

Tricalcium phosphate endosseous implants in dentistry: ultrastructural findings

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SUMMARY

β -Tricalcium phosphate magnesium substituted (β -TCMP), consisting of one part small unsintered and one part large sintered granules was placed in upper and lower jaw surgical cavities of monkeys. At light and transmission electron microscope the biopsies taken at 2, 4, 8 and 24 weeks showed that both sintered and unsintered granules were well tolerated by the host tissue and result biodegradable over time. β -TCMP may stimulate the direction of bone growth enhancing osteoblasts activity and new bone deposition in direct contact and in the micropores of the biomaterial. β -TCMP containing Mg ions reabsorbs slowly when in sintered granules form and rapidly as unsintered granules. When it is placed in bone cavities, if bone reabsorption is biologically necessary, the biomaterial can be reabsorbed with bone, while if osteogenesis is required β -TCMP provides a more durable matrix to support new bone growth.

KEY WORDS:

Tricalcium Phosphate – Endosseous Implants – Oral Surgery – Bone Regeneration – Ultrastructure.

RÉSUMÉ

Le β -phosphate tricalcique contenant à ions Mg (β -TCMP) et constitué à parts égales de fins granules non frittés et de larges granules frittés, est introduit dans des cavités chirurgicales pratiquées dans les maxillaires supérieurs et inférieurs de singes.

Des biopsies pratiquées à 2, 4, 8 et 24 semaines ont été examinées au microscope optique et électronique à transmission.

Les granules frittés et non frittés sont bien tolérés par les tissus hôtes et sont biodégradés avec le temps.

Les observations histologiques montrent aussi que le β -TCMP peut orienter la direction de la croissance osseuse en stimulant l'activité des ostéoblastes et le dépôt d'os nouveau aussi bien autour des biomatériaux que dans leur micropores. Le β -TCMP contenant les ions Mg est réabsorbé lentement lorsqu'il se présente sous la forme de granules frittés, et rapidement lorsqu'il est sous la forme de granules non frittés.

Lorsqu'il est introduit dans les cavités osseuses, si la réabsorption osseuse est biologiquement nécessaire, le biomatériau peut être réabsorbé avec l'os, tandis que si c'est l'ostéogénèse qui est sollicitée, β -TCMP fournit une matrice durable comme support à la croissance d'os nouveau.

MOTS CLEFS:

Phosphate tricalcique – Implants endosseux – Chirurgie orale – Régénérescence osseuse – Ultrastructure.

INTRODUCTION

β -TCP is often used as an alternative alloplastic material to conventional autogenous or allogeneous bone implants in dental, maxillo-facial, plastic and orthopedic surgery. It is biocompatible, biodegradable, easy to sterilize (Frame, 1980; Jarcho, 1981; Williams, 1987; Pizzoferrato, 1988) and may be used as fragments (Driskell *et al.*, 1973), granules or in various powder forms (Strub *et al.*, 1979) to fill bone defects resulting from disease, trauma or iatrogenic causes. Since β -TCP stimulates dentine growth (Franchi *et al.*, 1987) it is employed in conservative dental treatment. However β -TCP is especially used to fill periodontal (Levin *et al.*, 1974a; Levin *et al.*, 1974b; Barney *et al.*, 1986; Bowers *et al.*, 1986), palatal (Mors and Kaminski, 1975) and periapical bone defects (Roberts and Brilliant, 1975; Coviello and Brilliant, 1979). Once placed in calcifying tissues, β -TCP is readily enveloped first by osteoid tissue and subsequently by newly formed bone (Bhaskar *et al.*, 1971; Cutright *et al.*, 1972; Driskell *et al.*, 1973; Levin *et al.*, 1974a; Levin *et al.*, 1974b; Levin *et al.*, 1975; Nery and Lynch, 1975; Cameron *et al.*, 1977; Strub *et al.*, 1979; Metsger *et al.*, 1984; Snyder *et al.*, 1984; Barney *et al.*, 1986; Bowers *et al.*, 1986).

β -tricalcium phosphate magnesium substituted (β -TCMP), consisting of one part small unsintered granules and one part large sintered granules (Ruggeri *et al.*, 1987; Franchi and Ruggeri, 1989) was placed in upper and lower jaw surgical cavities. Periimplant tissue organization and in particular the β -TCMP/new bone interface were studied with light and electron microscopy.

MATERIAL AND METHODS

20 round bone cavities (diameter 4-5 mm) were created surgically using a diamond burr in the maxilla and mandible of Macacus Rhesus monkeys. The cavities were then packed with a mixture of sintered β -TCMP granules (average 250-350 μ m) and unsintered powder (granules size less than 50 μ m).

The animals were anaesthetised with Ketamine Chlorhydrate (Ketalar) i.m. and the β -TCMP was mixed with human fibrin (Tissucol) before use to ensure better handling and to enhance as soon as possible connective tissue organization. Once filled, all the bone cavities were then closed by suturing the mucoperiosteal flaps.

Periodic clinical and X-ray examinations showed that the ceramic material was gradually absorbed and replaced by newly formed bone tissue. In 2 animals 2 maxillar implants failed due to dehiscence of the mucoperiosteal flaps. The remaining 18 cavities were biopsied at 2,4,8 and 24 weeks for histological and ultrastructural examination.

