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Case Report

Tooth Transformer: A New Method to Prepare Sinus Lift Autologous Toothgrafts. Histologic and Histomorphometric Analyses of 4consecutive Clinical Cases

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Abstract

Introduction

Human dentin matrix could be successfully used for bone grafting procedure. It was well known that dentin grafts could induce osteoblasts proliferation. An innovative preparation method, using the dedicated automated device Tooth Transformer, which is able to transform autologous teeth in suitable grafting material, has been recently introduced. The aim of the present paper is to analyze the histologic outcomes in four human consecutive cases in which autologous tooth graft materials, starting from the whole tooth of the patient, was used for sinus lift regeneration.

Results

The bone defects were completely filled by newly formed tissue after 4 months of healing. The histologic analysis revealed no inflammatory or infective reactions against tooth graft. Tooth granules were surrounded by newly formed bone. Some tooth granules were incorporated in the bone trabeculae and they appeared partially resorbed. This fact testified that tooth graft underwent remodeling processes just like the native bone.

Discussion

Results from the present histologic case series analysis revealed that tooth graft appeared well integrated in the regenerative tissue without any inflammatory or infective reaction. The tooth of the patient may be used as autologous regenerative materials avoiding any foreign graft material.

Introduction

The tooth grafting procedure has been introduced by Urist et al. more than 50 years ago, when they discovered the osteo induction potential of demineralized dentin matrix[1-2]. More recently, Bessho et al. demonstrated the presence of bone morphogenetic proteins (BMPs) in human dentin matrix. In particular, it was observed bone formation and osteoblasts presence in rat muscle after demineralized human dentin matrix graft [3].

It was clear that both bone and dentin matrix contained fundamental growth factors for bone regeneration. It represented an efficient reserve of BMPs, bioactive growth factors (GFs), such transforming growth factor-B (TGF-B), which are well known to be involved in bone repairing processes [4]. Some authors theorized that the demineralization process allows better bone augmentation than non-demineralized dentin [5].

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Moreover, the chemical composition of bone and dentin was almost the same with the presence of an inorganic portion made of hydroxyapatite and an organic one, mainly composed by collagen type 1 and other secondary proteins. Heterologous or alloplastic grafting materials, on the other hand, are been used for bone augmentation procedures from more than 35 years but they works as mechanical scaffold for host cells and do not offer any osteoinduction stimulus [6-7-8-9]. The efficacy and safety of autogenous partially demineralized dentin matrix prepared onsite, for clinical application in bone regeneration procedures related to implant dentistry; including socket preservation, alveolar ridge augmentation, and maxillary sinus floor augmentation were recently demonstrated in some human studies [10-11].

Recently, an innovative medical device (TT TOOTH TRANSFORMER SRL, Via Washington, 59 – Milan, Italy) to obtain suitable tooth graft materials starting from the whole tooth of the patient was introduced to the market. This machine ensure completely automated disinfection, grinding and demineralizing processes without any possible mistake induced by human manipulation of the process. This new device represents an advanced system in the area of tissue engineering because it is able to process and transform extracted tooth into useful bone graft material in a short time. The graft material, produced starting from the whole tooth, showed high wett ability that allowed an easy handling and positioning on host site. A previous case series described the successful clinical outcomes of bone regeneration after autologous tooth grafting using this new device and demonstrated the complete filling of bone defects by hard tissue without any complications [12]. The present paper aims to describe the histologic and histomorphometric analyses of regenerated tissue after innovative autologous tooth grafting procedures in 11 consecutive case of socket preservation.

Materials and Methods

The whole extracted tooth was first cleaned from residual calculus using piezoelectric instrument. The root surface was polished using diamond burs with abundant irrigation. Any filling materials (gutta-percha, composite, etc) were carefully removed from the tooth. The tooth was cut in small pieces and they were inserted in the mill of the device.

A small box containing disposable liquids was inserted in the device in its correct position (indicated by arrows). According to the manufacturer, these solutions guarantee maximum release of BMP-2 and collagen as well as a decontamination of the root. When all the components were inserted and the cover of the machine was closed, the device was started using the generals witch button. Demineralized dentin graft was ready in 25 minutes to be placed in the patient's mouth (TT TOOTH TRANSFORMER SRL, Via Washington, 59 – Milan, Italy).

