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Clinical and Biologic Observations of Demineralized Freeze-Dried Bone Allografts in Augmentation Procedures Around Dental Implants

Cobi J. Landsberg, DMD*/Ayala Grosskopf**/Miron Weinreb, DMD**

To evaluate the possibility of regenerating bone around endosseous dental implants, 32 implants were placed into postextraction sockets or other bony defects, and human demineralized freeze-dried cortical bone powder (DFDB) mixed with tetracycline was packed around the exposed parts. Implants were covered with expanded polytetrafluoroethylene (e-PTFE) membranes for 4 to 6 months until abutment connection, unless the membranes were prematurely exposed and had to be removed. Bone biopsies from nonsupporting regenerated bone were taken from some of the patients and examined histologically. Whenever complete coverage was maintained throughout the healing period (4 to 6 months), complete bone regeneration resulted. Early membrane removal mainly resulted in partial bone regeneration. Histologically, regenerated bone consisted of particles of devitalized bone in contact with newly formed woven or lamellar bone with some connective tissue around them. Osteogenic activity was even present 1 year post-grafting. Thus, DFDB is capable of promoting bone formation around dental implants if complete flap coverage and the membrane presence can be maintained throughout the healing phase. (INT J ORAL MAXILLOFAC IMPLANTS 1994;9:586-592)

Key words: allograft, augmentation, demineralized, freeze-dried bone, implants

Recent animal¹⁻³ and human⁴⁻¹⁵ studies have demonstrated the possibility of regenerating bone around dental implants by using the principle of guided tissue regeneration (GTR). To regenerate bone around exposed parts of a dental implant, a membrane barrier is placed over the implant and associated bony defect, thus secluding a space to be repopulated exclusively by cells originating from bone tissue. Several methods have been proposed for maintaining this space during the entire healing period.⁵⁻¹⁵ The use of

miniscrews temporarily placed in the bony defect while supporting the membrane through the entire healing period before implant placement has been suggested.^{6,9} The use of synthetic materials, such as porous hydroxyapatite, as space maintainers was also suggested.¹⁰ Biologically and clinically, it is desirable to have a defect filled only with bone, rather than with a combination of bone and a synthetic inert graft material.⁷ Therefore, intraoral autogenous bone grafts¹¹ or demineralized freeze-dried bone (DFDB) particles are preferred.¹¹⁻¹⁵ Additional studies are required to determine the role of these different materials in bone regeneration around dental implants and to confirm their compatibility with the biologic principles of osseointegration.

In a recent animal study, Becker et al³ claimed that using human DFDB under an expanded polytetrafluoroethylene (e-PTFE) membrane had no significant clinical advantage over the use of a membrane alone in promoting bone around immediate extraction socket implants. Histologically, the authors did not find any evidence of osteogenic activity around

*Department of Periodontology, The Maurice and Gabriela Goldschleger School of Dental Medicine, Tel Aviv University, Tel Aviv, Israel.

**Department of Oral Biology, The Maurice and Gabriela Goldschleger School of Dental Medicine, Tel Aviv University, Tel Aviv, Israel.

Reprint requests: Dr Cobi J. Landsberg, Department of Periodontology, The Maurice and Gabriela Goldschleger School of Dental Medicine, Tel Aviv University, Tel Aviv 69978, Israel.

implants that were treated with a combination of DFDB and membrane. In contrast, bone regeneration was clinically significant when similar bone defects around immediate implants were treated by membrane alone. It was concluded that placement of DFDB allograft (DFDBA) under the membrane may not be necessary.

This article presents clinical and histologic observations carried out at second-stage implant surgery to evaluate human DFDBA when used in conjunction with augmentation procedures around dental implants.