The samples were fixed in glutaraldehyde diluted in 2% phosphate buffer, pH 7.0. Some samples were decalcified in an EDTA aqueous solution and subsequently treated for embedding in paraffin. The other specimens were divided into 2 groups, one being decalcified in an EDTA aqueous solution. Both these groups were then post-fixed in 1% osmium phosphate buffer, dehydrated in alcohol and embedded in epoxy resin for transmission electron microscopy study. The paraffin fixed samples were stained with Hematoxylin Eosin, Azan Mallory, Masson's Trichrome and Sirius Red. The semi-thin sections for TEM study were stained with Toluidine Blue while the thin sections were treated with Uranyl Acetate and Lead Citrate.

RESULTS

Host tissue response was not seen to differ between upper and lower jaws. After 2 weeks a characteristic well vascularized granular tissue with many cells enveloping both the sintered β -TCMP granules and unsintered powder was present. In particular, blood vessels with ample lumen, fibroblasts, granulocytes and few polynucleate cells with macrophages were prevalently arrayed around the sintered granules. At TEM, cytoplasmic processes, whole cells, collagen fibrils as well as finely granular electron dense material were seen to be lodged in the micropores of these sintered granules (Fig. 1, 2, 3, 4). The cytoplasm of polynucleate cells and macrophages in the newly formed tissue contained ceramic material presumably residues of the unsintered powder (Fig. 1, 5).

At 4 weeks the granulation tissue appeared well organized into fibrous connective tissue; the polynucleate cells and macrophages were practically absent and the unsintered powder largely absorbed. Compared to their original appearance the sintered β -TCMP granules were now of differing shapes and irregular sizes. In the Sirius Red stained samples, they appeared enveloped and surrounded by collagen fibrous bundles arranged parallel to the granule surface. At this stage, the newly formed tissue contained

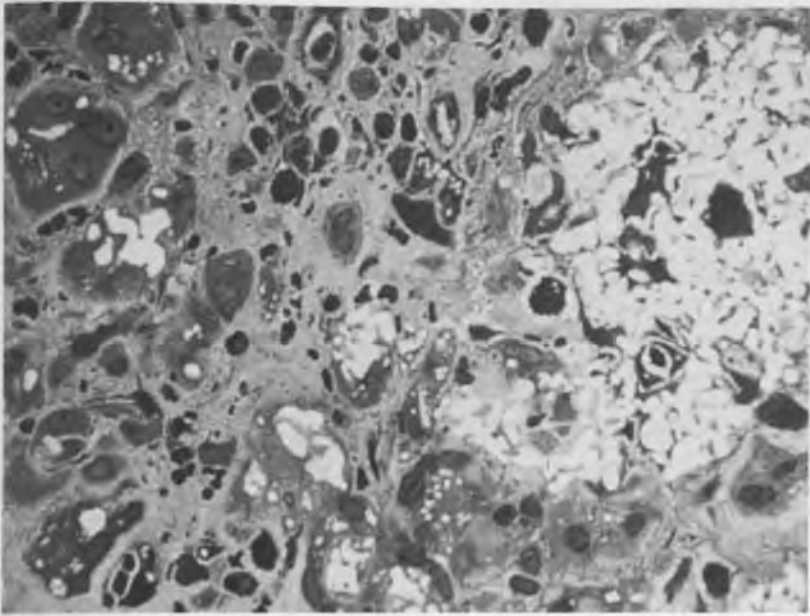


Fig. 1 - β -TCMP endosseous implant after 2 weeks. On the right a sintered granule with cellular processes colonizing the micropores and surrounded by polynucleate cells and macrophages. Several polynucleate cells and macrophages in the granulation tissue show cytoplasmic residues of unsintered powder (450 \times).

Fig. 1 - Implant endosseux de β -TCMP après 2 semaines. A droite, un granule fritté en rapport avec un processus cellulaire colonisant les micropores et entouré par des cellules plurinucléées et des macrophages. Plusieurs cellules plurinucléées et plusieurs macrophages du tissu de granulation contiennent des résidus intracytoplasmiques de poudre non frittée (Coupe semi fine, bleu de toluidine. Grossissement: 450 \times).

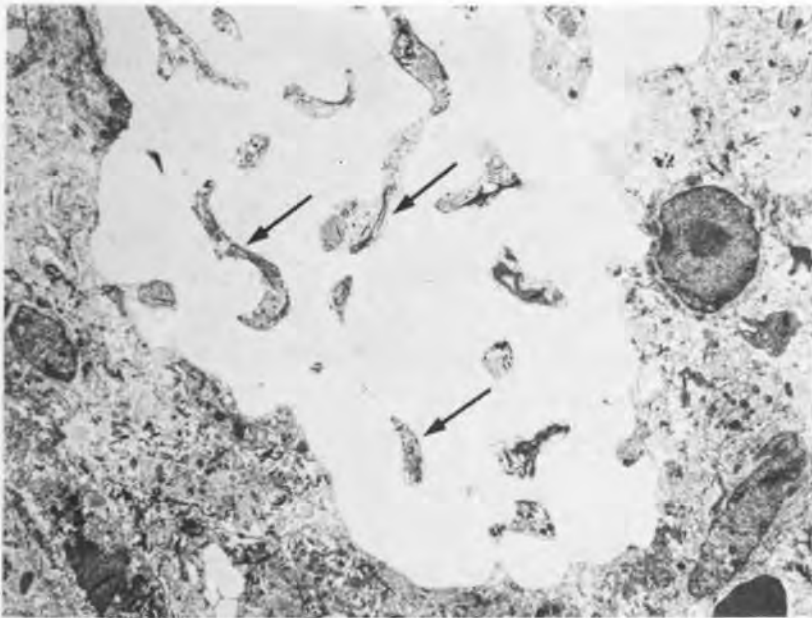


Fig. 2 - β -TCMP endosseous implant at 2 weeks. EDTA decalcified specimen. TEM micrograph shows the presence of cytoplasmic processes, collagen fibrils and finely granular material into the network of micropores of a sintered β -TCMP granule (Arrows) (2000 \times).

