JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 6 (1995) 680–684

Resorption of corals implanted in diffusion chambers

J. CH. FRICAIN
UFR Odontologie, Place de la Victoire – 33000 Bordeaux, France
CH. BAQUEY, B. DUPUY
INSERM-U. 306, 146 rue Léo Saignat – 33076 Bordeaux, France

Natural coral is a resorbable bone substitute currently used in osseous surgery. The action of cellular and interstitial fluids has been incriminated as a possible agent for coral resorption but it has not been possible to discriminate the importance of either factor. The aim of this study was to compare the resorption speed of the coral implant in contact only with biological fluids (coral dishes placed inside diffusion chambers closed with two filter membranes 1.2 μm pore size) or in contact with biological fluids and cells (coral dishes placed inside diffusion chambers closed with the above filters but with holes made with a 22 G needle or coral dishes in direct contact with soft tissue). Qualitative (SEM) and quantitative (gravimetric) results showed that the implants in contact with cells were resorbed faster than those in contact only with biological fluids. The cells in contact with the implant were mainly multinucleated giant cells and some were Trap + . TEM showed multinucleated cells with a ruffled border but without a clear zone or intracytoplasmic inclusions distinguishing them from osteoclasts. With only biological fluids, the latter intervened to a moderate extent in the resorption of coral implants in which the cellular action appears to be dominant. However, this action does not seem to be attributable to osteoclasts.

1. Introduction

Bone autografting has been widely used in orthopaedic surgery [1]. It does, however, have drawbacks including limited availability and donor site morbidity. To limit the use of bone autografts, various mineral materials have been used as bone substitutes: tricalcium phosphate [2], hydroxyapatite [3] and more recently natural coral [4, 5] for its properties of osteoconduction and resorption [6].

Questions still remain, however. Cellular and interstitial fluids have been proposed as possible agents for coral implant resorption in contact with bone or soft tissue, but 'the chemical dissolution of the coraline substance is not separated from the activity of the absorbing cells' [7].

The aim of this study was to determine the influence of interstitial fluids on the resorption of coral implants situated subcutaneously, and protected or not in diffusion chambers. To assess the phenomenon, histologic tests, scanning and transmission electron microscopy (SEM and TEM) were performed for qualitative analysis and the gravimetric method was used for quantitative analysis.

2. Materials and method

Discs (2 mm high, 4 mm diameter) were shaped in exoskeletons of *Porites astreoides* coral species. The mineral part of this coral had a uniform, well interconnected structure possessing an average pore size of 154 ± 2 μm and a volumetric porosity of 43.5 ± 2%. Some discs were placed inside diffusion chambers made of two filter membranes (1.2 μm pore size) glued to the two faces of lucite rings (Milipore Corporation, Bedford, MA 01730) and in diffusion chambers with holes made with a 22 G needle (DC H). Implantations of discs totally protected (DC), partially protect (DC H) or not (D) in diffusion chambers were performed under the skin of the back of OF1 mice. At four and eight weeks, the implants were retrieved and embedded using the paraffin or methylmethacrylate (polymerization at −20 °C) methods. Slices were stained with HES and intracellular tartrate-resistant acid phosphatase activities were estimated using a kit (Sigma, Kit 386-A) using naphtol AS-BI and fast garnet GBC. For SEM some discs were dried at 150° and coated with gold. Next, they were observed and photographed with an Hitachi S 2500 electron microscope. For TEM analysis, samples were fixed, decalcified in 7.5% EDTA, dehydrated and embedded in epoxy resin. Ultrathin sections were obtained using a diamond knife and stained with uranyl acetate and lead citrate. Observation was carried out using an electron microscope. Gravimetric measurements were performed on blocks after repeated immersions in a sodium hypochlorite solution in order to eliminate soft tissue.