Materials and Methods

Twenty-two patients, aged 32 to 71 years, who were partially edentulous and free of systemic disease were included in the study. Patients received routine periodontal treatment including oral hygiene instructions, scaling and root planing, with surgical procedures when indicated. Each patient was provided with one to two implants; a total of 32 implants were placed in 26 sites, which were either postextraction sockets or other jawbone defects. Three types of implants were used: Brånemark (Nobelpharma AB, Gothenburg, Sweden), Integral (Calcitek, Carlsbad, CA), and Screw-Vent (Dentsply Implant Division, Encino, CA).

Each patient received antibiotic treatment that included either doxycycline (200 mg 1 hour before surgery and 100 mg once daily for the next 10 days), or amoxicillin (1,000 mg 1 hour before surgery and 500 mg 3 times daily for the next 10 days). Analgesic treatment (diflunisal 1,000 mg or naproxen 550 mg) was also administered before surgery and afterward as needed.

In 9 of 26 surgical procedures, the eversed crestal flap design was used¹⁵; in the remaining procedures, a crestal approach was used, but flap eversion was not attempted during suturing. Each implant site was thoroughly debrided of granulation or connective tissue with sharp curettes and, whenever possible, perforation of cortical bone in the region to be subsequently covered by the membrane was achieved. After implant placement, human demineralized freeze-dried bone particles (Tissue Bank, University of Miami, Florida) mixed with tetracycline-HCl powder (Teva, Israel, 4:1 ratio) were gently packed around the coronal exposed parts of the implants. An expanded polytetrafluoroethylene (e-PTFE) membrane (W.L. Gore, Flagstaff, AZ) was trimmed, soaked in a 50 mg/mL solution of tetracycline/saline and placed precisely over the area of augmentation with the stiffer occlusive portion covering the defect area and the flexible occlusive portion extending 3 to

4 mm around the periphery of the defect. Every attempt was made to ensure complete flap coverage of the wound.

The membrane was removed at the time of abutment connection, which was generally 4 to 6 months postsurgery. In cases where the occlusive portion of the membrane became exposed, patients were instructed to use 2% chlorhexidine irrigations 3 to 4 times daily and were placed on antibiotic treatment and weekly checkups. At 6 weeks postsurgery, these membranes were removed. If the nonocclusive portion of the membrane became exposed, the membranes were removed immediately. Where bone regenerated over the implant cover screw, bone chisels, surgical blades, curettes, and carbide or diamond burs were used to remove the excess bone. Whenever possible, biopsies from nonsupporting regenerated bone (ie, bone not in contact with the implant) were taken for histologic evaluation at this stage. In one case, bone biopsy was taken 1 year after implant placement.

Bone biopsies were fixed in 10% buffered formalin, demineralized in 5% formic acid for 48 hours, and embedded in paraffin wax. Five-micron-thick sections were cut throughout each biopsy and some were stained with hematoxylin and eosin. Stained sections were photographed under bright-field or polarized-light illumination.

Results

Clinical. Clinically, the height and volume of hard tissue formed around the implants correlated with the success in achieving complete flap coverage of the wound. In all 11 procedures in which complete flap coverage was achieved and maintained during the entire healing period (4 to 6 months), there was complete bone regeneration (ie, along the entire length of the implant) (Figs 1 to 3). Frequently, bone formed over the cover screws. In most cases, there was also significant gain in buccolingual bone width adjacent to the implants. The newly formed bone seemed more vascularized than the preexisting cortical bone, but was hard enough to necessitate the use of sharp instruments for biopsy removal. Of the 15 cases in which incomplete flap coverage resulted in early membrane removal, complete bone regeneration was achieved in 4 only, while most (8 cases) resulted in incomplete bone regeneration. Two cases displayed bone loss around the implants and one implant became mobile and had to be removed. In all procedures in which bone regeneration occurred, tight contact between the newly-formed bone and the implant surface was always present and probing along the implant was not possible.

Figs 1a to 1f Photographs of the clinical procedure used in this study.

