Light and Laser Scanning Microscopy analysis of hydroxyapatite used in periodontal osseous defects in man: evidence of a different resorption pattern in bone and soft tissues.

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SUMMARY

Hydroxyapatite (HA) is a highly biocompatible material that recently has been shown to undergo biodegradation. The mechanisms of this phenomenon are unclear, and humoral and cellular events have been thought to be implicated. In the present study HA particles were put into infraosseous defects on teeth that were to be extracted for prosthetic reasons and then retrieved after a 1 year period. The specimens were processed with the cutting grinding system. Results show a very sharp difference of the biodegradation processes, related to the tissues that surround the HA particles. HA in tight contact with mineralized bone showed no evidence of degradation or resorption, while on the contrary, in the areas where bone loose connective tissue was present, it was possible to observe HA crystals detached and scattered in cells cytoplasm or extracellular fluids. This dissolution and resorption phenomenon were observed also by Laser Scanning Microscope (LSM) in fluorescent mode. These differences in degrees of degradation between bone and loose connective tissue could be due to the small amount of interstitial fluid present in mineralized bone and the greater flow of fluid through connective tissue.

KEY WORDS:

Biodegradation - Hydroxyapatite - Laser Scanning Microscopy - Multinucleated cells.

RÉSUMÉ

L'hydroxyapatite (HA) est un matériel hautement biocompatible qui s'est révélé récemment capable de subir une biodégradation. Le mécanisme de ce phénomène n'est pas clair, et semble impliquer une participation humorale et cellulaire. Notre recherche a porté sur la mise en place de particules d'HA dans des défects intraosseux en rapport avec des dents qui doivent être extraites pour des raisons prothésiques, et sur leur étude suite à leur ablation un an après. Les résultats ont démontré des différences marquées dans

le processus de dégradation en rapport avec les tissus qui entourent l'HA. Les particules d'HA qui sont en contact avec l'os minéralisé ne présentent pas de signes de dégradation ou de résorption, tandis que dans les régions où du tissu conjonctif était présent, il est possible d'observer des cristaux d'HA détachés et dispersés dans le cytoplasme des cellules et dans le liquide extracellulaire. Ces phénomènes de dissolution et de résorption ont été également étudiés au Microscope Electronique à Balayage au Laser en fluorescence. Ces différences dans le degré de dégradation observés dans l'os et dans le tissu conjonctif lâche pourraient être dues à la faible quantité de liquide interstitiel dans l'os minéralisé et à la quantité plus grande de flux liquidien dans le tissu conjonctif.

MOTS CLÉS:
Biodégradation - Hydroxyapatite - Microscope à Balayage au Laser - Cellules Multinuclées.

INTRODUCTION

One of the aims of periodontal therapy has been the elimination of periodontal pockets by inducing the formation of new bone. Several surgical techniques have been proposed over the years for therapy of infrabony defects. Autogenous bone grafts seem to have very good bone regenerating capabilities, but their use is limited by the necessity of an additional surgical procedure and by the difficulty of getting a large amount of donor tissue. For these reasons many attempts have been done to find various types of synthetic graft material.

One of the most widely used material for this purpose is hydroxyapatite (HA).

It is a highly biocompatible material and it comprises 60 to 70% of bone tissue and 97% of enamel (Jarcho, 1986).

It is usually said to be nonresorbable even over a long term period (Hoogerndoorn et al., 1984, Klein et al., 1989); on the other hand Donath (1990) states that there are no HA ceramics that are unresorbable, with the only thing that is different being their resorption rates.

Tricalcium-phosphate (TCP) resorbs in a more or less unpredictable fashion when implanted in hard and soft tissues (Kent, 1986) and data show that resorption rates of TCP between soft and hard tissues implant sites are different, being much more swift in bone (Jarcho, 1986).

Aim of the present investigation was a study of the resorption patterns of HA used in human periodontal defects.

MATERIALS AND METHODS

Three volunteer patients, who had given their informed consent, participated to this study.