3. Results

At one and two months, 2.5 to 11 μm membranes encapsulated the retrieved coral discs (D) and no major differences in histological aspect were noted. Inside

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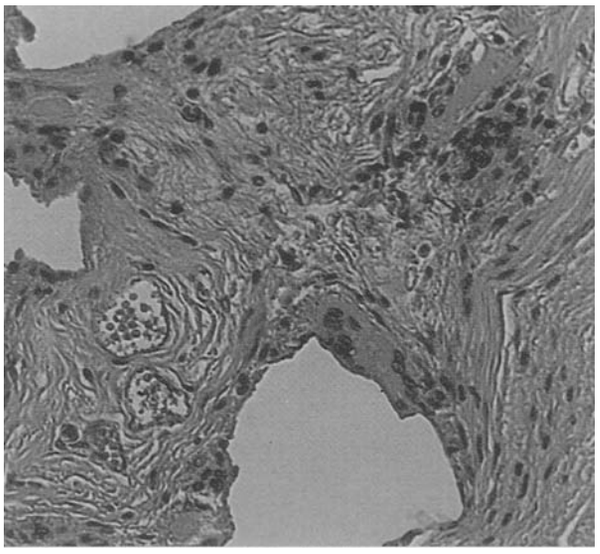


Figure 1 Four weeks after D implantation (HES stain) white area indicates the ghost of coral after decalcification process. On the coral surface there are numerous multinucleated giant cells. At a distance from the coral, there are fibroblasts in a well-vascularized connective tissue (original magnification 20 ×).

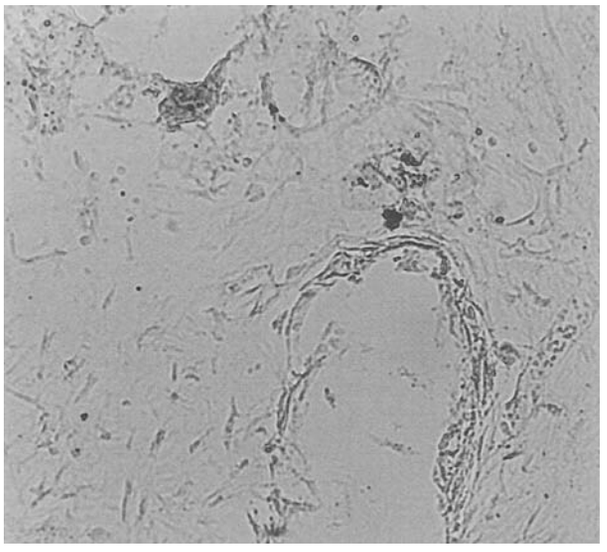


Figure 2 Four weeks after D implantation, tartrate-resistant activity was seen on a few multinucleated giant cells (original magnification 20 ×).

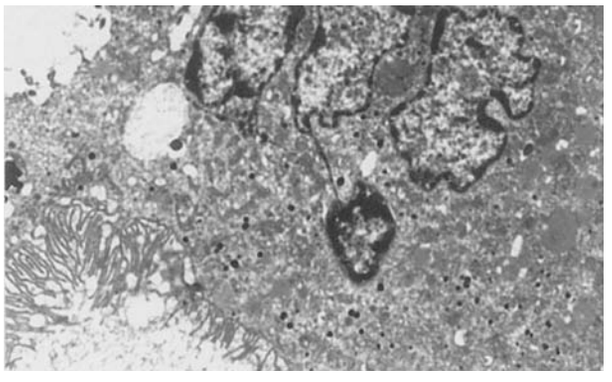


Figure 3 TEM micrograph of a multinucleated giant cell with a ruffled border on the opposite side from the coral (white area). There is no clear zone and few cytoplasmic inclusions (original magnification 3600 ×).

the coral pores, a connective well vascularized tissue appeared to be growing. In contact with the mineral, numerous fibroblasts and some multinucleated giant cells were observed (Fig. 1). Some multinucleated cells were strongly TRAP-positive (Fig. 2).

On coral implants retrieved from diffusion chambers (DC), neither fibres nor cells were observed. MET showed some multinucleated giant cells with a ruffled

