Ultrastructural characteristics of connective tissue around porous hydroxyapatite hypodermic implants in rats

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Adults Sprague-Dawley rats were implanted with porous hydroxyapatite (Interpore 200®), following a procedure different from that of Kawaguchi et al (1992). Instead of implanting hydroxyapatite (Ha) in periodontal osseus defects, we introduced Ha-implants in the hypodermis of rats. Animals were sacrificed on days 30, 90 and 150 (six in each stage). The interface between the Ha and connective tissue was studied by transmission electron microscopy, with the aim of understanding the biocompatibility and mechanisms of union of both parts.

The connective tissue reaction to the Ha implant was characterized by fibrovascular proliferation, with abundant fibroblasts, macrophages, multinucleated giant cells, and by the formation of a capsule surrounding the implant. The multinucleated giant cells were observed in the interface along all stages and exhibited: a) a progressive increase in mitochondria, ribosomes, rough endoplasmic reticulum and vesicles containing particles of Ha; and b) an electronlucent material of variable aspect in vesicles contained in their cytoplasmic expansions. The prominent cytologic aspects of the multinucleated giant cells in the juxta-Ha zone may indicate that both, the biocompatibility and the intimate union between connective tissue and Ha, are strongly dependent on the presence of these cells.

Key words: *biocompatibility, connective tissue reaction, multinucleated giant cells, porous hydroxyapatite.*

INTRODUCTION

The successful utilization of porous hydroxyapatite (Ha) in the correction of bone malformations and defects gives to this material an undeniable therapeutic value (1, 6, 8). Although bone tissue or Ha implant are usually utilized for this type of implant, it is inevitable that these grafts should be in contact with or will be invaded by connective tissue (CT). Any eventual inflammatory or foreign body reaction will be readily seen in the soft tissue. The histologic changes of the CT towards Ha implants are basically expressed by a fibrovascular proliferation, containing abundant fibroblasts, macrophages, multinucleated giant cells (MGC) and a collagen connective capsule surrounding the implant (4, 10).

The aim of this study was to inquire on the multiple reactions of CT to Ha implants in the rat, with emphasis on the ultrastructural aspects of the MGC involved in the process. We believe that the striking cytologic changes we observed in the interface may help to explain the biocompatibility of the whole system, including the union of the CT to the Ha-implant.

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MATERIAL AND METHODS

Interpore 200®, a product of Interpore International (Irvine, CA, USA), consists of a marketed porous Ha, obtained from coral skeletons by means of a hydrothermic treatment (15). This porous microcrystalline implant is described as a bioactive, non-degradating, unabsorbable, non-toxic material with osteoconducting capability (1, 5, 21).

Small fragments of this material were surgically implanted into the hypodermis of the back of 20 adult female Sprague-Dawley rats (weighing around 300 g). Tissue specimens were obtained from the three implants performed in each of the six animals, sacrificed on days 30, 90 and 150. For control purposes, two additional rats were sacrificed on days 15 and 360.

The blocks of tissue were fixed in 3% glutaraldehyde for 4 hours and overfixed for 90 minutes in 2% osmium tetroxide and then, included in Epon 812. Ultrathin sections grids for transmission electron microscopy were stained with uranyl acetate and lead citrate and observed in a Zeiss 109 electron microscope. Sections of 1 m thickness for optical microscopy were stained with methylene blue and hematoxylin-eosin. Observations and photomicrographs were performed by means of a Nikon Labophot using a Kodak T max 100 film.

RESULTS

30-day implants. They were surrounded by a fine fibrous capsule. Connective-vascular stalks were seen invading the cavities of the porous material, showing many fibroblasts and macrophages in different stages of development (Fig 1A, B). Numerous MGC, with chromophobic or finely vacuolated cytoplasm and a profuse number of vesicular nuclei, were disposed close to the surface of the cavities of the Ha. A conspicuous chromophilic band was observed in close relationship with the surface of the implant, sending thin extensions that invaded the microducts present in the Ha (Fig 1B). The study with transmission electron microscopy showed that this peripheral band corresponded to a finely granular and filamentous cytoplasm, containing abundant mitochondria, some profiles of rough endoplasmic reticulum (RER) and Ha particles (Fig 1C). This band showed also many filopodes extending in various directions (Fig 1D).

