Tomodensitometric and Histologic Evaluation of the Combined Use of a Collagen Membrane and a Hydroxyapatite Spacer for Guided Bone Regeneration: A Clinical Report

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In this report, the problems of insufficient bone and soft tissue after extraction of maxillary incisors were addressed concurrently prior to endosseous implant placement, by combining the use of a diphenylphosphorylazide–cross-linked Type I collagen membrane and a resorbable space-making biomaterial composed of 200-µm porous hydroxyapatite granules blended in Type I collagen and chondroitin-4-sulfate. Upon flap reflection 8 months postsurgery, the horizontal deficiencies were almost completely resolved, membranes completely resorbed and the defects filled with hard, bonelike tissue, with a few superficial hydroxyapatite granules. Histologic evaluation of the bone biopsies obtained at the implantation sites revealed dense, well-reconstructed alveolar bone with a few traces of hydroxyapatite granules that had been completely resorbed. Tomodensitometric evaluation indicated that bone regeneration ranged from 14% to 58%, with an average bone gain of 29.77%. Four nonsubmerged ITI titanium implants placed in the augmented bone have been in function for more than 5 years, with no clinical or radiographic signs of hard or soft tissue breakdown. Bacterial sampling at dental sites with periodontitis 1 month prior to periodontal therapy and at implant sites for up to 30 months demonstrated rapid colonization of implant surfaces by periodontopathogens without causing any detrimental effect to implant integration.
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Key words: bone regeneration, collagen membrane, guided tissue regeneration, human histology, hydroxyapatite, oral implant, resorbability

A prerequisite for the successful placement of oral implants is the availability of sufficient jawbone volume. Placement of endosseous implants in areas in which the dimensions of the jawbone are equal to or less than those of the implant results in parts of the implant surface not being covered by bone. This may lead to recession of the soft tissue, with denudation of the implant surface, which in turn will mechanically irritate the soft tissues and prevent proper plaque control. It is also preferable to strive to fully cover the implant surface to improve retention and long-term prosthetic prognosis. In a large bone defect, it could also be impossible to place an implant with good primary stability or in an appropriate position for restoration-driven implant placement.[1] In such situations, in which reduced bone volume is observed as a result of advanced periodontitis, trauma, infection, atrophy, malignancies, or developmental malformations, ridge enlargement based on the

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guided bone regeneration (GBR) approach[2] could be carried out.

In large, non–space making defects, it is desirable to have the defect filled with bone rather than solely restoring volume and/or bone contour with dense connective tissue or nonresorbable materials such as dense hydroxyapatite (HA), especially if osseointegrated implants are to be used to support a prosthesis. However, most membranes used for GBR have a tendency to sag into large non–space making defects, and if nonresorbable support materials are used at the site to prevent the collapse of the membranes, they may prevent osseointegration from occurring or form a weak union that would fracture under loading stress.

This report presents clinical, tomodensitometric, and histologic results for the use of a combined, resorbable, spacekeeping biomaterial composed of 200-µm homogenous porous HA granules, Type I collagen, and chondroitin-4-sulfate and a biodegradable diphenylphosphorylazide– (DPPA) cross-linked[3] Type I collagen membrane, to regenerate a postextraction maxillary labial ridge deficiency prior to endosseous implant placement. Both the membrane and the space-making biomaterial have been previously evaluated in animal experiments[4–8] and clinical trials.[9–13]

Report of Treatment

A 41-year-old white male patient was referred for the treatment of 4 highly mobile maxillary incisors that were impeding mastication (Fig 1). The patient requested that implants be used to replace the mobile teeth. The history of the patient revealed that since age 30, he had experienced recurring periodontal abscesses and pain involving the maxillary incisors, which exhibited severe destruction of the soft and hard supporting tissues. Therefore, the long-term prognosis of these teeth was considered to be hopeless, and it was decided to extract and replace them with an endosseous implant–supported fixed partial prosthesis. However, the patient was informed that there would most likely be only a very small volume of bone available after the extractions and that the lost bone volume would need to be reconstructed prior to implant placement.