The present case series, included four patients (3 male and 1 female), age ranged between 45 to 60 years old. All patients were in good health conditions and nonsmokers. In all cases the patient need sinus lift regeneration procedures. In all cases, the graft was covered by a resorbable porcine pericardium membrane (BEGO Implant Systems GmbH & Co. KG, Wilhelm-Herbst-Straße, Bremen, Germany). An immediate post-operative radiological check was performed. Each patient underwent clinical examination after 10 and 30 days in order to evaluate the healing process. After 4 months of healing period, all patients underwent a surgical re-entry session for dental implant insertion. The osteotomic sites were prepared using a trephine drill of 3 mm inner diameter that allowed retrieving a bone sample for each osteotomy.

The specimen were immediately fixed in 10% neutral buffered formalin and processed for histologic analysis. After dehydration, the specimens were infiltrated with a methyl-methacrylate resin from a starting solution 50% ethanol/resin and subsequently 100% resin, with each step lasting 24 hours. After polymerization, the blocks were sectioned and then ground down to about 40 microns. Toluidine-blue staining was used to analyze the different ages and remodeling pattern of the bone. The histomorphometric analysis was performed by digitizing the images from the microscope via a JVC TK-C1380 Color Video Camera (JVC Victor Company, Yokohama, Japan) and a frame grabber. The images were acquired with a 10x objective over the entire implant surface. Subsequently, the digitized images were analyzed by the image analysis software IAS 2000 (Delta Sistemi, Roma, Italy). For each section the 2 most central sections were analyzed.

Results

The bone defects were completely filled by newly formed tissue after 4 months of healing. In all cases a complete filling by hard tissue was evident by clinical and radiographs observation. The healing of soft tissues after grafting procedures was free of complications. No active or chronic infective processes were observed. Histomorphometric data of bone defects after bone regeneration were summarized in (Table 1). Clinical outcomes were showed in a previous case series study.12 Histomorphometric analysis showed a mean bone volume percentage (BV%) of 36,28+/-9,77and a residual graft percentage (RG%) of 14,61 ± 9,37. The newly formed tissue, observed during the surgical re-entry (after 4 months of healing), showed a compactness similar to the medium-density bone. No graft particles in sub mucous connective tissues were found during flap elevation procedures. The regenerated tissue aspect was almost homogeneous and tooth particles or grains were not distinguishable. A D3 tactile bone density during harvesting drilling procedures was supposed.

Table 1: Radiological and histomorphometric data of each case.

Sinus lift	1.4	Uniform radiolucency	31,8	22.3
Sinus lift	1.6	Uniform radiolucency	41,52	8,73
Sinus lift	2.6	Uniform radiolucency	40,51	14.09
Sinus lift	1.5	Uniform radiolucency	46,11	25,28
Sinus lift	1.6	Uniform radiolucency	21,46	2,64
Mean value			36,28+/-9,77	14,61 ± 9,37

The histologic analysis revealed no inflammatory or infective reactions against tooth graft. Tooth granules were surrounded by newly formed bone. Woven bone and numerous roundish osteocytes were also visible. In the medullary bone area was present large vascular canals. Some tooth granules were incorporated in the bone trabeculae and they appeared partially resorbed. This fact testified that tooth graft underwent remodeling processes just like the native bone. In some cases, dentin granules appeared completely incorporated in woven bone and surrounded by osteoid tissue layer in development. Some more coronal granules were surrounded by fibrous tissue.

Discussion

Autogenous bone has been considered the gold standard in bone regeneration procedures for many years. However several studies have highlighted some problems related to the use of autologous bone such as donor site morbidity, severe pain or patient hospitalization [13]. In addition, the long term stability of autologous bone graft has been investigated for many years and some authors found a high reabsorption rate [14]. To overcame the bone resorption, other authors suggested to mix autogenous bone to xenograft particles [15]. An ideal grafting material should be stable and, at the same time, should promote the bone forming cells proliferation and bone apposition [16]. Xenograft and alloplasticbone substitutes have been used for many years with success in oral implantology and many authors described that these materials represent an efficient mechanical support for cells migration but they were not able to induce the osteogenesis process [17-18-19-20]. In addition, the chemical or physical processes to eliminate any organic residuals, which all xenograft materials are subjected, destroyed all proteins that are fundamental in bone regeneration promotion. Furthermore, we cannot completely exclude the possibility of man-animal cross-infection by prions.