The patients presented 5 to 10 mm deep infraosseous mandibular defects on teeth that were to be extracted for prosthetic reasons. Mucoperiosteal flaps were executed under local anesthesia to expose the defects and after having removed all the granulation tissue present, the HA particles were put into the defects and lightly compressed to the brim of the bone walls. The teeth were extracted and the HA retrieved after 1 year. The specimens were fixed, immediately upon removal, by immersion in 10% neutral buffered formalin, dehydrated in an ascending series of alcohols and infiltrated and polymerized in Technovit 7200 VLC resin. The final preparation of the histologic slides was performed by the cutting-grinding technique (Donath and Breuner, 1982). The thin (10-20 μ) ground sections were stained with toluidine blue, basic fuchsin and methylene blue and were investigated with normal transmitted and polarized light.

RESULTS

Low-power view showed a good bone-HA contact in almost all the HA granules observed (Fig. 1). Nevertheless some of this granules were immersed in gingival connective tissue, surrounded by a fibrous

capsule, or in contact with bone marrow cells. No inflammatory reaction was seen in this areas, or in bone tissue. At high power magnification it was possible to see that different resorption rate patterns were present. In the areas in which the HA was in contact with bone soft tissues or gingival connective tissue, it was possible to see HA particles detached from the bulk HA and scattered in cytoplasms or extracellular fluids (Fig. 2). The shape of these detached particles was not uniform, and they showed different diameters. The particles had a morphology of small irregular fragments, aggregated inside the cell cytoplasm or dispersed in extracellular fluids. Many macrophages were seen near or directly adherent to the HA. In many areas it was possible to detect the presence of osteoblasts or of thin amounts of newly formed bone. Connective fibers seemed sometimes to originate from the HA. Macrophages were completely absent from the portions of the interface where it was possible to see osteoblasts or osteoid matrix. HA that was in tight contact with mineralized bone showed a very smooth surface with no evidence of dissolution and resorption (Fig. 3). The shape of these particles was almost uniform with the morphology of HA crystals (Fig. 4). In some fields it was possible to see cell-mediated resorption similar to Howship lacunae (Figg. 5-6-7). At the interface with bone laser scanning microscopy showed an unbroken line of autofluorescent material, with a fluorescence similar to that of osteocyte lamina limitans, interposed between HA crystals and bone.

Fig. 2: High power view of HA particles in bone marrow spaces. Note the roughness of the surface and the small HA crystals (arrowheads) detached from the HA particle (basic fuchsin-methylene blue) Bar = 50 μ.

Fig. 2: Aspect à plus fort grossissement de particules d'HA dans les espaces médullaires osseux. On remarque la rugosité de la surface et des petits cristaux d'HA détachés de la particule d'HA (fuchsine basique-bleu de méthylène) Barre = 50 μ.

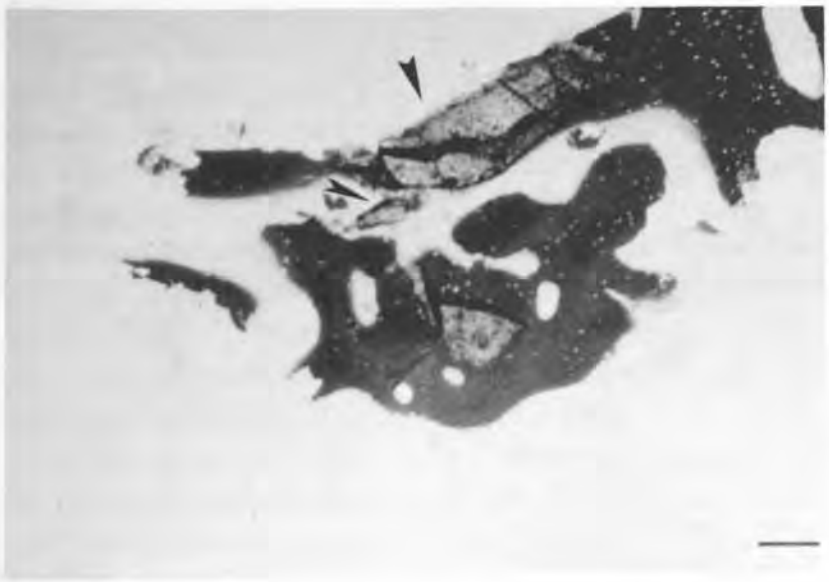

Fig. 1: Overview of HA particles (arrow) from patient n° 1 (basic fuchsin-methylene blue) Bar = 250 μ.