90-day implants. The surrounding connective capsule showed increased fibrosis, which was connected with the fibrovascular stalks that were located preferentially at the center of the holes or tunnels of Ha (Fig 2A). In between these stalks and the walls of the tunnels, a rich cellular population persisted, including fibroblasts in different stages of maturity, plasma cells, and a profusion of macrophages, some of them of large size, with huge phagosomes filled with Ha particles (Fig 2B). MGC showed large nuclei, mitochondria and vacuoles, containing either electronlucent material or Ha particles (Fig 2C).

The above described juxta-Ha cytoplasmic band was wider, more chromophilic and neater than that observed at 30 days (Fig 2D), showing an increased number of mitochondria and vacuoles filled with either electronlucent or fine granular material (Fig 3A).

150-day implants. The external capsule and the fibrovascular stalks attained greater definition and maturity (Fig 3B). The cellular population was mainly composed of fibroblasts and large macrophages containing voluminous phagosomes with particulated Ha. Plasma cells were also numerous. The MGC had reached a strikingly large size and had more nuclei (Fig 3B). The chromophilic cytoplasmic band was neater, and showed more vacuoles containing either homogeneous or finely granular material, or Ha particles. Profiles of RER cisternae and clusters of ribosomes were also present (Fig 3C).

15- and 360-day implants. Two additional rats were sacrificed on these days to reach a better understanding of the role of the MGC in the whole process. At 15 days, several MGC were already present and also some epithelioid-like cells were observed (Fig 3D). At 360 days, the MGC continued to be in close contact with the surface of the Ha (Fig 3E).