The results of the present study demonstrated that the values of BV% after bone regeneration procedures are super imposable to those that the international literature attributes to other grafting materials in humans [21-22]. In addition, the RG% recorded of 14, 61 ± 9,37 was lower than that reported for common used xenograft materials [23]. This datum testified that the tooth graft underwent physiologic bone remodeling phenomena and, at the same time, supported bone regeneration. A previously published literature review, analyzing 108 studies about autogenous teeth used as graft material, reported an implant survival rate of 97.7% but found that the dehiscence of the wound was a frequent complication [24]. Another animal study showed an accelerated bone healing in defects treated by autogenous demineralized dentin matrix and PTFE membrane in respect with PTFE membrane alone [25]. Many authors demonstrated that demineralized dentin is able to maintain intact the autogenous growth factors (such as osteopontin, dentin sialoprotein and BMP) and, for this reason, could induce bone formation (osteoinduction) [26-27-28].

It was also demonstrated that these growth factors, such as insulin-like growth factor (IGF), bone morphogenetic protein-2 (BMP-2) and transforming growth factor (TGF-β) are preserved over the time allowing to use for bone regeneration autologous tooth preserved for years (i.e. previously extracted wisdom tooth or deciduous teeth) [29]. The phenomenon of dent alveolar alkalosis, often seen after tooth replantation, is an excellent explanation of the osteoinductive properties of demineralized dentin matrix are that acts as a slow-releasing carrier of bone morphogenic proteins (BMP).

While osteo conduction means that bone grows on a surface, osteo induction could be explained as the process by which osteogenesis is induced and it is a phenomenon regularly seen in any type of bone healing process. It implies the recruitment of immature cells and the stimulation of these cells to develop into preosteoblasts [30]. The autologous tooth graft, described by the present paper, may induce osteoblasts proliferation and bone induction and, in the same time, eliminating any risk of cross-infection (such as prions infections).

The innovative preparation technique, to transform autologous teeth in suitable grafting material, allowed preserving the organic autologous components, removing any contaminants (to avoid inflammatory or infective reactions), and preparing the inorganic part to be easily colonized by osteoblasts. The demineralization process is required for freeing the various growth factors and proteins, since the release of the growth factors is sometimes blocked by the presence of hydroxyapatite crystals [31].Through the reduction of the mineral phase, demineralization support the release of such growth factors from the tooth matrix [32]. An in vitro study, testing the graft material obtained by this new device starting from whole tooth,

demonstrated that the demineralization process lead to an increase of BMP-2 bioavailability [33]. The same authors, in a subsequent study, showed that the demineralization treatment made by Tooth Transformer in deciduous teeth led to a dramatic decrease in relative Ca and P content while preserving native protein conformation and activity [34]. Furthermore, the demineralization process led to a great rise in the bioavailability of BMP-2 that was also proved to be very effective in enhancing alkaline phosphatase activity, thus in the osteodifferentiation of SAOS-2 cells in vitro. These studies demonstrated the complete absence of bacteria in tooth graft treated by Tooth Transformer method and that the BMP-2 content, found in all demineralized tooth even in deciduous teeth extracted many years before, is very effective in inducing cell osteodifferentiation.

