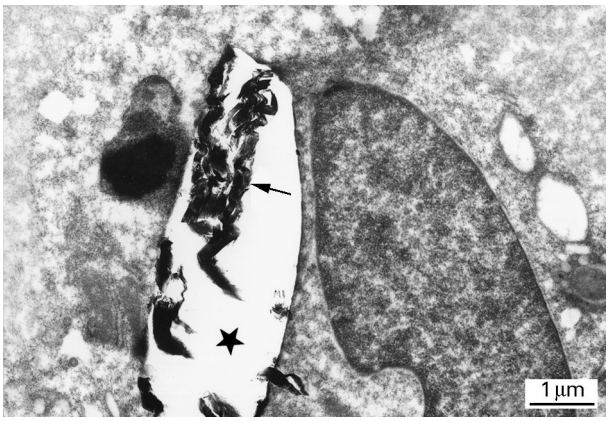

Figure 12 Transmission electron micrograph of a macrophagic cell with coralline material (arrow) present in an intracytoplasmic vacuole 29 mon after implantation of the coral–collagen biomaterial. The empty space (star) corresponds to a preparation artefact.

be located at a distance from the mineralized bone tissue (Fig. 11). Finally, after the longest implantation period of 29 mon, large vacuoles containing coralline crystals are noticed in the cytoplasm of a macrophage-like cell (Fig. 12).

4. Discussion

Several studies, devoted to the osteogenic potentialities of natural $CaCO_3$ have already been performed. Thus, Yukna [6], concluded that resorbable coralline calcium carbonates implanted in human periodontal osseous defects provided identically favorable clinical results to other available periodontal grafting materials. On the other hand, Naaman Bou-Abboud *et al.* [7] concluded, after evaluation of the osteogenic potentialities of natural coral implanted into a non-osseous site, that the considered alloplastic biomaterial had no bone induction property. In connection with this study, Vuola *et al.* [8] compared the bone-forming ability of natural coral blocks implanted in rat latissimus dorsi muscle with and without autogenous bone marrow. Their results showed that bone was present only in implants containing bone marrow. Moreover, when comparing the natural coral implants with structurally similar derivates in the form of hydroxyapatite, the same authors noticed significantly higher bone formation in coral than in hydroxyapatite.

The three-dimensional features of the different coral species are thought to favor ingrowth of the host bone. In this respect, *Porites* and *Goniopora* would present a similar structure to trabecular bone whereas *Favites* and *Lobophyllia* would more resemble compact bone [9]. Fricain *et al.* [10] studied the influence of the structure of different corals on the resorption kinetics and pointed out that the speed of resorption increased with the open porosity of the coral, probably due to a higher surface area. For these reasons, in the present investigations the structural and crystallographic features of the biomaterial (Biocoral® gel) were thoroughly analyzed prior to implantation (Figs 1–3). Despite seemingly satisfactory radiographical and

histological evaluations of several implantation sites (Figs 4–6) several noteworthy cellular (Figs 7, 8 and 12) and crystalline (Figs 9–11) structures were found. The most important information of this study has to be related to the degradative character of the coralline material. Surprisingly, intact calcium carbonate without signs of mineralized bone depositions was still present in biopsies harvested after 7 (Fig. 9) and 12 (Figs 10 and 11) mon implantation. No particular differences can be noticed between Figs 2 (before implantation) and 9 (after implantation). Yet, it has been reported that natural coral would be completely resorbed after implantation times as short as 12 wk [11]. In these cases, biomaterial degradation would simultaneously be accompanied by new bone apposition [12]. In the present study, phagocytoses of coral particles was still noticed after 29 mon implantation (Fig. 12). According to Guillemin *et al.* [2], the biodegradation process was of enzymatic origin and particularly due to carbonic anhydrase. At this point, it must be emphasized that the previous reports were devoted to biomaterials constituted by coralline calcium carbonate alone, whereas the biomaterial investigated in this study was a coral–collagen composite. When this particular composite biomaterial was used for the filling of alveoli in animals, the implanted biomaterial was still present after 90 d implantation [13]. Thus, it seems questionable if the results related to natural calcium carbonate can be extrapolated to a composite biomaterial incorporating coralline material. Moreover, Frank *et al.* [14] reported that hydroxyapatite associated with collagen was less well incorporated in alveolar bone and was even able to cause localized and moderate inflammatory reactions compared to hydroxyapatite implanted alone. Anyhow, Louise and Borghetti [15] suggested that, even for clinical purposes, knowledge of the resorption rate of natural coral would be very interesting.

5. Conclusion

From the present study and analyses of specimens harvested after 7, 8, 9, 12 and 29 mon implantation and subjected to histological analyses, it can be concluded that mineral particles of the studied biomaterial still remain after long implantation periods. This composite biomaterial may thus be used in situations where neither incorporation nor dissolution of the implanted biomaterial are essential, i.e. maintenance of edentulous ridge volume. More and better documented reports are necessary before suggesting such type of material for grafting procedure (filling alveoli with coral–collagen biomaterial) prior to endosseous implantation.

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