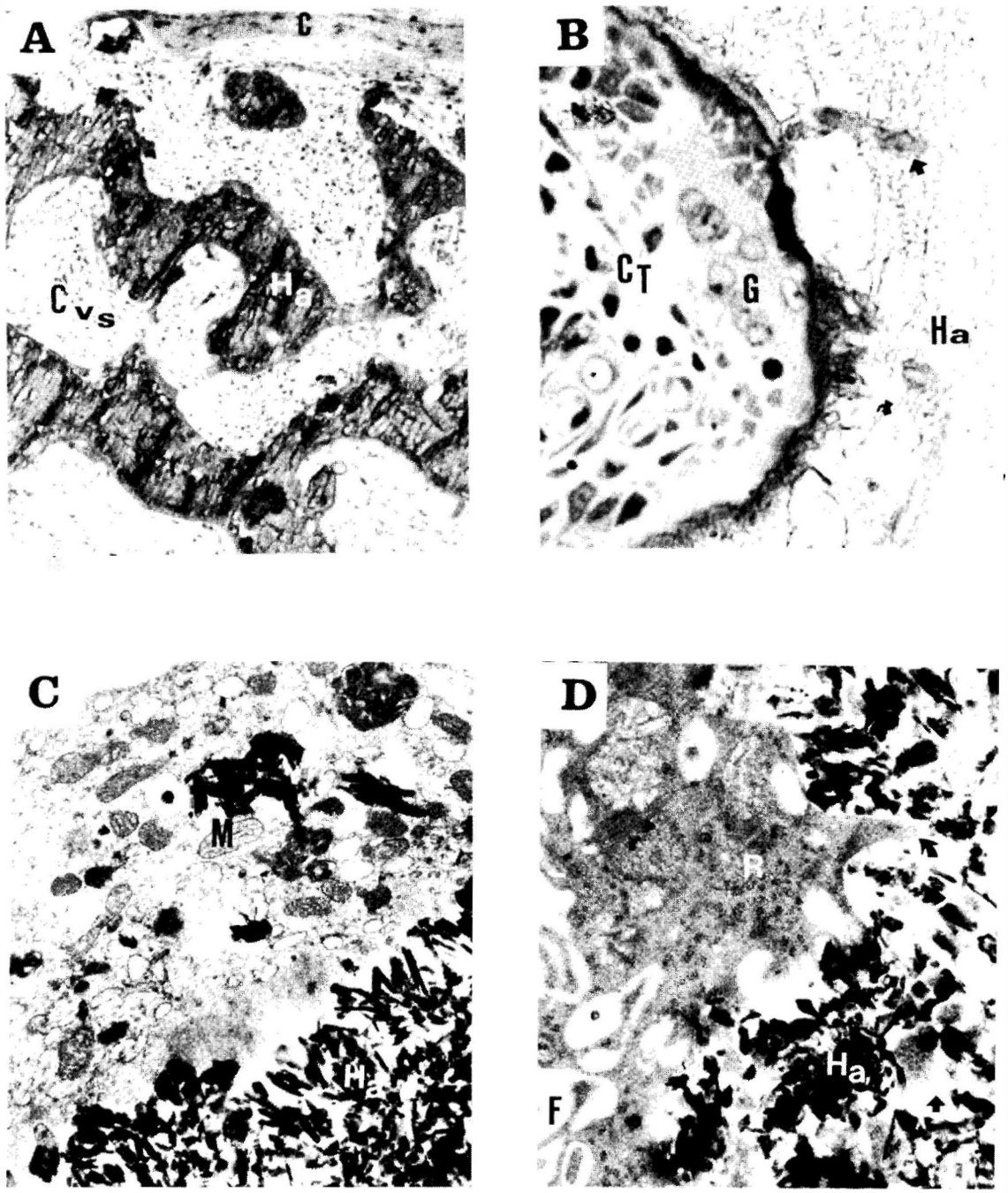

Fig 1. 30-day implants. **A.** Connective-vascular stalks (Cvs) inside the pores and spaces of hydroxyapatite frame (Ha). A segment of the external fibrillar capsule (C) is shown. It is noteworthy that the contact between Ha and the connective-vascular stalks is optimal. Optical microscopy. 40 X, original enlargement. **B.** Hydroxyapatite connective tissue interface. Conspicuous cytoplasmic chromophilic band in close contact with hydroxyapatite (Ha) showing branching projections from the multinucleated giant cells (G) into microducts of the frame (arrows). CT: connective tissue. Optical microscopy. 400 X, original enlargement. **C.** Cytoplasmic interface band-hydroxyapatite. Many mitochondria (M) and clusters of particulated hydroxyapatite (Ha) are shown within this band. TEM. 12,000 X, original enlargement. **D.** Magnified view of interface hydroxyapatite (Ha) adjoined band that shows branches of this band projected into the microducts of the frame (arrows). Clusters of ribosomes (R) and external filopodes (F) also shown. TEM. 20,000 X, original enlargement.

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DISCUSSION

Several authors have described the main histologic aspects of the interface between the Ha implant and the CT or bone, stressing the presence of MGC (1, 4, 5, 6, 21). Nevertheless, the role of these cells in establishing a close grasp with the implant and a derived biocompatibility has not been defined. We focused our study mainly on the cytologic features of the MGC, which may explain its possible functional role in the success of the implant (1, 2, 6, 10, 17, 18).

The CT reaction to the implant at 30 days can be described as of a granulation tissue in the early stage of the healing process, with the presence of MGC of foreign type and a fibrillar enwrapping capsule that spreads through connective vascular buds into the pores and tunnels of the implant. Lately (at 90 and 150 days) the reaction becomes mature and fibrotic. This reaction is equivalent to a cicatrization process.

The great cellular density around the Ha-implants was particularly striking at 90 and 150 days, showing abundant fibroblasts and many large macrophages with bulky phagosomes, filled with Ha particles. Therefore, it appears that the CT maintains reactional foci of cellular proliferation, which might be interpreted as a defensive reaction, finally yielding a cicatrization process, with the eventual absorption of the implanted material.

The presence of plasma cells in the reactive tissue increases with time, and suggests that some kind of immune response might be involved. This point is of great interest, since it is similar to the events recently found in silicone implants. In fact, although once thought to be inert, silicone implants induce chronic inflammation and strong immune responses which probably are of autoimmune nature and eventually might be considered as monoclonal gammapathies. Therefore, the implants of apparently inert material could produce not only local inflammatory reactions, but also systemic consequences, which should be carefully investigated in the future (14).