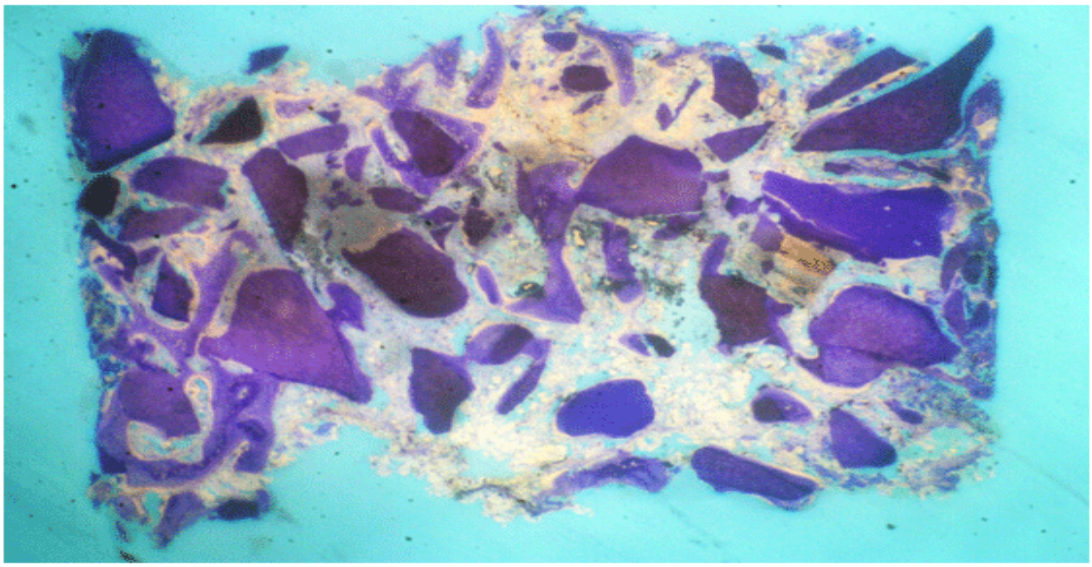

Figure 1. Overview of the biopsy at low magnification: tooth granules and newly formed bone were visible (Magnification 8x – Toluidine Blue).

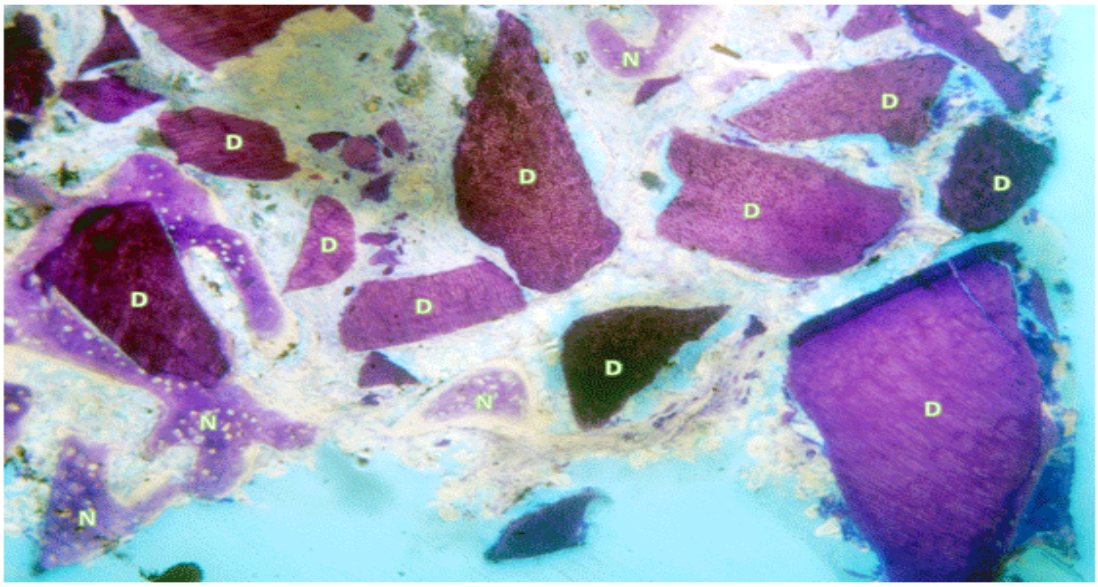

Figure 2. Newly formed bone trabeculae and graft particles were observed. (Magnification 25x – Toluidine Blue).