The other maxillary teeth were also periodontally involved and were treated prior to implant placement, since the pockets around the remaining teeth could act as reservoirs of microorganisms for the colonization of oral implants.[14] Bacterial sampling was performed 1 month before periodontal treatment on the maxillary right and left premolars, revealing a total microbiota of 10^6/mL with 20% *Porphyromonas*.

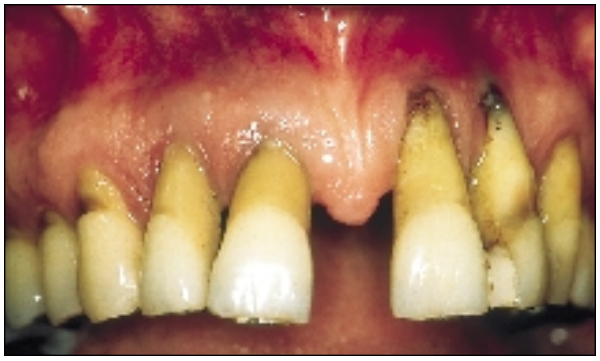


Fig 1 Preoperative view of the maxillary incisors. Note loss of periodontal supporting tissues.

The patient, who smoked more than 40 cigarettes per day, was given detailed information on the risks associated with smoking and agreed to follow a smoking cessation protocol,[15] since smoking has been considered to have a detrimental effect on regeneration[16] and can also contribute significantly to implant failure before or after loading.[17]

After adequate local anesthesia was administered, incisions were made on the ridge below the epithelial zones left by the apices of the 4 extracted incisors, and a combined split- and full-thickness flap was raised. Upon flap reflection, the bone loss appeared to be extensive, with residual sockets 4 to 7 mm deep that led to thin palatal bone, yet the bone appeared thickened in the proximal areas. After the bone defects were meticulously debrided, 2 DPPA–cross-linked Type I collagen membranes (Paroguide, Colética Corp, Lyon, France), available as 3 × 3-cm sheets, were trimmed, placed side by side, and adapted to completely cover the defect, overlapping the residual bone both apically and laterally by at least 2 mm. Because of the risk of collapse and sagging of the membranes into this large, non–space making defect, the space under the membrane was preserved using a resorbable biomaterial composed of 200-µm homogenous, porous granules of HA (88%), bovine Type I collagen (9.5%), and chondroitin-4-sulfate (2.5%) (Biostite, Colética Corp). The spacer was soaked in blood and overfilled the defect. Membranes were pulled down and inserted beneath the buccal flap. As a result of the partial thickness dissection of the mucosa, the soft tissue flaps were closed without tension using interrupted 4-0 resorbable sutures (Vicryl, Ethicon, Lyon, France). Because of the

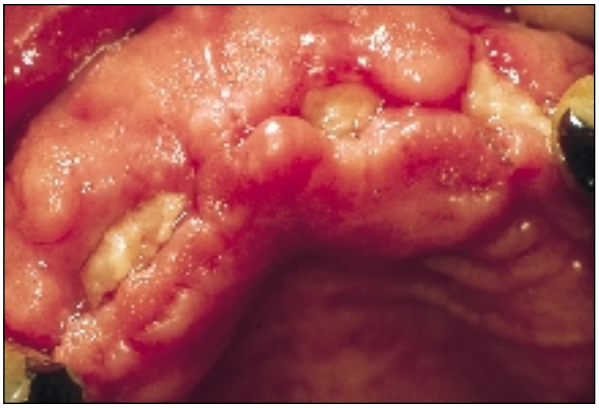


Fig 2a Clinical view after 15 days of healing, demonstrating the amount of membrane exposure.

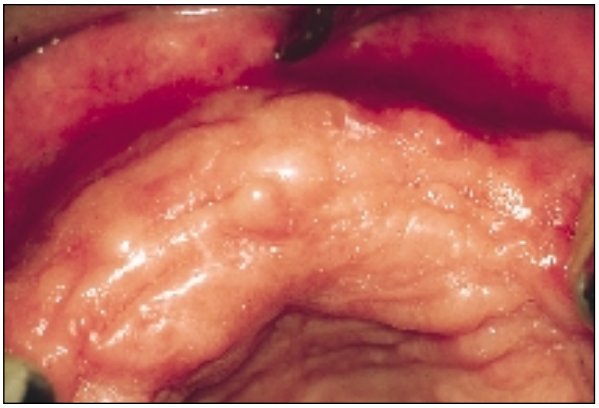


Fig 2b Tissue healing and maturation at 1 month postsurgery.