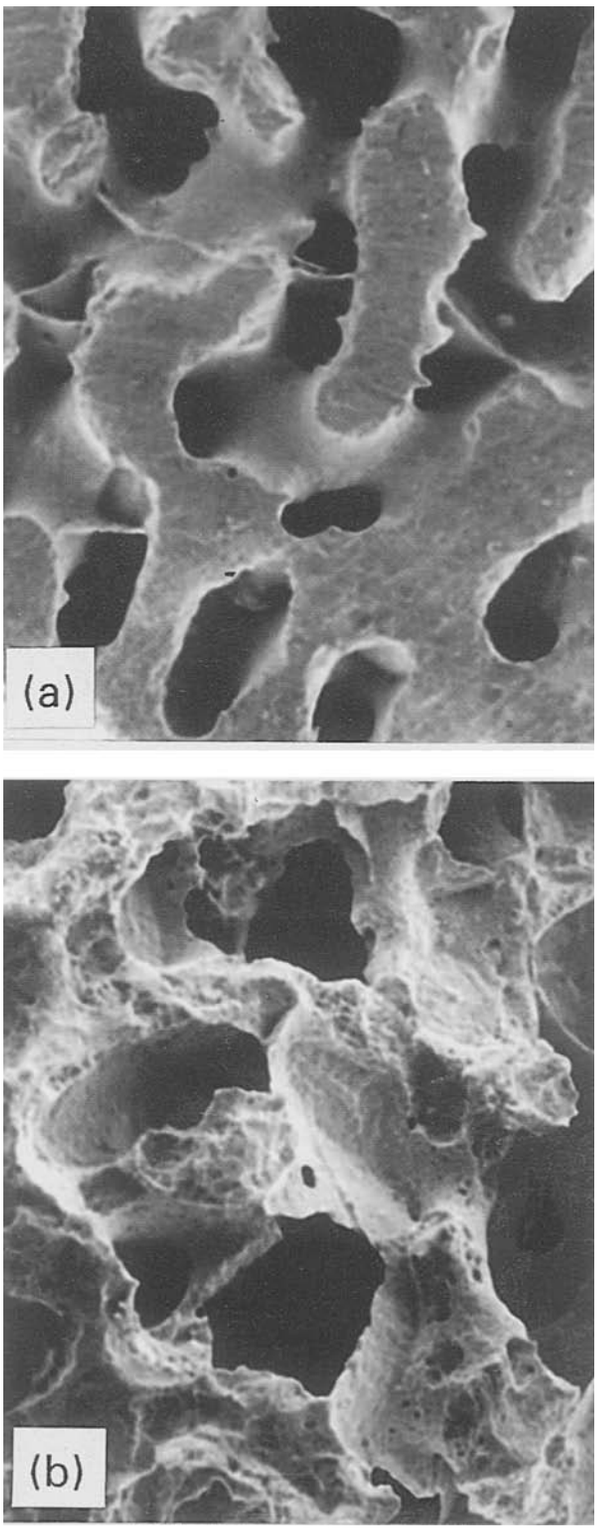


Figure 4 SEM micrograph of D before and one and two months after implantation (a, b, c). Coral destruction increased with implantation time; surface loosening of the microcrystals has destroyed the D architecture.

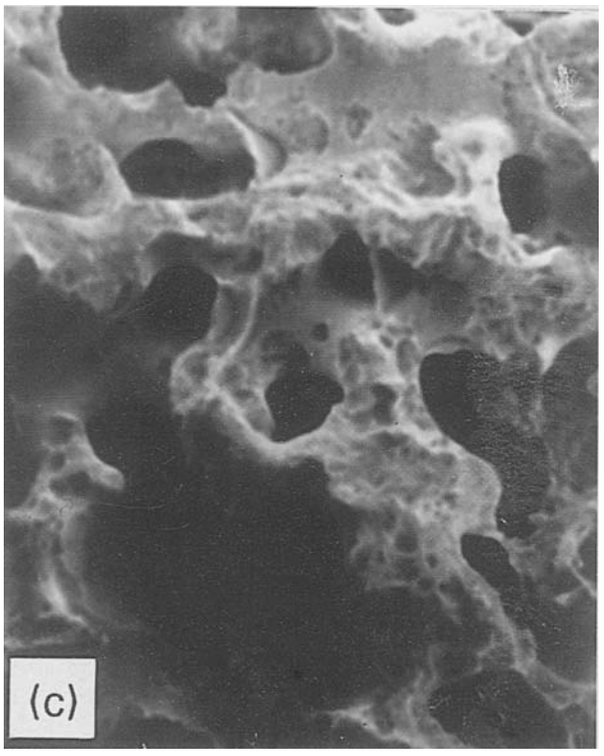
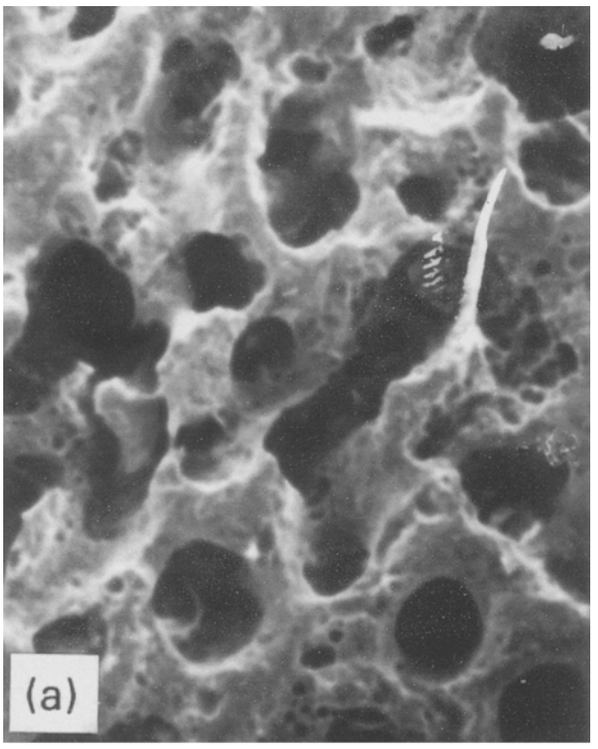