Fig. 2 - Implant endosseux de β -TCMP à 2 semaines. Décalcification par l'EDTA. La photographie en microscopie électronique à transmission montre la présence de processus cytoplasmiques, de fibrilles de collagène et un matériel finement granuleux (flèches) dans le réseau de micropores d'un granule fritté de β -TCMP (Acétate d'uranyle, citrate de plomb, Grossissement: 2000 \times).

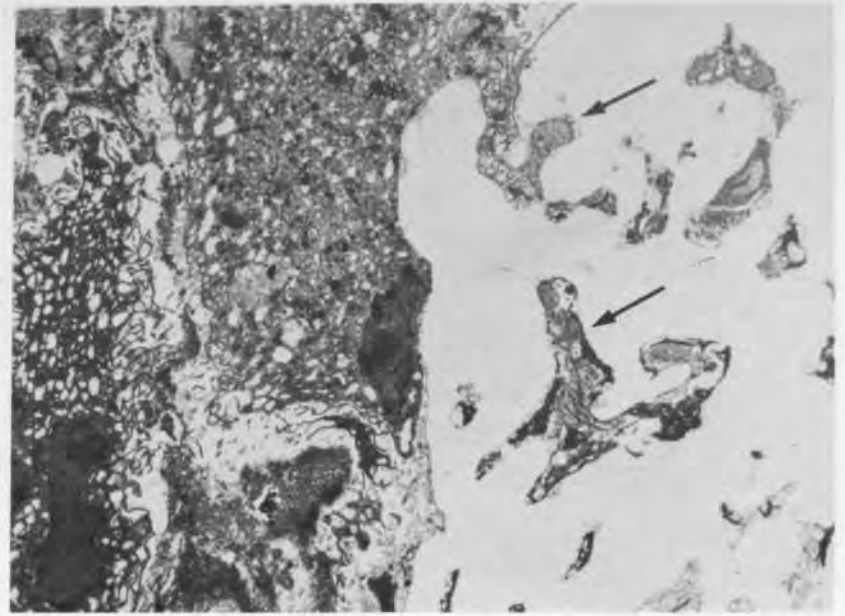


Fig. 3 - β -TCMP endosseous implant at 2 weeks. TEM micrograph of an EDTA decalcified specimen showing a granule (on the right) surrounded and enveloped by polynucleate cells and by collagen fibrils and granular material (Arrows) (2000 \times).

Fig. 3 - Implant endosseux de β -TCMP à 3 semaines. Image en microscopie électronique à transmission d'un spécimen décalcifié par l'EDTA montrant un granule (à droite) entouré et enveloppé par des cellules plurinucléées, par des fibrilles de collagène et du matériel granuleux (flèche) (Acétate d'uranyle, citrate de plomb. Grossissement: 2000 \times).

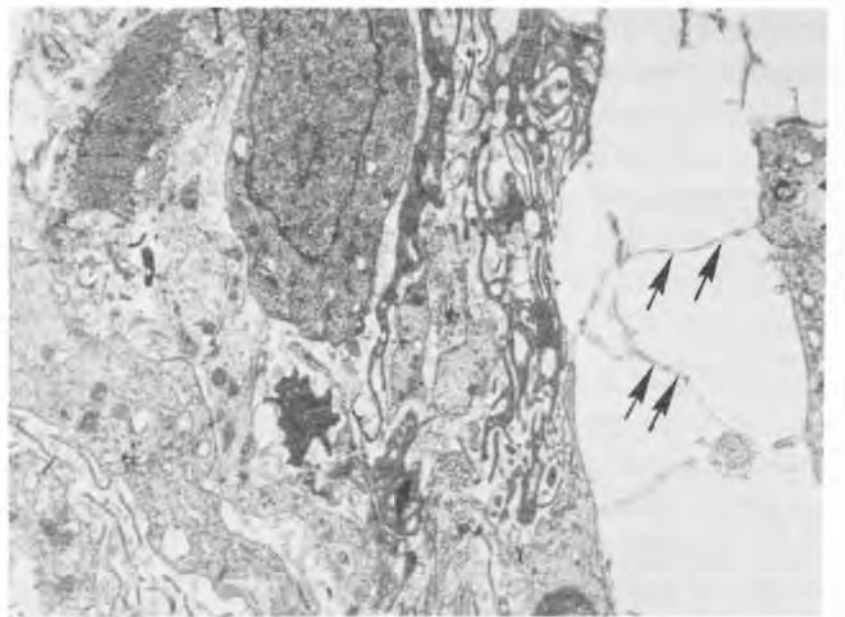


Fig. 4 - Electron micrograph of EDTA decalcified specimen of β -TCMP endosseous implant after 2 weeks. Cytoplasmic processes are present in the micropores of a sintered granule (on the right). 40-50 nm diameter canals (arrows) can also be observed running through the granule and forming a communicating network with the micropores. (4000 \times).