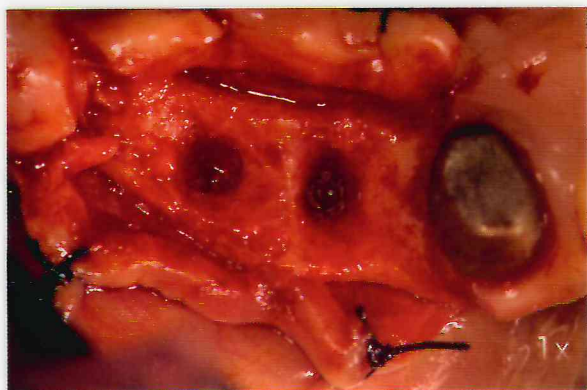


Fig 1a Postextraction sockets of the maxillary first and second premolars.

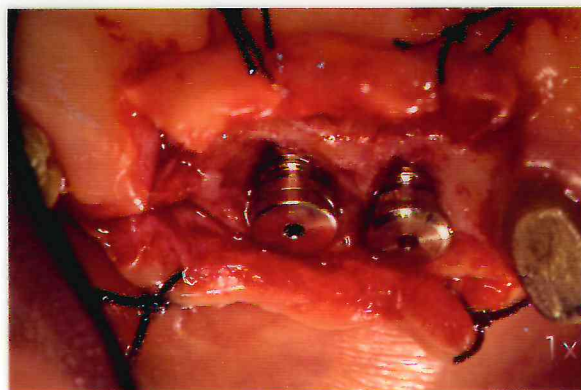


Fig 1b Brånemark implants with exposed coronal part on the palatal side.

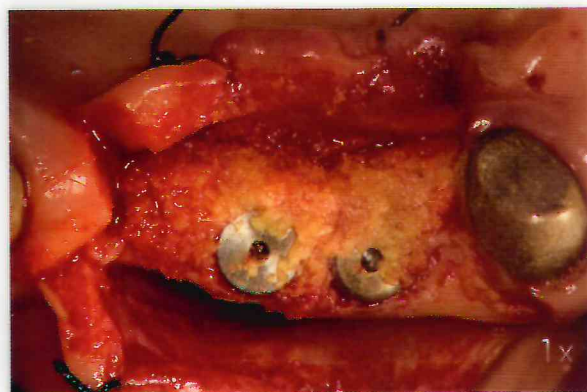


Fig 1c DFDB-tetracycline mixture packed around the exposed portion of the implants.

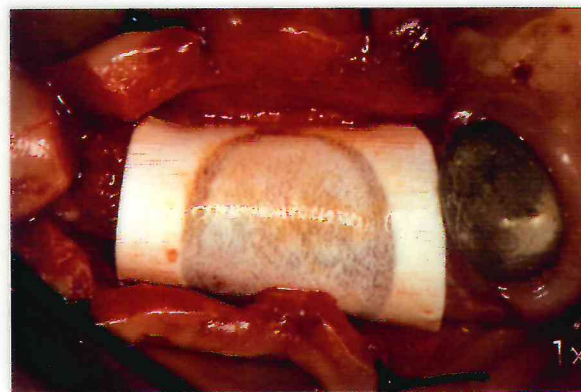


Fig 1d e-PTFE membranes positioned over the two implants.

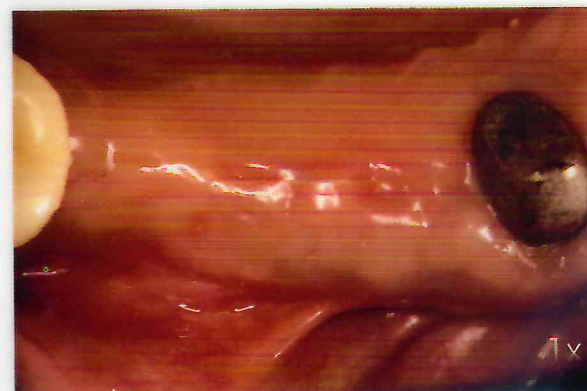


Fig 1e Complete flap coverage 5 months after placement; indentations are the result of pontic pressure.