Fig. 1: Aspect général des particules d'HA (flèche) du patient n° 1 (fuchsine basique-bleu de méthylène) Barre = 250 μ.

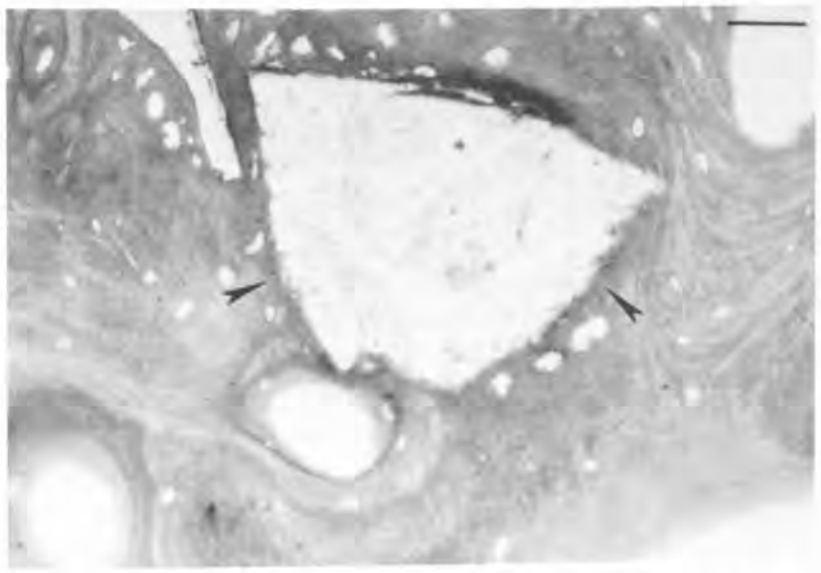

Fig. 3: High power view of an HA particle completely surrounded by mineralized bone. It is present a smooth surface (arrowheads) without degradation phenomena (basic fuchsin-methylene blue) Bar = 50 μ.

Fig. 3: Aspect à plus fort grossissement d'une particule d'HA complètement entourée d'os minéralisé. La surface est lisse, sans phénomènes de dégradation (fuchsine basique-bleu de méthylène) Barre = 50 μ.

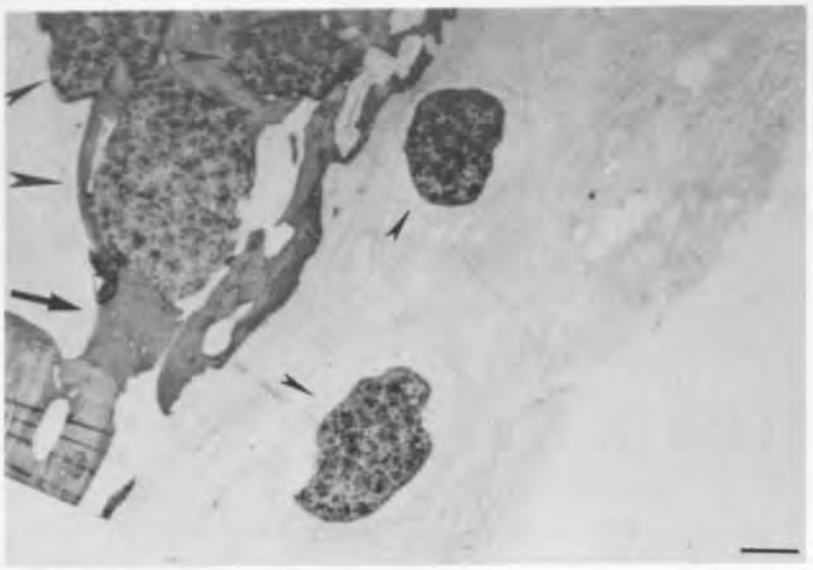

Fig. 4: General view of HA particles in patient n° 2. The HA particles (large arrowheads) are interspersed between mineralized bone (arrow) and bone marrow spaces (small arrowheads) (basic fuchsin-methylene blue) Bar = 250 μ.