Fig. 4 - Vue en microscopie électronique à transmission d'un spécimen décalcifié par l'EDTA d'un implant endosseux de β -TCMP après 2 semaines. Des extensions cytoplasmiques sont présentes dans les micropores d'un granule fritté (à droite). Des canaux de 40-50 nm de diamètre (flèches) peuvent être observés à l'intérieur du granule et formant un réseau communicant avec les micropores. (Acétate d'uranyle, citrate de plomb. Grossissement: 4000 \times).

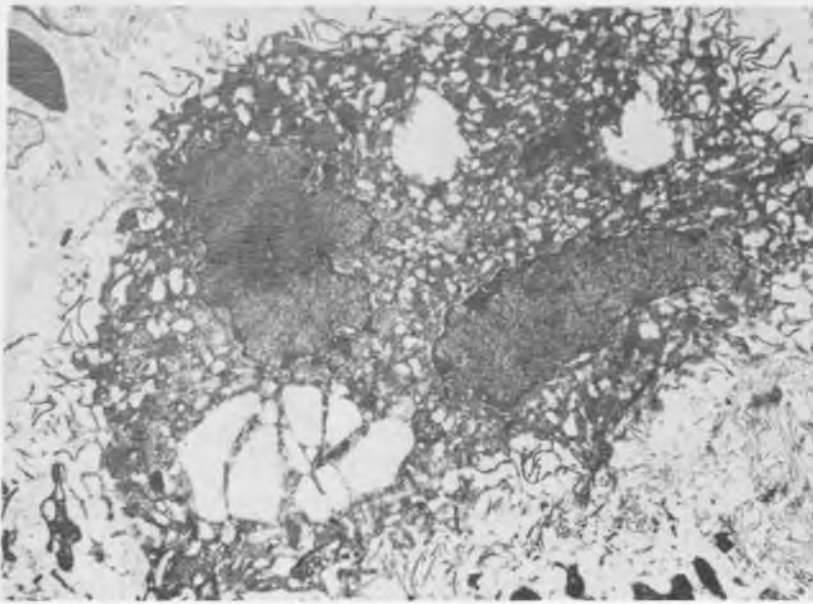


Fig. 5 - β -TCMP endosseous implant after 2 weeks. EDTA decalcified specimen. At TEM, a polynucleate cell containing unsintered powder residues in its cytoplasm is visible. (4000 \times).

Fig. 5 - Implant endosseux de β -TCMP après 2 semaines. Specimen décalcifié par l'EDTA. Au microscope électronique, on voit une cellule plurinucléée contenant des résidus de poudre non frittée dans son cytoplasme. (Acétate d'uranyle, citrate de plomb. Grossissement: 4000 \times).

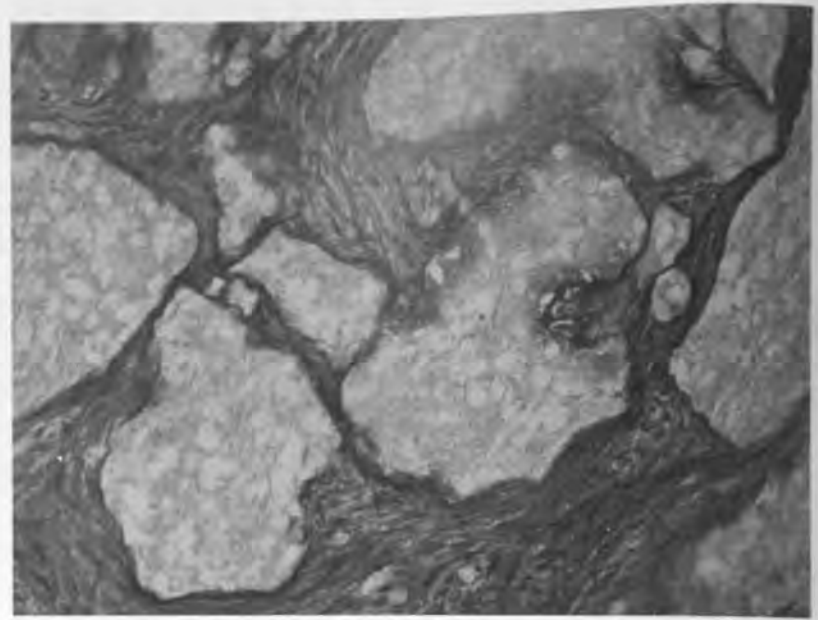


Fig. 6 - β -TCMP endosseous implant at 4 weeks. The sintered granules appear irregular in shape and enveloped by closely packed collagen fibre bundles, mature fibroblasts and globular cells. Sirius Red Staining. (180 \times).

Fig. 6 - Implant endosseux de β -TCMP après 4 semaines. Le granule fritté apparaît de forme irrégulière et enveloppé par des faisceaux de fibres de collagène fortement serrées, de fibroblastes matures et de cellules globulaires. Coloration au Rouge Sirius (Grossissement: 180 \times).

cells similar to mature fibroblasts in active protein synthesis and larger round cells similar to osteoblasts arranged at the surface of the granules (Fig. 6). The organic material observed in the micropores of the sintered granules at ultrastructural examination comprised collagen fibrils and finely granular electron-dense material. This communicating network of micropores was further integrated by a smaller network of canals whose diameter did not exceed 40-50 nm (Fig. 4,7).