The studies of Kawaguchi *et al* (6) were concerned with Ha implants in periodontal osseus defects. These authors stressed that the occurrence of MGC is one of the striking histologic features of the cellular reaction of bones against an Ha- implant. It is widely accepted that these cells are derived from the fusion of specialized macrophages, which are known as epithelioid cells (9, 17, 18). In our study, the large size and the increasing number of nuclei of these cells (Figs 2D and 3B) strongly suggests that the MGC are in a very active state. These MGC can be differentiated from the osteoclasts by the absence of ruffled-border, specific granules and by the lack of an osseus structure for their support (2, 3, 6, 12, 16, 19). Moreover, the presence of numerous mitochondria, abundant RER, ribosome clusters, inclusions of Ha-particles and vesicles containing material of variable electronlucence, shows that they are very active cells, in an evident stage of biosynthesis of substances with endocytic activity (Figs 2C and 3C).

Another important feature of the MGC is the cytoplasmic chromophilic band, intimately in contact with the surface of Ha, sending extensions to the microducts of the Ha-frame. This strongly stained band becomes more neat and chromophilic with time, and shows an increasing content of mitochondria, RER, ribosomes and vesicular structures containing sometimes particles of Ha, while others have a substance of homogeneous or granule-fibrillar aspect inside (Figs 3A and 3C). These vesicles are either a cellular secretion or necrobiotic material (13).

The term "biocompatibility" has been defined as the ability of an alien material to induce an appropriate host response in each specific application (20). We believe that the cytoplasmic band already described in the MGC, through its veiled expansions, may be important in establishing a firm grasp with the implant (Fig 2D). It is attractive to speculate that several cytokines, some hydrolytic enzymes, and even interleukin I, may be produced in this area (6, 7, 9, 17), helping to the biocompatibility of the implant. Such biocompatibility, based on the persistence and modulation of cytological structures, was observed both in the study of Kawaguchi *et al* (6) and in this investigation.