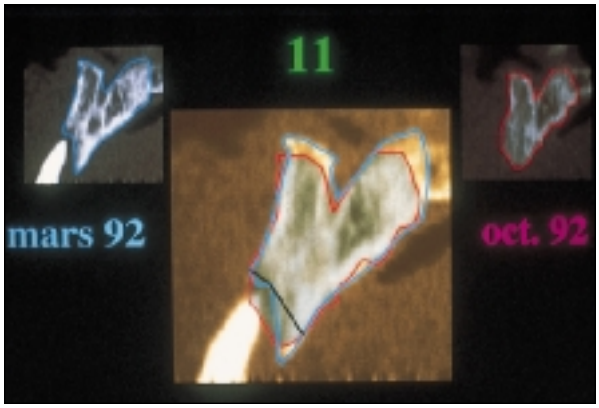


Fig 3 Bone surface measurements from CT scan data immediately after extraction (March 1992) and 7 months postextraction (October 1992) at the site of the right central incisor (tooth 11).

amount of grafting material required, the coverage was not complete in the extraction sites, leading to 5 to 7 mm of the membranes being exposed to the oral environment.

The patient was given appropriate postoperative instructions, prescriptions for amoxicillin (500 mg twice daily), paracetamol 500 mg (Doliprane, Théraplix, Paris, France; 500 mg 3 times daily), and oral antiseptic rinse (0.10% chlorhexidine digluconate 3 times daily). The patient was seen every 2 weeks during the first 3 months after surgery and every 2 months thereafter. A temporary removable prosthesis replacing 4 teeth was provided for the patient, previously relieved and relined with a soft liner (Fitt, Kerr, Paris, France) to eliminate trauma to the surgical site. The postoperative period was uneventful, and although 2 weeks after surgery the collagen membranes were partially exposed (Fig 2a), they did not cause any visible sign of inflammation or local trauma to the covering tissues. One month postsurgery, the soft tissue healing was complete, with no parts of the underlying biomaterials remaining visible (Fig 2b).

A volumetric analysis of the regenerated bone was performed using computed tomographic (CT) images obtained immediately after the extractions (March 1992) and after 7 months of healing (October 1992). Sections to be compared were chosen at the same level, the mesial aspect of the remaining canines serving as precise landmarks for corresponding sections. The CT images were scanned, and data were computerized, giving images (3 cm × 3 cm, 500 pixels/inch) with identical image surfaces (115 × 600 pixels). Sections were superimposed before and after treatment by matching the nonaugmented cortical bone contours (Fig 3). Bone limits of each section were traced in "Bézier" contour lines (Photoshop, Adobe, Mountain View, CA). The area of the selected surfaces was measured using image analysis software (Optilab, Graftek, Mirmande, France) and expressed as the percentage of the total surface of the lineage. At each section, the differences between the area immediately after extraction and 7 months postextraction were calculated as a percentage of bone regeneration (Table 1). The calculation of true bone regeneration at each site took into account both guided bone regeneration and bone loss resulting from secondary resorption after surgery. The bone regeneration ranged from 14% to 58%, the average bone gain being 29.77%.

To place the planned dental implants, a crestal incision was made and the flap elevated to expose the bone surface. The bonelike tissue appeared to be dense and covered in some areas by a few HA

Table 1 Bone Surface in Pixels on Computed Tomographic Images Before and After Guided Bone Regeneration

Site	Before treatment	After treatment	Bone increase (%)
Maxillary right lateral incisor	1595	2023	26.83
Maxillary right central incisor	1861	2948	58.41
Maxillary left central incisor	3526	4219	19.65
Maxillary left lateral incisor	2855	3260	14.19
Mean ± SD			29.77 ± 19.7