After 8 weeks newly formed bone trabeculae were observed in the β -TCMP filled cavities: osteocytes were evidenced in ample lacunae along with numerous osteoblasts. Very few unsintered powder residues surrounded by osteoid or bone tissue were observed near the trabeculae (Fig. 8,9). The sintered granules were of various sizes, though mainly small, and if located at the periphery of the cavity were either completely or partially enveloped in newly formed bone trabeculae. This new bone always appeared to impinge directly upon the β -TCMP surface: calcified collagen fibrils being observed even in the micropores of the granules themselves (Fig. 10,11). The sintered granules at the centre of the cavity were mainly surrounded by osteoblasts and osteoid tissue (Fig. 12,13) which in some areas, near the β -TCMP, showed the presence of calcification nuclei (Fig. 14).

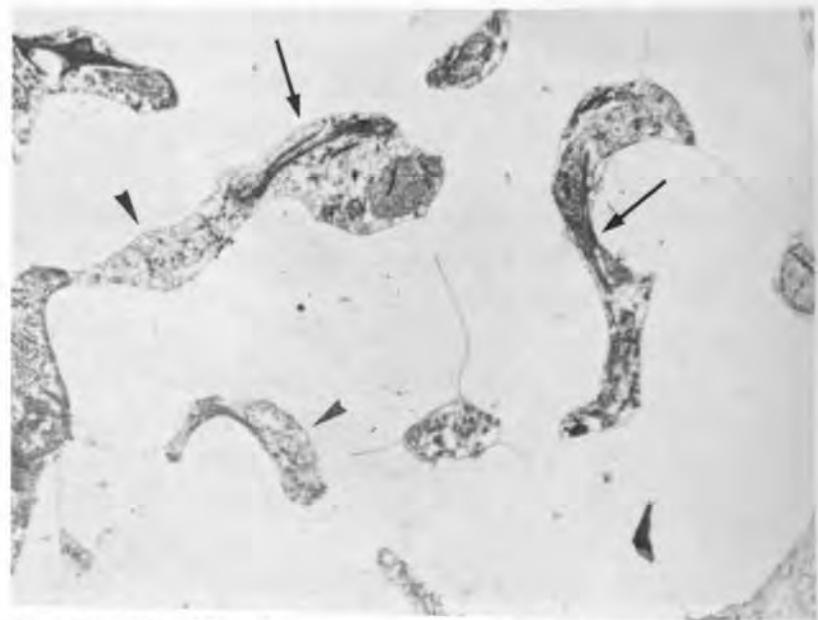


Fig. 7 - β -TCMP endosseous implant after 4 weeks. TEM micrograph of EDTA decalcified sample. Both the micropores and the small canals (diameter 40-50 nm) are occupied by collagen fibrils (arrows), cytoplasmic processes and granular material (arrow heads) (5000 \times).

Fig. 7 - Implant endosseux de β -TCMP après 4 semaines. Vue au microscope électronique d'un spécimen décalcifié par l'EDTA. Les micropores et les fins canaux (40-50 nm de diamètre) sont occupés par des fibrilles de collagène (flèches) des extensions cytoplasmiques et du matériel granuleux (têtes de flèches) (Acétate d'uranyle, citrate de plomb. Grossissement: 5000 \times).

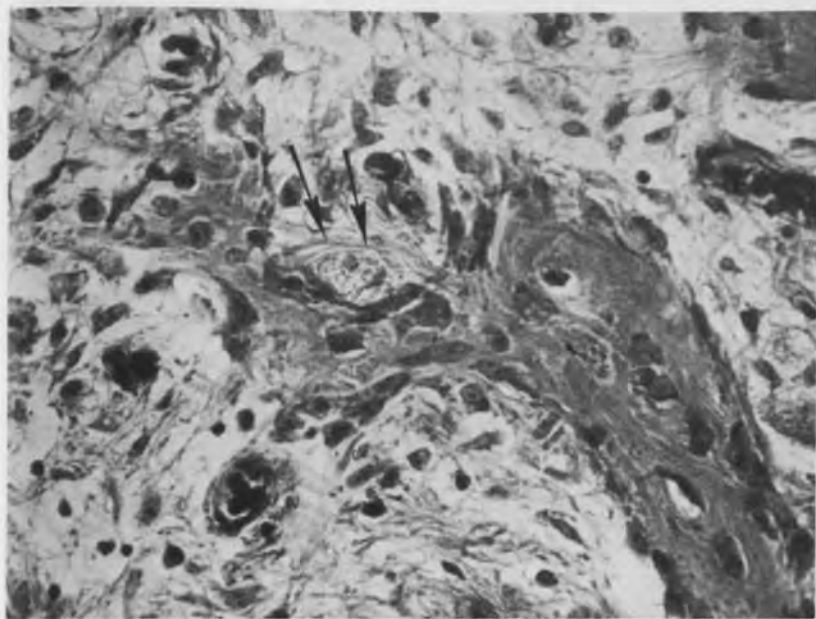


Fig. 8 - β -TCMP endosseous implant at 8 weeks. Newly formed bone trabeculae are observed near unsintered powder residues (arrows). Masson's Trichrome Staining. (400 \times).

Fig. 8 - Implant endosseux de β -TCMP à 8 semaines. Des travées osseuses nouvellement formées sont observées à proximité des résidus de poudre non frittée (flèches). Coloration au Trichrome de Masson (Grossissement: 400 \times).