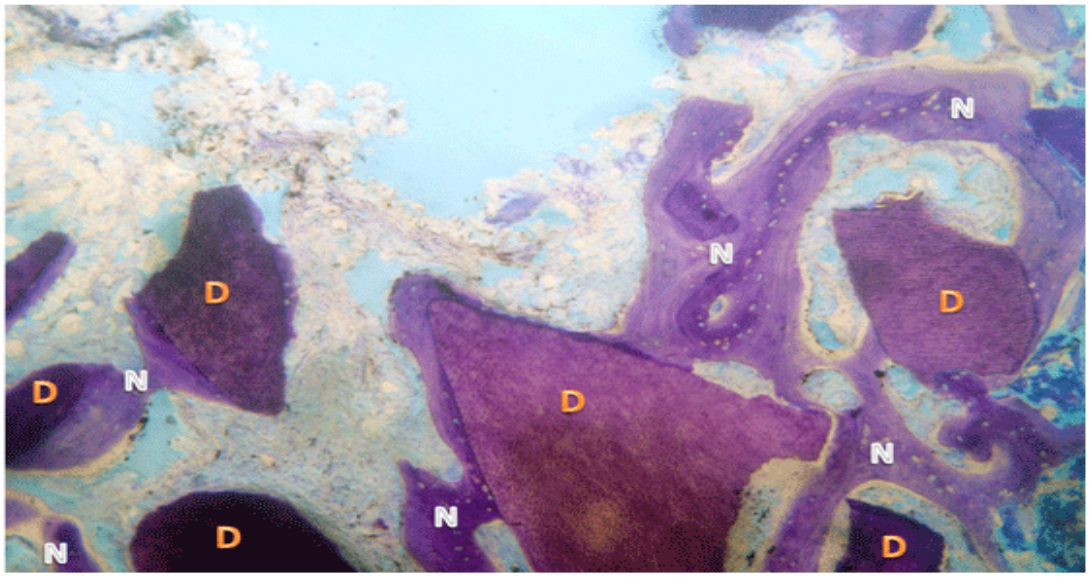

Figure 3. Woven bone and numerous roundish osteocytes were present.(N) Osteoid bands were also visible. Some dentin granules were incorporated in the bone trabeculae and they appeared partially resorbed. (D) The presence of this process demonstrated that dentin graft underwent remodeling processes just like the native bone. (Magnification 100x – Toluidine Blue).

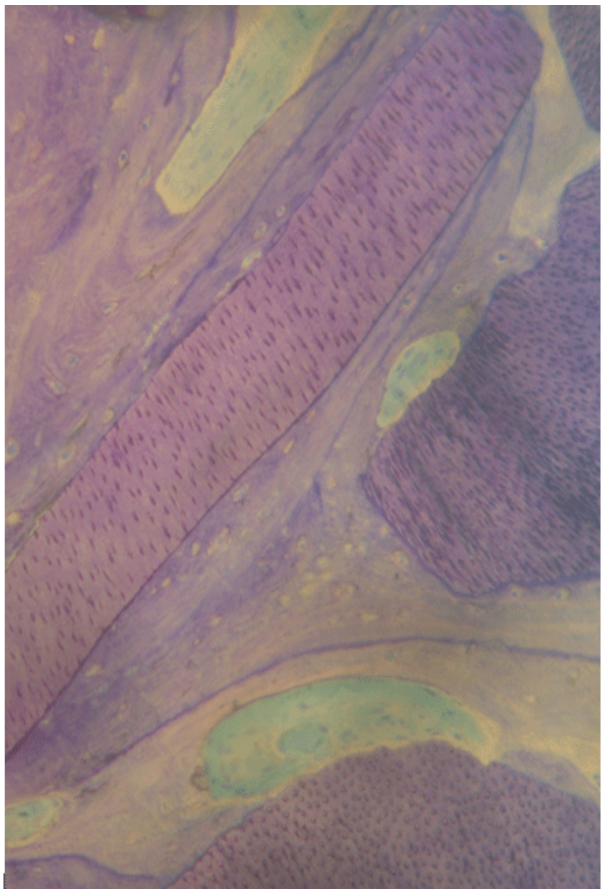

Figure 4. Dentin granule (200 x 500 μm) completely incorporated in the woven bone and surrounded by osteoid tissue layer. (Magnification 100x – Toluidine Blue)

Conclusions

he histological analysis of the present case series demonstrated bone regeneration and no inflammatory reactions around dentin granules. The graft, in all the cases analyzed, was subjected to the physiological bone remodeling phenomena, demonstrating an excellent integration with the host tissues. Future controlled and randomized studies with long follow up period are needed in order to better evaluate the potential of demineralized dentin autografts in bone regeneration field.

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