Fig. 4: Aspect général des particules d'HA du patient n° 2. Les particules d'HA sont éparpillées à l'intérieur de l'os minéralisé et des espaces médullaires (fuchsine basique-bleu de méthylène) Barre = 250 μ.

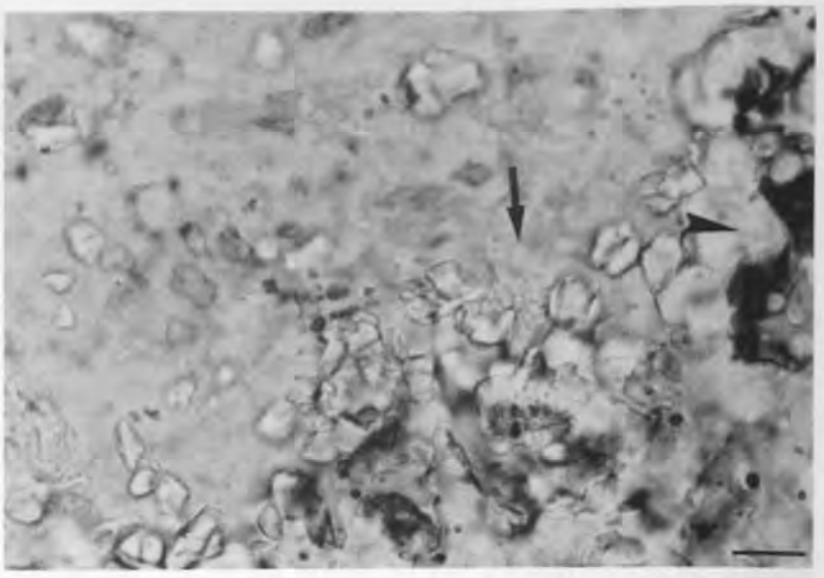

Fig. 6: HA particle almost completely immersed in bone marrow spaces. Big resorption lacuna (arrow) with initial bone mineralization (arrowhead) (basic fuchsin-methylene blue) Bar = 10 μ.

Fig. 6: Particule d'HA complètement plongée dans les espaces médullaires osseux. Grande cavité de résorption avec début de minéralisation de l'os (fuchsine basique-bleu de méthylène) Barre = 10 μ.

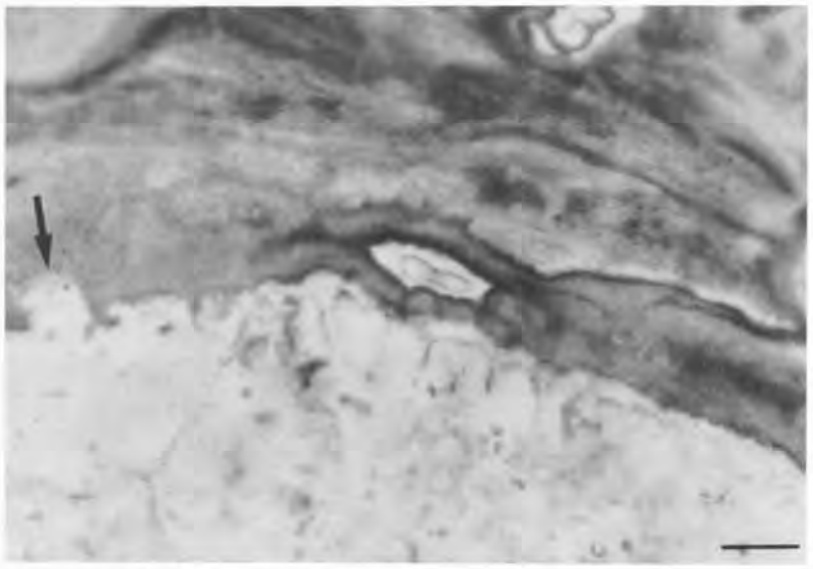

Fig. 5: High power view of HA particles in mineralized bone. Note the smooth edge of this area of the particles. Active osteoclastic-like resorption lacuna (arrow) (basic fuchsin-methylene blue) Bar = 10 μ.