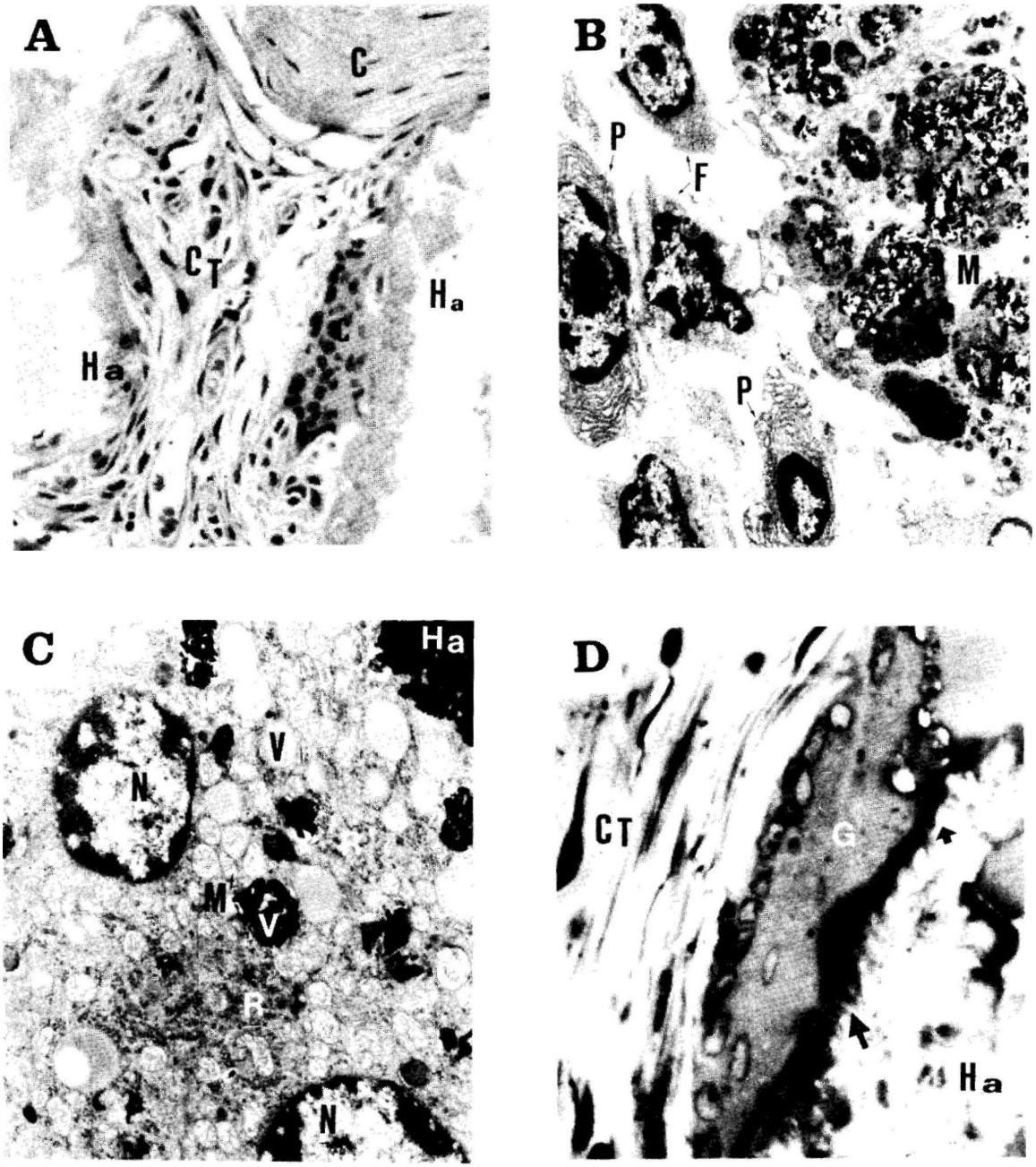

Fig 2. 90-day implants. **A.** Wrapping fibrillar capsule (C) and vascular connective content (CT) inside a space of the hydroxyapatite (Ha) frame. G: multinucleated giant cell. Optical microscopy. 200 X, original enlargement. **B.** Fibroblasts (F), two plasma cells (P) and a large macrophage (M) containing huge phagosomes with hydroxyapatite particles, localized inside a space of the Ha-frame. TEM. 3,000 X, original enlargement. **C.** Segment of a multinucleated giant cell apposed to hydroxyapatite (Ha). The cell contains two nuclei (N), clusters of ribosomes (R), many mitochondria (M) and several vesicles (V), some of them containing an electronlucent material and others, particles of hydroxyapatite. TEM. 7,000 X, original enlargement. **D.** Very large multinucleated giant cell (G) continuous to a striking chromophilic cytoplasmic juxta hydroxyapatite band (arrows). CT: connective tissue; Ha: hydroxyapatite. Optical microscopy. 400 X, original enlargement.