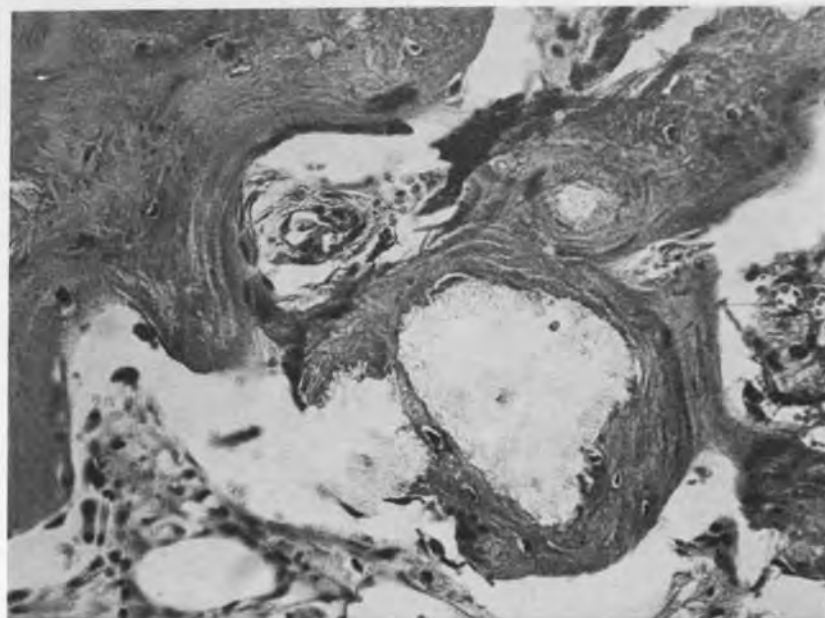


Fig. 10 - β -TCMP endosseous implant after 8 weeks. The sintered granules in the periphery of the cavity appear tightly enveloped in newly formed bone trabeculae. Masson's Trichrome Staining. (300 \times).

Fig. 10 - Implant endosseux de β -TCMP après 8 semaines. Les granules frittés à la périphérie de la cavité apparaissent enveloppés d'une manière serrée par des travées osseuses nouvellement formées. Trichrome de Masson. (Grossissement: 300 \times).

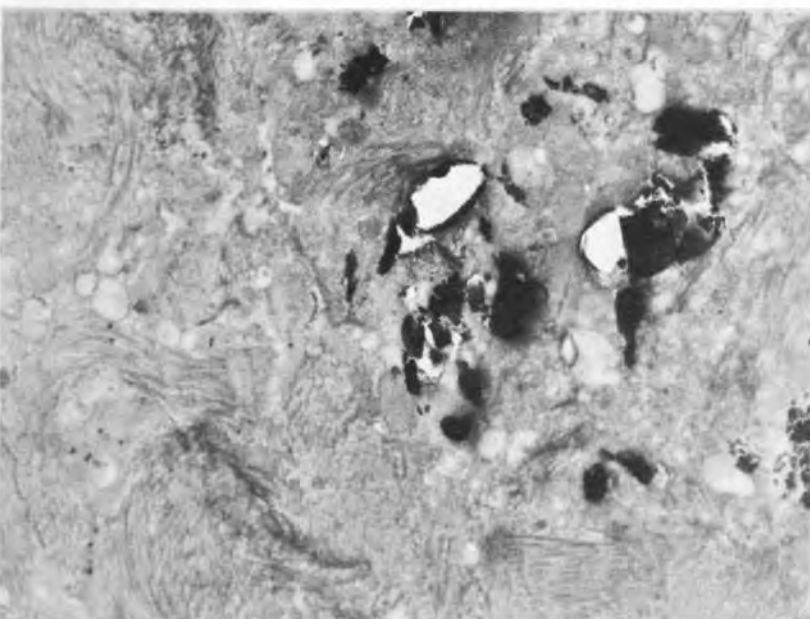


Fig. 9 - β -TCMP endosseous implant after 8 weeks. Undecalcified specimen unsintered powder residues appear surrounded by osteoid tissue. (8000 \times).

Fig. 9 - Implant endosseux de β -TCMP après 8 semaines. Spécimen non décalcifié. Résidus de poudre non frittée entourés par du tissu ostéoïde. (Acétate d'uranyle, citrate de plomb. Grossissement: 8000 \times).

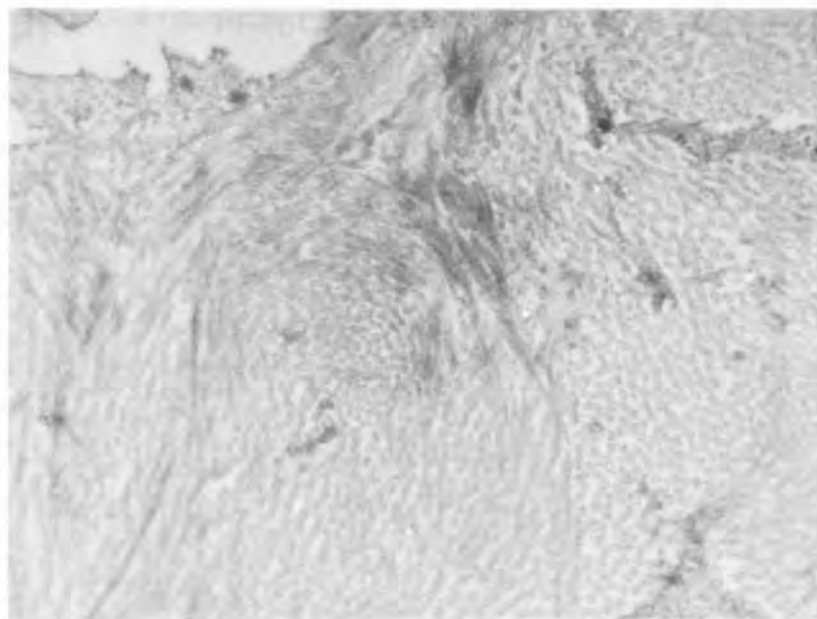


Fig. 11- EDTA decalcified specimen of β -TCMP endosseous implant after 8 weeks. The sintered granule (up on the left) appears enveloped and penetrated by newly formed bone (12.000 \times).