Fig. 5: Aspect à plus fort grossissement des particules d'HA à l'intérieur de l'os minéralisé. On remarque la marge lisse de cette zone de la particule. Cavité de résorption active ostéoblastic-semblable (fuchsine basique-bleu de méthylène) Barre = 10 μ.

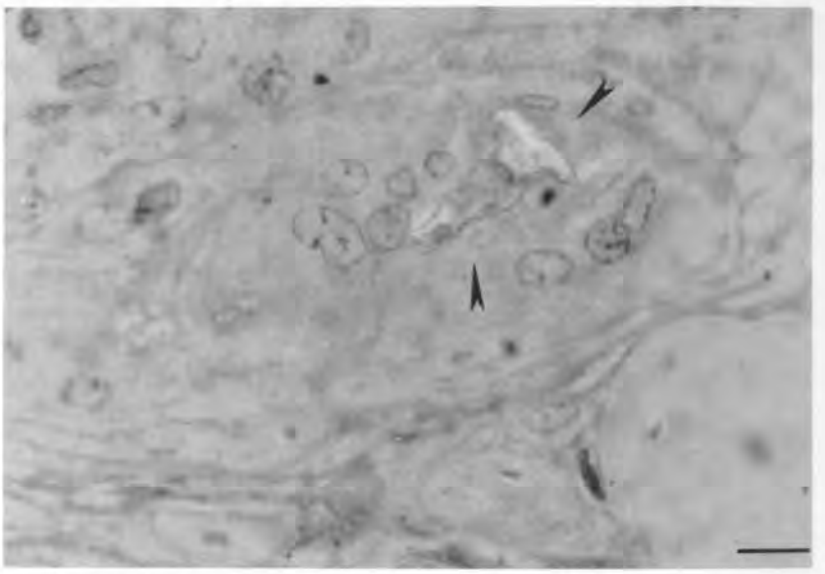

Fig. 7: High power magnification of a resorption lacuna. Small HA crystals are detached from the bulk of the HA material (arrowheads) (basic fuchsin-methylene blue) Bar = 10 μ.

Fig. 7: Aspect à plus fort grossissement d'une cavité de résorption. Petits cristaux d'HA détachés de la particule d'HA (fuchsine basique-bleu de méthylène) Barre = 10 μ.

The dissolution and resorptions phenomena were very clear with LSM. LSM fluorescent material was also present in the most part of the HA particles in a way similar to the basophilic material seen in light microscopy (Fig. 8).

This substance had a granular appearance with a polygonal shape very similar to HA crystal's morphology. A fracture line of the external side of the HA was detected, probably due to the sectioning process (Fig. 8).