Fig 1f Complete bone regeneration over the previously exposed parts of the implants after 5 months.

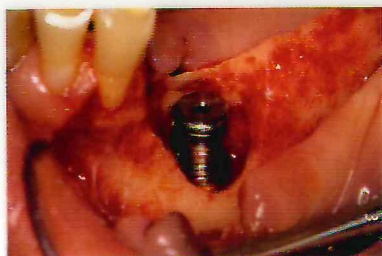
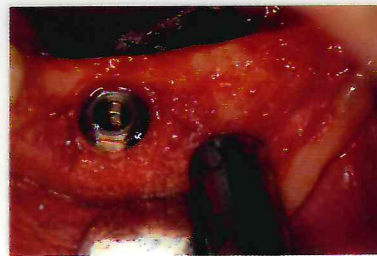


Fig 2a Brånemark implant in an extraction socket of a mandibular canine with a large peri-implant defect.



Figs 2b and 2c Complete bone regeneration around the implant 6 months postgrafting. Before (b, left) and after (c, right) exposing the implant head.

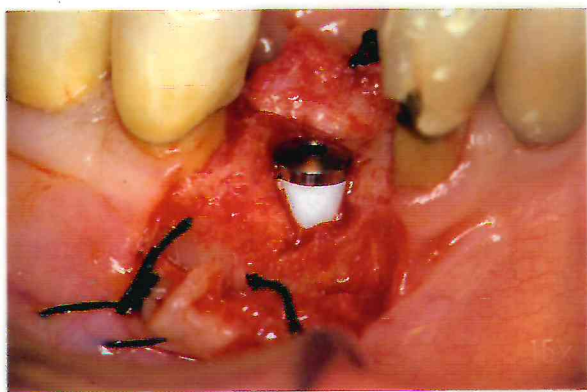


Fig 3a Integral implant placed 1 month postextraction of a mandibular canine.

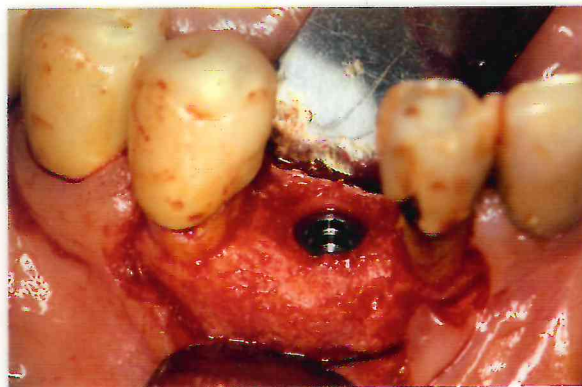


Fig 3b Complete bone regeneration around the implant and thickening of the buccal aspect 6 months postgrafting.

Histologic. The biopsies contained numerous areas of bone tissue surrounded by loose connective tissue and blood vessels. These areas were interconnected and, thus created a three-dimensional network of bone (Fig 4). They contained two types of bone: particles of devitalized bone containing empty osteocytic lacunae (DFDB remnants) and large areas of vital bone tissue with distinct osteocytes and either a woven (Fig 5) or a lamellar (Fig 6) architecture. These two types of bone were in direct contact with each other and thus represent new bone formed on the template of the grafted DFDB. In the biopsy taken one year postgrafting, DFDB particles were still present and osteoblasts engaged in formation of lamellar bone could still be seen (Fig 7).

Discussion

Freeze-dried bone allografts have been used clinically in orthopedic therapy since 1950. In the early 1970s, bone allografts were introduced for periodontal

therapy as an alternative source of graft material to autogenous bone. Since then, bone allografts have been routinely used. Both FDBA and periodontal DFDBA are clinically efficacious in the treatment of periodontal intraosseous lesions, resulting in significant reduction in probing depth, gain in clinical attachment, and bone fill. DFDBA is preferred over FDBA because of its potential as an osteoinductive graft material and its demonstrated potential in regenerating the periodontium in humans with^{16,17} or without^{18,19} the use of membrane barriers.