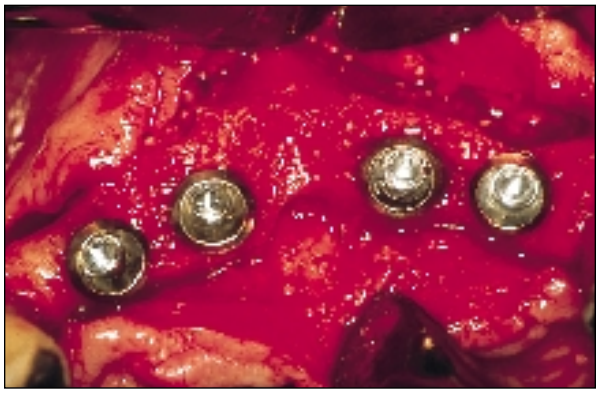


Fig 4 Four hollow-cylinder ITI dental implants are placed.

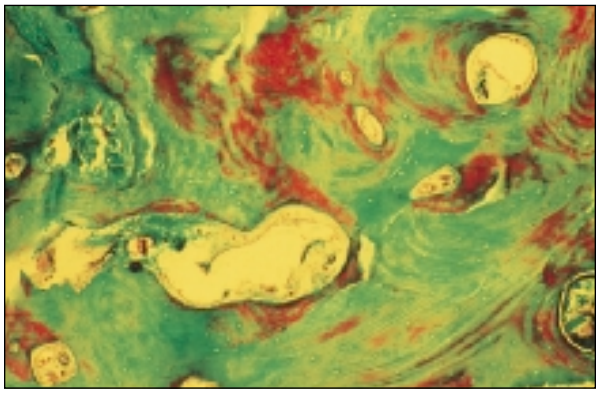


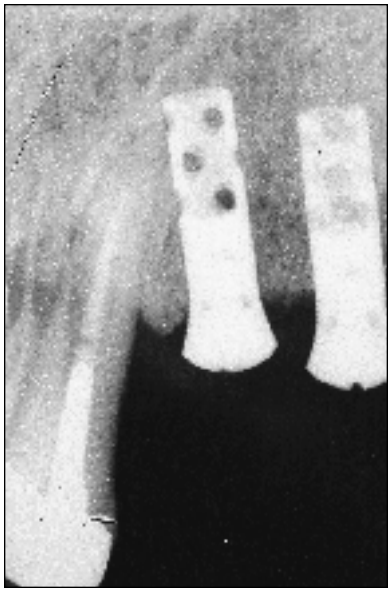
Fig 5 Enlarged view of the biopsy after 6 months of healing. Lamellar bone remodeling is occurring, with ducts that are well open (Masson/Goldner stain; original magnification ×63).

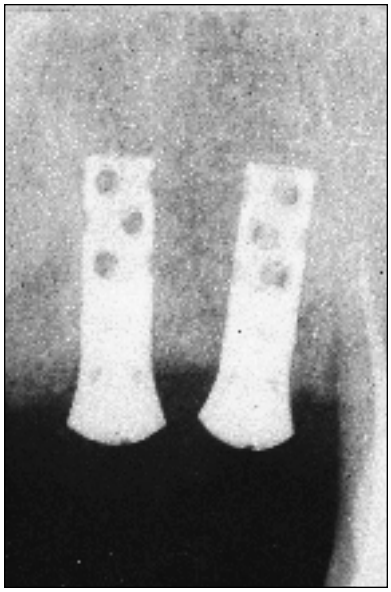
granules, which were embedded in the connective tissue. Prior to the placement of implants, a careful ridge contouring was performed to achieve a flat bone surface. Following this procedure, the bone surface appeared free from hydroxyapatite granules that were apparently superficial, excess, nonintegrated HA particles.

Before placement of the 4 nonsubmerged ITI hollow-cylinder dental implants (Institut Straumann AG, Waldenburg, Switzerland) (Fig 4) and with the informed consent of the patient, the bone cores of the 4 implant sites were removed and immediately placed in 10% neutral buffered formalin. After being embedded in acrylic resin, undemineralized 5-µm sections were stained with Masson/Goldner trichrome. Under optical microscopy, the histologic aspect of the regenerated bone showed rather high bone density for the maxilla, which is usually mostly cancellous with large medullary spaces (Fig 5). In these specimens, bone trabeculae showed signs of recent Haversian deposits that seemed to be ongoing in some areas; the other conspicuous finding was the total absence of the space-making material, which was completely resorbed on bone, leaving no trace whatsoever.