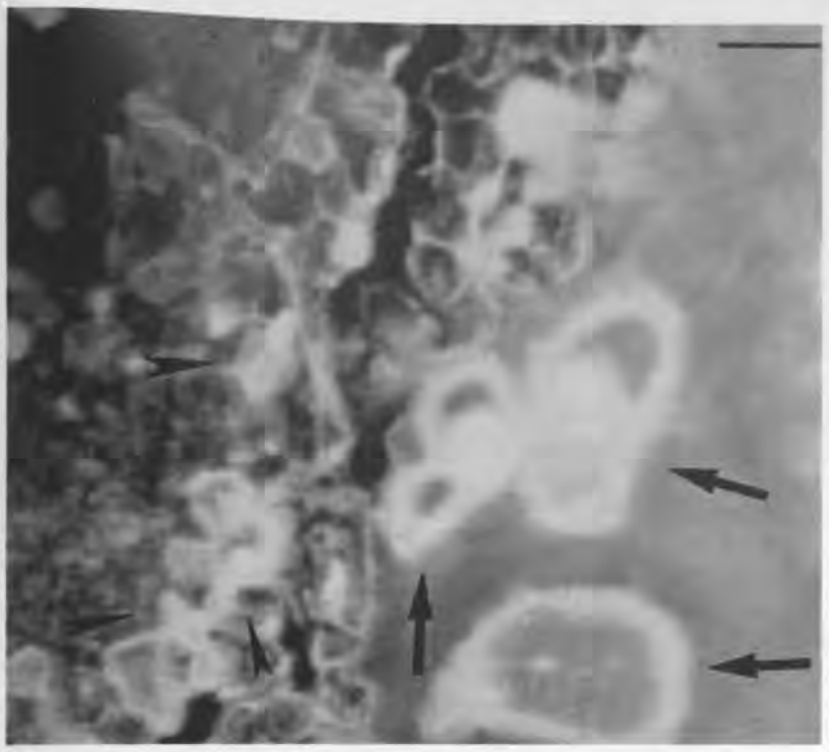

Fig. 8: LSM image in fluorescence mode. On the right side is present bone tissue and a marrow space. Note the presence of fluorescent material between HA crystals (arrowheads), and of osteocytes (arrows) (basic fuchsin-methylene blue) Bar = 10 μ.

Fig. 8: Image au LSM en fluorescence. A la droite il y a du tissu osseux et un espace médullaire. On remarque du matériel fluorescent à l'intérieur des cristaux d'HA dans la partie inférieure de la photo (fuchsine basique-bleu de méthylène) Barre = 10 μ.

DISCUSSION

Numerous factors have been implicated in host-biomaterials response. An important role is certainly played by solubility of the material, surface morphology, roughness and porosity, type and activity of cells (Gross et al., 1991). Most of the data have been obtained from experimental work in animals and little human material has been available to confirm those results (Craig et al., 1989): moreover results obtained from animals studies cannot easily be compared to those concerning chronic progressive periodontitis in humans (Orly et al., 1989). HA was thought to be nonresorbable (Hoogendoorn et al., 1984, Verburg et al., 1988, Klein et al., 1989), but recently many studies have shown that it can undergo degradation (Craig et al., 1989, Orly et al., 1989, Gross et al., 1991): this bioresorption seems to be proportional to the tricalcium phosphate (TCP) content (Kwong et al. 1989, Donath 1990). The mechanism of biodegradation of HA is yet unclear: it appears that it could be a result of a disaggregation of particles into crystals and a dissolution of crystals (Rey, 1990). Kwong et al. (1989) state that the bioresorption of calcium-phosphate ceramics begins with a solution-mediated microscopic break up of the crystals with subsequent cell-mediated removal of the fragments. Little is moreover known about the cell-mediated process of resorption (Kwong et al., 1989, Gross et al., 1991): Kawaguchi et al. (1992) have demonstrated the presence of multinucleated cells in the interface between HA and bone after implantation. Cells surrounding the implanted material have been shown to phagocytose individual particles or fragments of the material, with subsequent intracellular degradation in phagolysosomes (Kwong et al., 1989). Kawaguchi et al. (1992) have obtained morphological and cytochemical data that show that the multinucleated cells elicited by HA can be characterized as osteoclasts: other cells that have been shown to solubilize bone mineral in vitro include macrophages, monocytes, synovial cells and fibroblasts (Kwong et al., 1989). Kwong et al. (1989) have moreover shown that cell contact is essential for the cell-mediated crystal dissolution. Also in our material it has been possible to demonstrate multinucleated cells at the bone-HA interface, with HA crystals in the cytoplasm. One of the most striking results that we have had is the difference in the bioresorption rate of the HA between mineralized bone and marrow soft tissues. These results in man are comparable to those that we have had in animals (Piattelli et al.). According to Gross et al. (1991) interstitial fluid seems to play an important role in bioresorption of calciumphosphate ceramics, and our data could be well explained by the small amount of fluid that is present at the interface with mineralized bone and the relatively greater flow of fluid through loose connective tissue. This difference can thus be due to the possibility of HA being exposed to fluids, macrophages or osteoclasts, present in marrow spaces. On the other hand we observed that the production of bone at the interface by the osteoblasts seemed to put a stop to the further resorption of the ceramic, probably as no fluids or phagocytic cells could interfere with the interface. We could thus hypothesize that the resorption phenomena proceed as long as the HA is exposed to the fluids and to the marrow cells; a «race» between macrophages and osteoblasts could then be surmised.

ACKNOWLEDGMENTS

This work was partially supported by the Italian National Research Council and by the Ministry of University, Research, Science and Technology (MURST 60%).

The authors gratefully acknowledge the technical work done by Dr. Antonio Scarano, D.D.S.

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