Fig. 11 - Spécimen décalcifié par l'EDTA d'un implant endosseux de β -TCMP après 8 semaines. Le granule fritté (en haut et à gauche) apparaît enveloppé et pénétré par de l'os nouvellement formé. (Acétate d'uranyle, citrate de plomb. Grossissement: 12.000 \times).

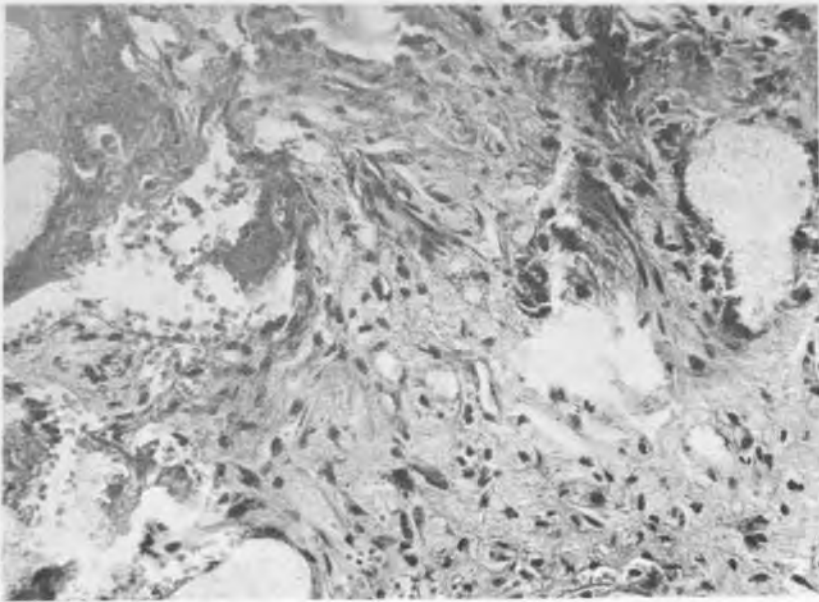


Fig. 12 - β -TCMP endosseous implant at 8 weeks. The granules at the center of the bone cavities are prevalently surrounded by osteoblasts and osteoid tissue. Masson's Trichrome Staining. (300 \times).

Fig. 12 - Implant endosseux de β -TCMP à 8 semaines. les granules frittés au centre des cavités osseuses sont essentiellement entourés par des ostéoblastes et du tissu ostéοide. Trichrome de Masson. (Grossissement: 300 \times).



Fig. 14 - Undecalcified sample of β -TCMP endosseous implant at 8 weeks. A sintered granule in the center of the bone cavity (on the right) appears surrounded by osteoid tissue with large calcification nuclei. (8000 \times).

Fig. 14 - Echantillon non décalcifié d'un implant endosseux de β -TCMP à 8 semaines. Un granule fritté dans le centre d'une cavité osseuse (à droite) apparaît entouré par du tissu ostéοide avec un noyau largement calcifié. (Acétate d'uranyle, citrate de plomb. Grossissement: 8.000 \times).

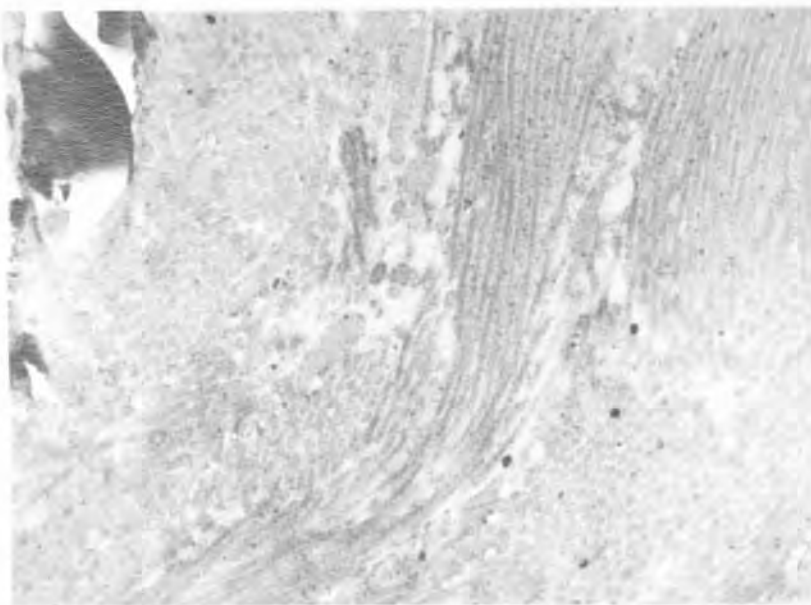


Fig. 13 - Undecalcified specimen of β -TCMP endosseous implant after 8 weeks. TEM micrograph shows a sintered granule in the center of the cavity surrounded by osteoid tissue. (12.000 \times).

