A novel procedure to process extracted teeth for immediate grafting of autogenous dentin.

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Running title: extracted teeth for immediate grafting of autogenous dentin.

Key findings: Autogenous mineralized dentin particulate that is grafted immediately after extractions should be considered as the gold standard for socket preservation, bone augmentation in sinuses or filling bone defects.

Abstract:

Background: Extracted teeth are still considered a clinical waste and therefore discarded. It is evident that chemical composition of dentin is similar to that of bone. After tooth reimplantation it is replaced by bone tissue, followed by root resorption and ankylosis, being integrated into the surrounding alveolar bone. Here, we present a novel procedure in a clinical setting that employs freshly extracted teeth, processing them into a bacteria-free particulate dentin,then grafting immediately into extraction sites or bone deficiencies in the jaws.

Methods: The procedure consists of reducing any restorations, caries, calculus or plaque. The clean and dry tooth, mostly dentin, is immediately grinded and sorted in a specially designed 'Smart Dentin Grinder'. The dentin particulate of 300-1200um is selected through a special sieve system. The sorted particulate dentin is immersed in basic alcohol cleanser in a sterile container, dissolving all organic debris and bacteria. Then, the particulate is washed by sterile saline. The bacteria-free particulate dentin is ready for immediate grafting into freshly extracted sockets or into bone voids.

Results: During the period of 2 years, more than 100 procedures were performed, mostly preservation of alveolar bone. In those patients implant insertion was possible already after 2-3 month after grafting of autogenous dentin. On x-rays and biopsy of grafting sites a solid dense dentin-bone composite is found. No wound healing complications were seen.

Conclusion: It seems that autogenous mineralized dentin particulate that is grafted immediately after extractions should be considered as the gold standard for socket preservation, bone augmentation in sinuses or filling bone defects.

Key words: Smart dentin grinder and sorter, autogenous particulate dentin graft, socket preservation.

Introduction:

In most cases, after tooth extraction alveolar bone undergoes remodeling with net bone loss which endangers esthetic reconstruction[1]. In fact, osteoprogenitor cells in conjunction with scaffold and osteogenic factors are used to create bone tissue both in vitro and in vivo [2,3]. These engineered bone grafts have been shown to poses the capacity for osteogenesis, but also for osteoconduction and even bioactivity. Ideally, the engineered bone should form a structural and functional connection with the host bone, also termed as physical connectivity[2]. Unfortunately, vascularization of engineered bone tissue remains a major obstacle in achieving a clinically sized bone grafts. Autogenous bone graft is currently considered the safest and most efficient procedure because it exhibits bioactive cell instructive matrix properties and is non-immunogenic and non – pathogenic. It is well accepted that membranous cortical bone harvested from the lower jaw or cranial bones are preferred harvesting sites, in comparison to iliac crest bone, because they remodel in much slower pace[4]. However, at the donor site risk of infection and morbidity are possible complications. It is well known that jaw bones, alveolar bone and teeth develop from cells of the neural crest and that many proteins are common to bone, dentin, and cementum[5]. It is therefore not surprising that dentin that comprise of more than 85% of tooth structure can serve as native bone grafting material. Interestingly, Schmidt-Schultz and Schultz[6] found that intact growth factors are conserved even in the collagenous extracellular matrix of ancient human bone and teeth. In fact, this study provided additional proof that hydroxyapatite (HA) mineral of bone and dentin is protecting their organic matrices, like type I collagen and most non collagenous proteins from being exposed to enzymatic digestion. In previous reports, a method for processing bovine dentin into particulate and sterile grafting material for preservation of alveolar bone was described and used in animal studies[7,8,9] . It is therefore evident that teeth become grafts that are slowly and gradually replaced by bone[10]. Moreover, studies have shown that dentin matrix that is processed from extracted teeth is considered as optimal autogenous graft material in defects of jaw bone and in preserving socket after extraction[7,10,11,12,13].