Figure 4 (Continued)



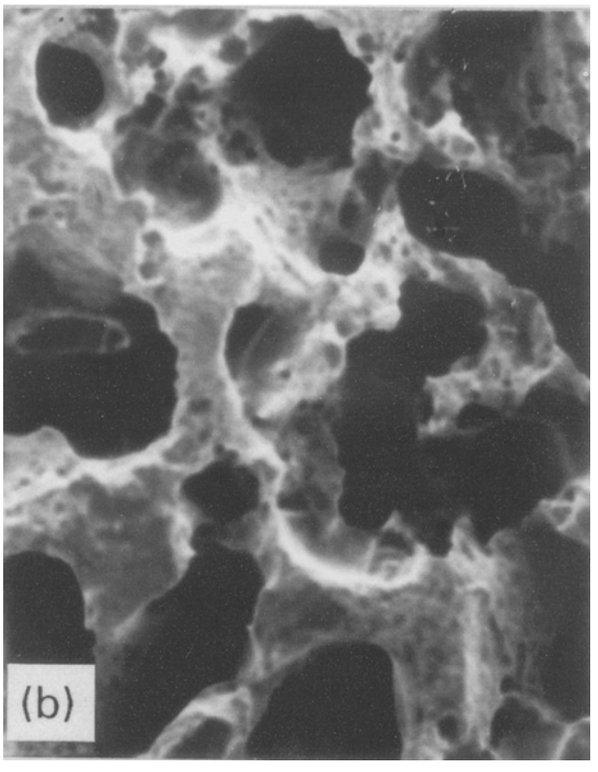


Figure 5 SEM micrograph of DCH one and two months after implantation (a, b). Coral destruction has increased with time. The implant appears altered with loss of the morphology of some pores.

border on the opposite side from the coral, no clear zone and a few intracytoplasmic inclusions (Fig. 3).

By SEM analysis at one month, (D) and (DCH) samples showed alteration on their surfaces but pore morphology was preserved (Figs 4a, 5a). At two months, the implants appeared more altered with loss of the morphology of some pores (Figs 4b, 5b). Coral disappearance was not uniform and it looked as if a crumbling process was taking place. At one and two months, (DC) samples presented the same type of alteration but much smoother and more uniform, without any disappearance of pore morphology (Fig. 6 a–b).

On the external surface of the membrane filters, there was a vascularized fibrous tissue characteristic of a light foreign body reaction (Fig. 7). The internal surface had preserved its integrity but after two months, some cytoplasmic expansion had crossed the membrane (Fig. 8).

At one and two months, the mass losses in (D) samples were 13.83 ± 3.26 and $38.21 \pm 7.98\%$ respectively ($P < 0.01$ Wilcoxon test). For the same periods, the mass losses in (DC) samples were 0.61 ± 1.2 and 3.5 ± 2.21 respectively $p < 0.22$ and $p < 0.01$ Wilcoxon test) and for (DCH) 5.36 ± 2.90 and 28.7 ± 5.3 ($p < 0.01$ and $p < 0.02$ Wilcoxon test).