DFDBA may also be used in conjunction with membrane barriers to successfully promote bone regeneration around endosseous dental implants.¹¹⁻¹⁵ In contrast, Becker et al,³ in a controlled dog study, showed that only a limited amount of bone height could be gained around exposed implant threads treated by e-PTFE membranes and human DFDBA. These authors could not find any evidence of bone formation within the allografted material. The apparent inability of DFDBA to induce bone formation in their study was explained by the use of

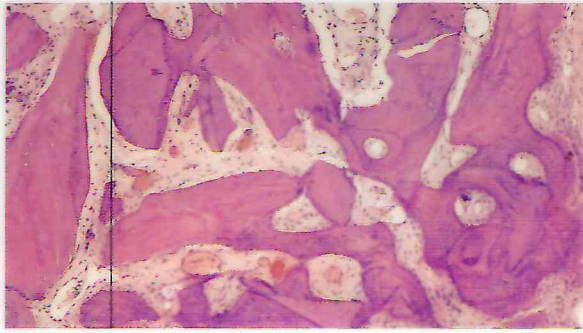


Fig 4 (Above) Low-power photomicrograph of a bone biopsy taken 5 months postgrafting, showing a network of bone with loose connective tissue in between (hematoxylin-eosin stain, original magnification $\times 125$).

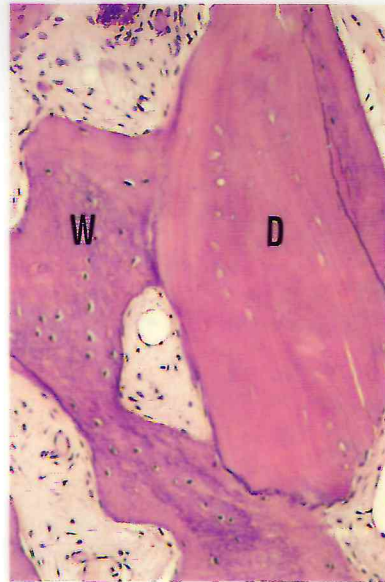


Fig 5 (Right) High-power photomicrograph of the lower left corner of the bone biopsy shown in Fig 4, depicting DFDB particles (D) in contact with newly-formed woven bone (W) (hematoxylin-eosin stain, original magnification $\times 375$, bright-field illumination).



Fig 6a Photomicrograph of DFDB particles (D) in contact with newly-formed lamellar bone (L) in a bone biopsy taken 5 months post-engraftment (hematoxylin-eosin stain, original magnification $\times 375$, bright-field illumination).

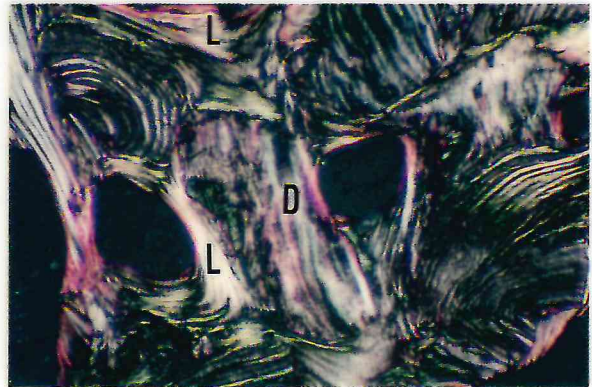


Fig 6b The lamellar structure of the new bone is evident in the polarized-light image.