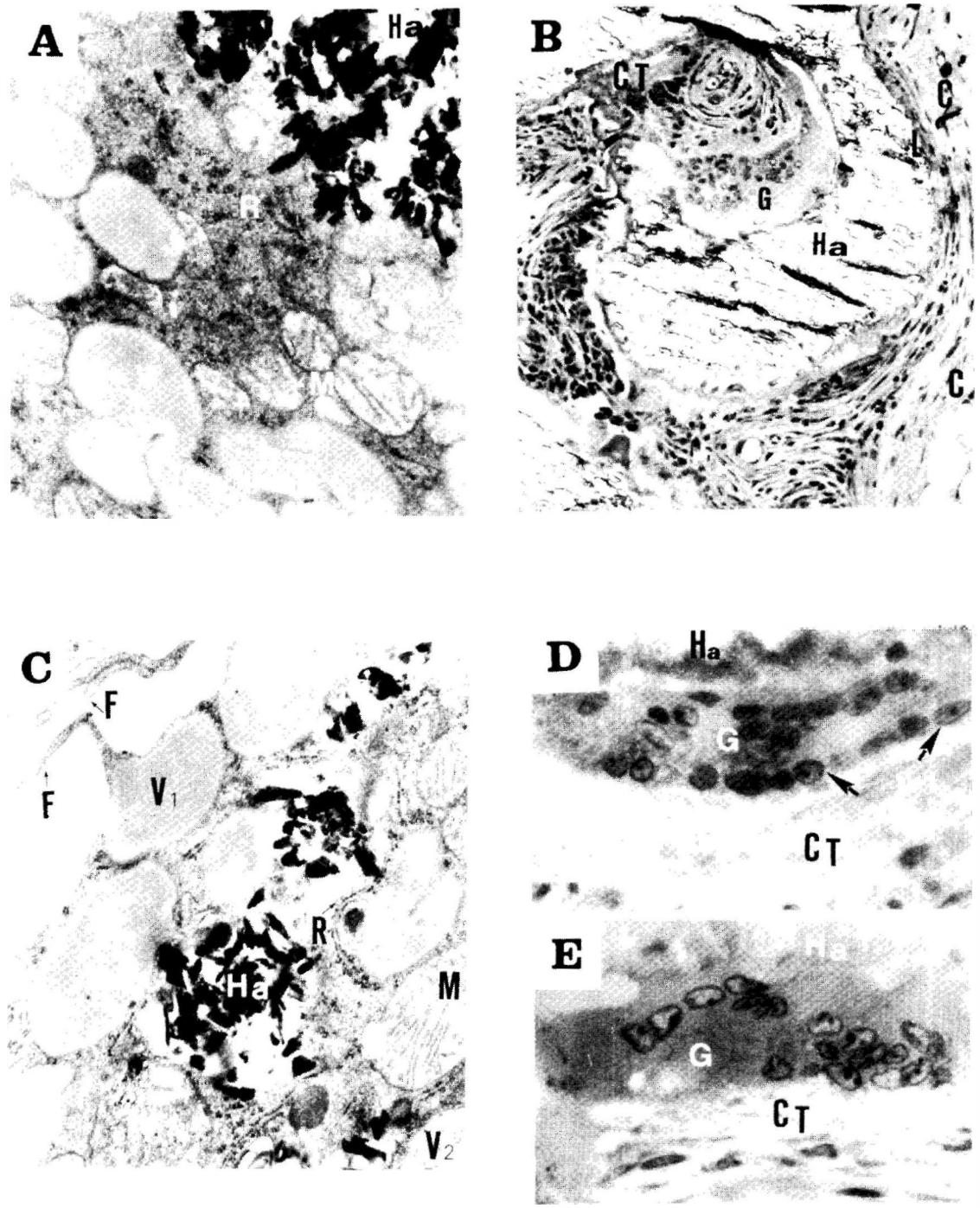

Fig. 3. A. 90-day implant. Cytoplasmic juxta hydroxyapatite stalks. Numerous mitochondria (M), clusters of ribosomes (R) and several vacuoles (V) with electronlucent material of various aspects. Ha: hydroxyapatite. TEM. 20,000 X, original enlargement. **B.** Topographic aspect of 150-day implant. A more mature and fibrous surrounding capsule (C) with intra frame stalks is shown. G: multinucleated giant cell; Ha: Hydroxyapatite; CT: connective tissue. Optical microscopy. 100 X, original enlargement. **C.** 150-day implant. Cytoplasmic band juxta hydroxyapatite. Mitochondria (M) and several vesicles, some of them containing particles of hydroxyapatite (Ha) and others, electronlucent material either homogeneous (V_1) or particulated (V_2). Profiles of rough endoplasmic reticulum (R) and filopodes (F) also shown. TEM. 20,000 X, original enlargement. **D.** 15-day implant. Assembling epithelioid cells (arrows) forming a multinucleated giant cell (G). Ha: Hydroxyapatite; CT: connective tissue. Optical microscopy. 400 X, original enlargement. **E.** 360-day implant. Multinucleated giant cell (G) close to the frame of hydroxiapatite (Ha). CT: conective tissue. Optical microscopy. 400 X, original enlargement.

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