4. Discussion

After implantation in the bone site, the coral appeared to be resorbed progressively and replaced by bone [6–7]. Previous authors have considered osteoclasts as the factor responsible for coral resorption. Owing to carbonic anhydrase (CA), osteoclasts produce H^+ions ($CO_2 + H_2O \overset{CA}{\leftrightarrows} HCO_3^- + H^+$) which are concentrated directly on the extracellular environment with the help of one or several proton pumps [8]. The drop in pH was a direct result of the dissolution of the coral. Previous studies [9] have demonstrated that coral in contact with soft tissue is resorbed, and that the speed of resorption varies according to the porosity of the material and the implantation time. These results demonstrate that the coral is resorbable in contact with both bone and soft tissue, but do not allow the action of biological fluids to be

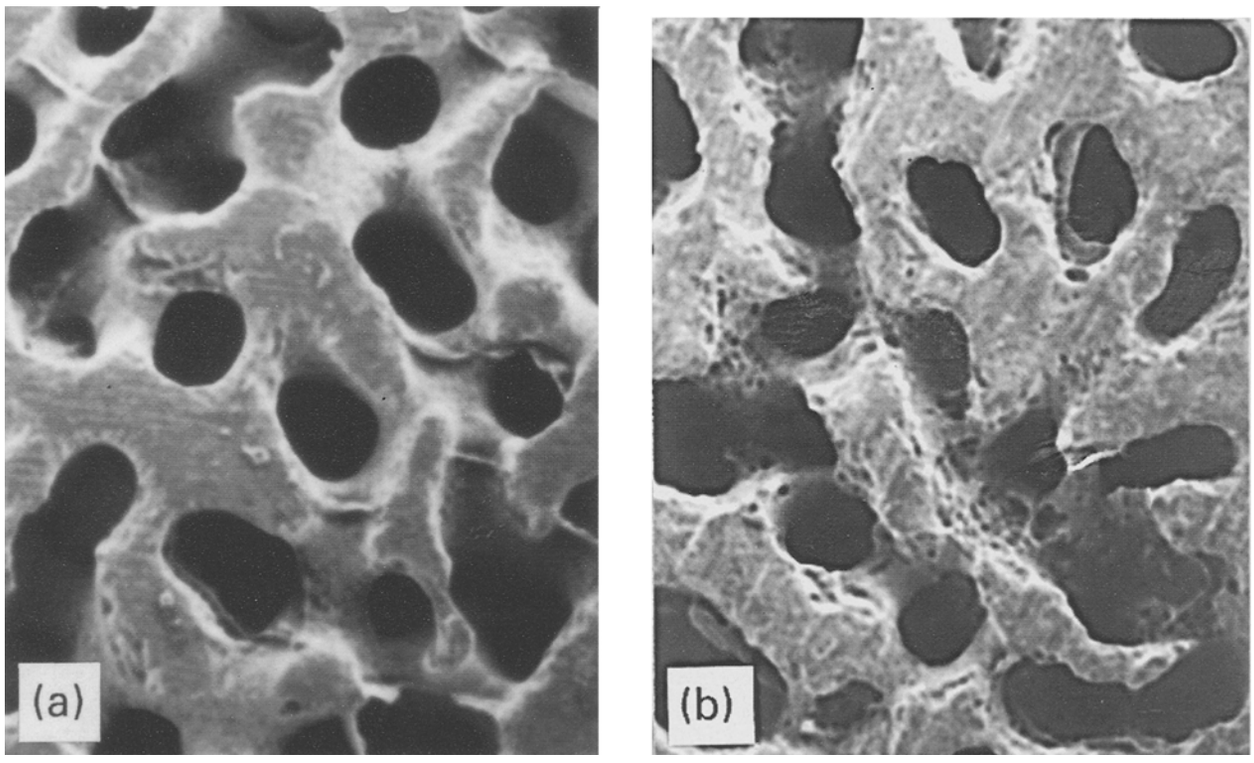


Figure 6 SEM micrograph of DC one and two months after implantation. Resorption has increased with time but pore morphology is preserved.