Fig 7 Photomicrograph of bone biopsy taken 1 year postgrafting, showing DFDB particles (D) and osteoblasts (O) forming lamellar bone (L) (hematoxylin-eosin stain, original magnification $\times 375$, bright-field illumination).

human material on dogs, by the possible presence of limited levels of bone morphogenetic proteins in commercially available DFDB, or by the lack of vascular supply when bone grafts are placed over exposed titanium.³ In view of the improved results obtained in the same study when e-PTFE membranes were used either alone or in combination with growth factors, the use of DFDB both as a bone inductive material adjacent to implants and as a space maintainer beneath membranes was questioned.

In the experiments reported herein, it was observed, both clinically and histologically, that when human DFDBA is used for augmentation procedures around implants in humans, it is effective for both space maintenance beneath the membrane and bone induction, provided that complete membrane coverage during the entire 4- to 6-month healing process is maintained. The significant bone volume achieved in most cases suggests that DFDB may also have significant bone conduction properties that guide bone-forming cells to reach the inferior aspect of the membrane when it is placed significantly away from the implant and the "floor" of the defect. Thus, it seems that DFDBA may promote bone formation in augmentation procedures through its osteoconductive and osteoinductive properties, as well as maintaining a suitable space under the membrane. However, the relative contribution of each of these suggested properties of DFDB to successful bone fill needs further investigation.

It has been shown that late removal of the augmentation material may enhance bone promotion around dental implants better than early removal.^{1,5-9,20,21} In our study, it was evident that whenever the membrane could be retained unexposed in situ throughout the healing period (4 to 6 months), bone regeneration was complete. This corroborates a recent dog study which showed maximal (100% fill) bone formation if the membrane remained submerged during a 16-week healing period.²¹ However, as in our study, early removal of exposed membranes resulted in partial bone fill. This observation may be attributed to possible wound contamination if the exposed membrane is not promptly removed.²⁰ Furthermore, once the membrane is removed, the wound may become exposed to physical and/or biochemical interferences to the regenerating bone that may lead to disintegration of the new bone and its significant resorption. Histologically, we observed significant bone formation at 4 to 6 months postgrafting. However, maturation of the newly formed bone may take longer (as observed in the 1-year biopsy). Therefore, it is suggested that the membrane not be removed earlier than 4 to 6 months postsurgery.

Summary

This study suggests that DFDBA may promote bone formation around dental implants. Successful bone promotion using DFDB can be correlated with: (1) complete soft tissue coverage during the entire healing process, providing ideal conditions for wound stabilization and preventing bacterial and chemical contamination; (2) maintenance of the membrane for the entire healing process, allowing optimal conditions for "long-term" guided bone regeneration; and (3) incorporation of tetracycline into the graft material, possibly contributing to infection control and to bone promotion via its anticollagenolytic properties.

Although this report is limited to a small number of patients and biopsies, it still sheds some light on the clinical potential of DFDBA in such procedures and encourages further investigation. □

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Résumé

Observations cliniques et biologiques des allogreffes d'os lyophilisé déminéralisé dans les procédures d'augmentation.

On condensa autour des zones exposées de 32 implants placés dans des sites alvéolaires post-extractionnels ou autres défauts osseux de la poudre d'os cortical humain lyophilisé déminéralisé (DFDB) mélangé avec de la tétracycline, afin d'évaluer la possibilité de régénération osseuse autour des implants dentaires endo-osseux. Les implants furent recouverts de membranes de polytétrafluoroéthylène étiré (e-ptfe) pendant 4 à 6 mois, jusqu'à connexion des piliers, à moins que ces membranes aient été exposées prématurément et déposées. Des biopsies osseuses d'os régénéré non-support furent récoltées chez certains patients et examinées histologiquement. Chaque fois qu'une couverture complète pouvait être maintenue tout au long de la période de guérison (4 à 6 mois), une régénération osseuse complète se produisit. La dépose prématurée de la membrane aboutit à une régénération osseuse partielle. Histologiquement, l'os régénéré consistait de particules d'os dévitalisé en contact avec un os immature néo-formé ou bien d'os lamellaire avec un peu de tissu conjonctif autour des particules. L'activité ostéogénique était présente même un an après la greffe. Ainsi, le DFDB est capable de promouvoir la formation d'os autour des implants dentaires si le recouvrement du lambeau reste complet et si la présence de la membrane est maintenue tout au long de la période de guérison.