Fig. 13 - Spécimen non décalcifié d'un implant endosseux de β -TCMP après 8 semaines. La vue microscopique électronique montre un granule fritté au centre d'une cavité entouré par du tissu ostéοide. (Acétate d'uranyle, citrate de plomb. Grossissement: 12.000 \times).



Fig. 15 - β -TCMP endosseous implant after 24 weeks. The cavities filled with β -TCMP evidence bone tissue undergoing remodelling with isolated sintered granule residues (arrows) (100 \times).

Fig. 15 - Implant endosseux de β -TCMP après 24 semaines. Les cavités sont remplies par un tissu osseux en voie de remodelage avec des résidus de granules frittés isolés. (Flèches) Trichrome de Masson. (Grossissement: 100 \times).

The samples taken after 24 weeks evidenced remodelling bone tissue uniformly distributed throughout the cavity. Isolated sintered granule residues were, however, still present (Fig. 15).

DISCUSSION

Sintered and unsintered β -TCMP granules are well tolerated by the host tissues: at 2 weeks the endosseous cavities filled with the biomaterial present a slightly inflammatory infiltrate characterised by few polynucleate cells and macrophages. In accordance with previous research (Levin *et al.*, 1974a; Cameron *et al.*, 1977; Klein *et al.*, 1985; Barney *et al.*, 1986; Franchi et Ruggeri, 1989), these cells disappear almost completely after only 4 weeks. In this study the sintered β -TCMP granules were absorbed over a period of 2 to 24 months (see also Cutright *et al.*, 1972; Ferraro, 1979), while the unsintered granules, being small and thus easily phagocytized (Pizzoferrato, 1979), were reabsorbed completely within 8 weeks. The micropores of the sintered granules were occupied by a network of cytoplasmic processes of the phagocytizing cells, fibroblasts and other mesenchymal cells as well as other organic material including collagen fibrils and glycoproteins. Each granule is riddled with a network of canals 40-50 nm in diameter which link up with the larger micropores: this intercommunicating network appears invaded by cells, collagen fibrils and macromolecules. This colonized network increase considerably the active surface area of each β -TCMP granule which is in contact with the newly formed periimplant tissue.

After 4 weeks the granulation tissue around the granules has become a fibrous connective tissue with characteristic dense collagen fibril bundles lying parallel to the surface of the sintered β -TCMP granules. Between these fibrils a few blood vessels are observed along with numerous mature fibroblasts and active protein-synthesising osteoblasts are present lying on the granule surface. After 8 weeks the last traces of unsintered powder appear near the newly formed bone trabeculae. The sintered granules at the centre of the cavity are surrounded by osteoid and new bone tissue. The granules nearest the host bone are completely englobed in bone lamellae which impinge directly upon the sintered β -TCMP surface. Like other biomaterials (Williams, 1987; Ganales *et al.*, 1986), β -TCMP is confirmed as susceptible of biointegration also by the presence of calcified collagen fibrils in the porous network of each granule.

The fact that the bone growth front moves from the periphery towards the centre of the cavity is a demonstration that β -TCMP does not induce new bone formation by activating undifferentiated cells (Urist and Strates, 1973), but rather acts as an «oste conductor» or «osteostimulator» of already differentiated cells. Similar research on the effect of biodegradable materials (Getter *et al.*, 1972; Levin *et al.*, 1974a; Meffert *et al.*, 1985) suggests that physiological bone reabsorption is slowed on account of the presence of the biomaterials. More probably, in fact, collagen matrix production is enhanced in the presence of Ca, PO₄ and Mg ions released by the β -TCMP granules. This material in fact is produced by heating biological apatites and furthermore contains Mg ions (Baravelli *et al.*, 1984), a natural component of all bone which regulates the precipitation of hydroxyapatite crystals in some calcification processes (Jense and Rowles, 1957; Le Geros, 1974; Gron and Campen, 1967; Bigi *et al.*, 1980; Williams, 1987).

An environment saturated with these ions will provide accelerated precipitation of mineral ions into the matrix, thus promoting centripetal calcification from the bone border at the outer edges of the cavity towards the centre. Evidence of β -TCMP's osteogenic properties is the early appearance of calcification nuclei in the osteoid tissue nearest the granule surface. Like other research (Bhaskar *et al.*, 1971; Cutright *et al.*, 1972; Levin *et al.*, 1974a, 1975; Nelson *et al.*, 1977; Turnbull *et al.*, 1985; Ragezi *et al.*, 1985; Barney *et al.*, 1986; Hyakuma *et al.*, 1988) this study also found that osteostimulation was accelerated when the biomaterial is implanted near freshened bone. However further clinical and experimental studies of the osteogenic properties of β -TCMP are required.

CONCLUSIONS

- 1) Sintered and unsintered β -TCMP granules proved to be well tolerated by the host tissues and result biodegradable over time if implanted into endosseous cavities of the upper or lower jaw.
- 2) β -TCMP can be considered an «oste conductor» and «osteostimulator» guiding the direction of bone growth from the periphery of the endosseous cavity towards the centre and enhancing osteoblast activity and new bone deposition.
- 3) The newly formed bone appears to be in direct contact with the surface of the β -TCMP granules. No connective tissue was evidenced between new bone and biomaterial.

4) β -TCMP containing Mg ions reabsorbs slowly when in sintered granules form and rapidly as unsintered granules. This β -TCMP mixture can be considered a biomaterial whose effect depends on the particular tissue requirements. If bone reabsorption is necessary the biomaterial can be reabsorbed with bone, while if osteogenesis is required β -TCMP provides a more durable matrix to support new bone growth.

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