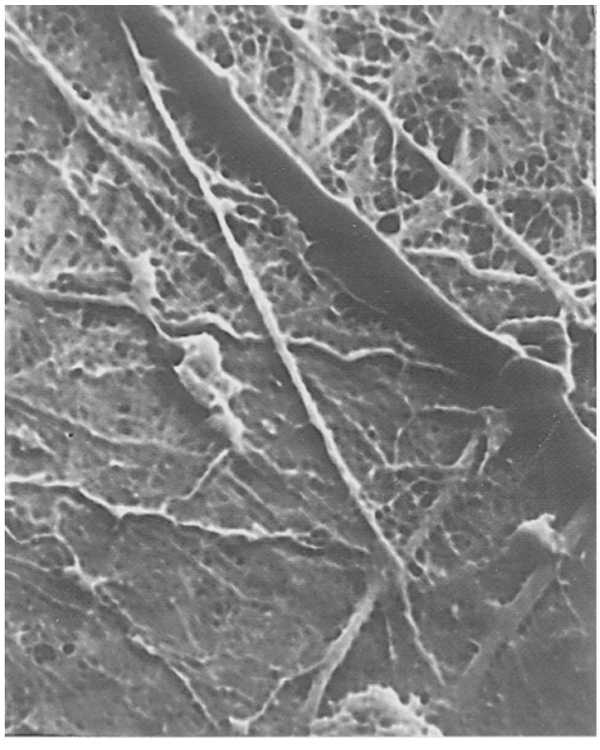


Figure 7 SEM of the external surface of the membrane filters one month after implantation. There is a vascularized fibrous tissue characteristic of a foreign body encapsulated membrane.

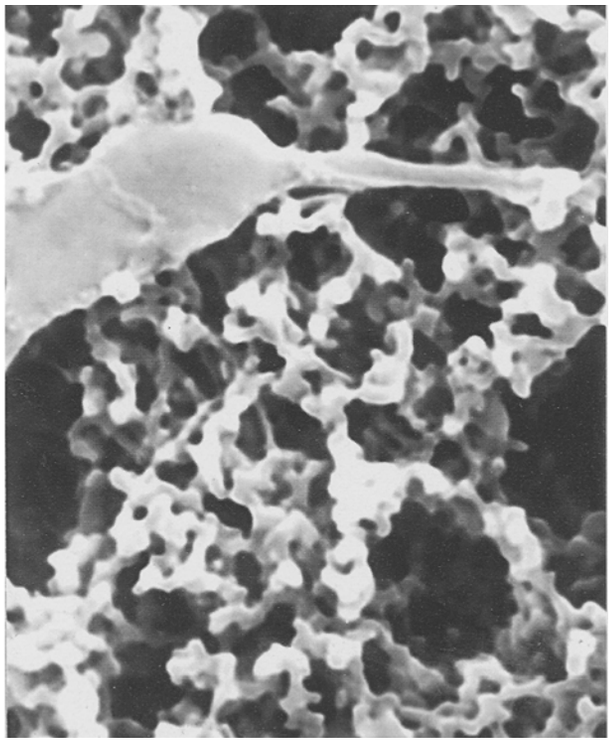


Figure 8 SEM of the internal surface of the membrane filter two months after implantation. There are a few cytoplasmic expansions crossing the membrane, but there is no cell and the integrity of the filter is preserved.

distinguished from that of cells in contact with the implant.

To allow implant biological fluid contact and to prevent contact between cell and implant, coral implantations inside the diffusion chamber (DC) were carried out. SEM of the membrane closing the chambers, demonstrated the integrity of the inner side and the presence of fibrous tissue vascularized on the outer side. The presence of vessels showed a permanent renewal of the plasmatic components and, therefore of the biological fluids.

After one and two months of implantation, the samples (D) and (DCH) were destroyed at the surface, that demonstrating a non-uniform process of erosion observed in a previous study [9] for the same periods. The samples (DC) appeared much less altered, indicating a much slower erosion process attributable to the absence of cell contact. The role of the different cells in the mechanisms of resorption is not yet clear. Many, multinucleated giant cells were observed in contact with the material, but only a few were TRAP +. However, this is not a solid enough criterion on which to assimilate these to osteoclasts [10], especially as TEM showed multinucleated cells with a ruffled border but with little intracytoplasmic inclusion and the absence of the clear zone characteristic of osteoclasts. Furthermore, it is not, however, possible to rule out the fact that these cells are involved in the resorption of coral, as has been shown with regard to bone tissue [11].

Quantimetric results showed that the implants (D) were resorbed at a faster rate than (DCH) owing to their more recent contact with the cells, but also owing to the mechanical action of tissues which could play a part by aiding the disintegration of coral. The implants (DC) in contact with biological fluids were resorbed very slowly. In addition, on these implants no deposit of carbonated apatite was observed, unlike with hydroxyapatite [12]. This is certainly due to the different chemical structures of these materials.

5. Conclusion

When only biological fluids were in contact with the implant, resorption of coral, in which the cellular action appear to be dominant was low. However, this action cannot seem to be attributed to osteoclasts.

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Received 29 June
and accepted 4 July 1995