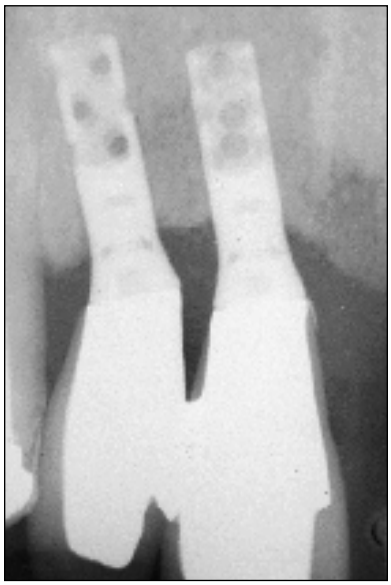
Since nonsubmerged dental implants were chosen to rehabilitate the patient, abutments were connected without a second surgical operation. The patient was rehabilitated with 4 single crowns. The regenerated hard tissues have remained stable under functional loading of the dental implants for more than 5 years without any clinical signs of failure. There was no evidence of suppuration, increased probing depths, pain, or progressive loss of osseous support around the implants. Radiographic examination at the day of placement (Fig 6) and after 5 years in function (Fig 7), demonstrated no peri-implant radiolucency and stable crestal bone levels, thus confirming the clinical observations.

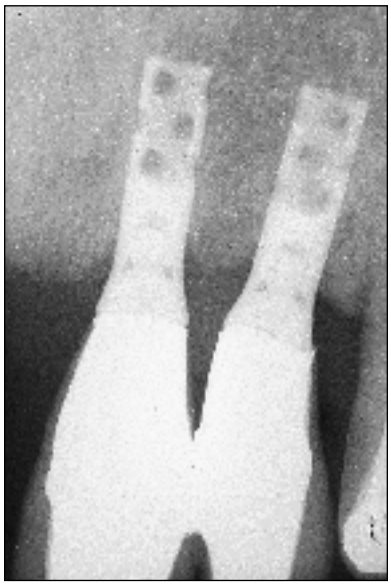
Bacterial sampling was performed at 15 days, 3 months, 12 months, and 30 months after implant placement. Two paper points (Mynol, Block Drug, NJ) were inserted in the peri-implant sulcus, and transferred into 3-mL Schaedler broth completed with vitamin K3 and agar (0.2%) (Biomérieux, Crapone, France). Dilutions were spread on Chocolate Polyvitex (1%) Bacitracine (50 IU/mL) agar plates (Biomérieux) and inoculated on Columbia agar plates enriched with 5% defibrinated sheep blood (Biomérieux). The Chocolate Polyvi-





Figs 6a and 6b Radiograph of the 2 implants in the left *(left)* and right *(right)* anterior maxilla immediately after placement.





Figs 7a and 7b Radiograph of the 2 implants in the left *(left)* and right *(right)* anterior maxilla 5 years after placement.

tex Bacitracine agar plates were incubated for 5 days at 37°C in an atmosphere containing 5% CO_2 in air and the Columbia agar plates for 10 days at 37°C in anaerobic conditions (oxygen not exceeding 2%). At day 15, the total bacterial load was 10^4/mL, with a clear predominance of *Streptococcus* (88%). At 3 months, the bacterial load was 10^5/mL, containing 24% *Fusobacterium* and 56% *Peptostreptococcus*. At 12 months, bacteriologic results were stable, with a total bacterial load of 1.5×10^5/mL and 14% *Peptostreptococcus*. At 30 months, total microbiota was still 10^5/mL, with 20% *Peptostreptococcus* and 3% *Prevotella.*