Zusammenfassung

Klinische und biologische Beobachtungen nach Verwendung von demineralisiertem, gefriergetrocknetem Knochen zur Knochenaugmentation

Um die Möglichkeit der Knochengeneration im Bereich von enossalen Implantaten zu beurteilen, wurden 32 Implantate in Extraktionsalveolen oder andere knöcherne Defekte eingebracht. Die im Bereich von exponierten Implantatanteilen entstandenen Defekte wurden mit einem aus humanem, demineralisiertem, gefriergetrocknetem, corticalem Knochen und Tetracyclin bestehenden Implantatmaterial aufgefüllt. Die Implantate wurden mit Polytetrafluoroethylen(e-PTFE)-Membranen abgedeckt. Die Einheilungszeit lag zwischen 4 und 6 Monaten, wonach die Implantateröffnung erfolgte. Vorzeitig exponierte Membranen wurden entfernt. Während der Implantateröffnung wurden bei einigen Patienten Knochenproben im Bereich von regeneriertem, nicht stützendem Knochen entnommen und histologisch untersucht. Falls während des gesamten Einheilungszeitraumes (4 bis 6 Monate) die Membran vollständig von Weichgewebe bedeckt war, kam es zur vollständigen Knochenregeneration. In Fällen mit vorzeitig entfernter Membran fand nur zum Teil eine knöcherne Regeneration statt. Histologisch bestand der regenerierte Knochen aus devitalisierten Knochenpartikeln, welche in Kontakt mit neugebildetem Geflechts- oder lamellärem Knochen und umgebendem Bindegewebe standen. Sogar ein Jahr nach Knochenaugmentation war noch eine osteogenetische Aktivität vorhanden. Die Beobachtungen zeigen, daß humaner, demineralisierter, gefriergetrockneter, corticaler Knochen zur Knochenbildung im Bereich von Implantaten fähig ist. Voraussetzung ist, daß eine vollständige Weichgewebendeckung im Bereich der Membran während der gesamten Einheilungsphase erhalten werden kann.

Resumen

Observaciones clínicas y biológicas de aloinjertos de hueso desmineralizado y deshidratado por congelación utilizados en los procedimientos de aumento de hueso

Se colocaron 32 implantes en los alveolos de dientes recientemente extraídos, o en áreas que presentaban defectos óseos para evaluar la posibilidad de regenerar hueso alrededor de los implantes endóseos. Las partes expuestas fueron cubiertas con polvo de hueso cortical humano desmineralizado y deshidratado por congelación (HDDC), mezclado con tetraciclina. Los implantes fueron cubiertos con membranas de politetrafluoroetileno expandido (PTFE-e) por un periodo de 4 a 6 meses hasta la conexión del pilar (componente transepitelial), a no ser que las membranas se hubieran expuesto prematuramente y se hubieran tenido que quitar. Se tomaron biopsias del hueso regenerado no soportado de algunos pacientes, y se examinaron histológicamente. Cuando las membranas permanecieron cubiertas completamente durante el periodo de cicatrización (4 a 6 meses), hubo regeneración ósea completa. Cuando las membranas fueron removidas prematuramente el hueso se regeneró parcialmente. Histológicamente, el hueso regenerado consistió de partículas de hueso sin vitalidad en contacto con hueso lamelar o medular recientemente formado con algún tejido conectivo alrededor de éstos. Se presentó actividad osteogénica aún después de un año de colocar el injerto. Por lo tanto, el HDDC es capaz de promover la formación ósea alrededor de los implantes dentales si es posible mantener la cobertura completa del colgajo y la membrana en su lugar, a través de la fase de cicatrización.