Discussion

This report has described the successful reconstruction of a large non–space making defect with the combined use of a DPPA–cross-linked collagen membrane that was prevented from collapsing into the defect by a space-keeping biomaterial composed of porous hydroxyapatite granules, Type I collagen, and chondroitin-4-sulfate. Since the initial successful clinical trials of alveolar ridge augmentation by HA,[18] several animal studies have provided histologic evidence of the long-term biocompatibility of particulate HA and of its favorable interac-

tion with both soft tissue and bone.[19,20] However, only a few human histologic reports[21,22] are available concerning the outcome of HA or other alloplastic bone-grafting materials. In most of these studies, HA was demonstrated to be osteoconductive and osteophilic, but not osteogenic. Moreover, the HA particles were seldom resorbed. Rather, the histologic evidence has shown either fibrous encapsulation of HA granules or integration of the particles into osseous tissue. In contrast, the most conspicuous finding from the bone biopsies in this study was the total degradation of the hydroxyapatite particles, along with their replacement by a relatively dense, homogenous osseous tissue. Several factors might have influenced the tissue reaction and the degradation of the space-keeping material. One of these factors, as shown by studies relevant to granule size and HA crystalline structure, is the evenly shaped 200-µm granule dimension, which determines bioresorption, biodegradation, and bone formation.[4,6,21,22]

Macroporosity has also been shown to be an important factor for the osseous substitution of ceramics.[23] Moreover, stabilization of HA particles within a collagen matrix appears to be a favorable attribute for osseous ingrowth,[24] since bone has a low tolerance for micromotion.[25] The addition of glycosaminoglycans into Type I collagen-reconstituted matrices has been demonstrated to modify cell proliferation and migration and induce the expression of a differentiated phenotype.[26] Indeed, it has been shown that the addition of chondroitin-4-sulfate, a glycosaminoglycan, may take advantage of the combined effect of these molecules on cellular growth and mineralization, promoting mineralization of 3-dimensional collagen matrices when seeded with bone-derived cells in vitro.[27]

When physical barriers are used, resorbable rather than nonresorbable membranes are preferred, since they obviate the need for a second surgery solely for the purpose of membrane removal and eliminate the risk of possible late complications associated with permanent membrane retention. Various resorbable materials have been evaluated for membrane preparation. Among these is collagen, the most important structural protein component of the body; it is naturally bioresorbable and has proven efficacious as a biomaterial.[28] The use of collagen as a biomaterial has been advocated based on several factors, such as its favorable role in cellular development,[29] wound healing, and blood coagulation.[28] Several techniques have been proposed for collagen crosslinking,[30] with the disadvantage for some, such as the glutaraldehyde technique, of partial inclusion of the crosslinking agent in the collagen, which renders the biomaterial partly cytotoxic.[31] The recently developed DPPA technique[3] achieves natural cross links between peptide chains without leaving any foreign product in the protein of collagen. The DPPA–cross-linked collagen membrane used in this patient treatment has been previously evaluated in animals[7,8] and in humans.[9–13] The present report confirmed the excellent tissue integration and biocompatibility of this collagen membrane, since despite the fact that primary closure was not obtained and several areas of the membrane were exposed to the oral cavity, no adverse tissue reactions were observed, and 1 month postsurgery, superficial soft tissue healing was completed. In contrast, exposure of nonresorbable expanded polytetrafluoroethylene membranes and their premature removal could jeopardize bone regeneration.[32]

The presence of microorganisms in implant sites is more important in partially edentulous than in completely edentulous patients, since it appears that adjacent teeth seem to play a role in the periimplant microbiota.[33] Similar microbiota has been observed in zones of progressive periodontitis and failing implant sites.[34] Bacterial sampling performed in this report demonstrated that potential pathogens of periodontal disease can rapidly colonize implant surfaces without causing additional bone loss at 3 years. Similar observations were reported by Leonhardt et al.[35]

Conclusion

The results from this report are based on only 4 sites in a single patient and cannot be considered as universal. The use of resorbable, space-keeping, 200-µm homogenous porous HA granules to support a collagen membrane allowed optimal bone regeneration for the placement of 4 nonsubmerged implants, which have been in function for more than 5 years. The resorbability of the type of HA used in this report, if confirmed by other studies, could be beneficial for regenerating bone prior to the placement of implants, whenever a membrane requires support. The genuine benefit contributed by the barrier per se, or the achievement of the same results without the placement of a barrier, remains to be answered.

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