Currently, all extracted teeth are considered a clinical waste and therefore are simply discarded. Recently, several studies reported that extracted teeth from patients that undergo a process of cleaning, grinding, demineralization and sterilization is a very effective graft to fill alveolar bone defects of same patient[10,12,13]. Since it is prepared from autogenous teeth that underwent cleaning and sterilization it is eliminating the risk of an immune reaction that may lead to rejection. However, this procedure is much time consuming. It includes demineralization by acid and then sterilization of demineralized dentin matrix and therefore the particulate demineralized autogenous tooth dentin is ready for use hours and days after extraction. The authors claim for excellent bony healing by osteoinduction and osteoconduction, similar to demineralized bone (DBM). We present here a modified procedure, in a clinical setting that employs freshly extracted teeth, by recycling them into a bacteria-free particulate autogenous mineralized dentin for immediate grafting. A ‘Smart Dentin Grinder’™ was devised to grind and sort extracted teeth

into a specific size dentin particulate. The 300-1200 μm dentin particulate is then immersed for 10 minutes in a non toxic chemical cleanser that dissolves all surface organic debris in order to achieve a bacteria-free particulate mineralized dentin for immediate augmentation into freshly extracted teeth sockets, bone defects and sinus augmentation procedures. This novel procedure is indicated mainly in cases when teeth are extracted because of periodontal reasons, extraction of partially or totally impacted teeth, especially most of wisdom teeth. Also, teeth that are extracted for orthodontic reasons can be employed for grafting of bone defects. The main advantage of the procedure is that immediately after extraction, teeth are processed and ready to be grafted back into extraction site or any other need for bone grafts, during 15-20 minutes. Teeth that underwent root canal fillings should not be employed in this procedure because of foreign materials contamination. On the other hand, crowns and fillings can be reduced and clean dentin of tooth crown can be processed for immediate grafting.

Methods

Procedure from tooth extraction to grafting of particulate dentin:

Teeth without root canal fillings that are extracted due to advanced periodontal bone loss or other indications like wisdom teeth or orthodontics indications, are prepared for immediate grafting. Immediately after extraction, restorations like crowns and fillings should be cut off or removed. Also carious lesions and discolored dentin or remnants of PDL and calculus should be reduced by tungsten bur (Figure 1a & 1b). We find that high speed tungsten carbide burs are most efficient for this process. The roots could be split in case of multi-rooted teeth. Clean teeth including crown and root dentin are dried by air syringe, put into a grinding sterile chamber of a newly designed 'Smart Dentin Grinder'™ (Figure 2a). The 'Smart Dentin Grinder'™ (SDG) is capable in 3 seconds to grind the roots and then by vibrating movement of the grinding chamber for 20 seconds the particles of less than 1200 μm fall through a sieve to a lower chamber that keeps particles between 300-1200 μm (Figure 2b). The particles less than 300 μm fall into a lower drawer. This fine particulate (less than 300 μm) is considered as a non-efficient particulate size for bone grafting. This grinding and sorting protocol is repeated to grind the remaining teeth particles left in grinding chamber. In the collecting drawer chamber dentin particles between 300 - 1200 μm are collected (Figure 2b). The particulate dentin from the drawer is immersed in basic alcohol for 10 minutes, in a small sterile glass container. The basic alcohol cleanser consists of 0.5M of NaOH and 30% alcohol (v/v), for defatting, dissolving all organic debris, bacteria and toxins of the dentin particulate. The efficiency of the cleanser to dissolve all the organic debri from dentin particulate including dentin tubules is demonstrated in Figure 3. The SEM picture shows wide open and clean tubules after 10 minutes of cleanser treatment (Figure 3c). After decanting the basic alcohol cleanser, the particulate is washed twice, in sterile phosphate buffered saline (PBS). The PBS is decanted leaving wet particulate dentin ready to graft into freshly extracted sockets or into alveolar bone defects or in procedures of augmenting maxillary sinus. The process from tooth extraction until grafting takes approximately 15-20 minutes. It

should be noted, that the efficiency of selecting the dentin particulate of specific size for grafting is more than 95%. It is obvious that the volume of the particulate dentin is more than twice of the original root volume. Alternatively, the wet particulate can be put on a hot plate (140^0C) for 5 minutes and the dry bacteria-free particulate autologous dentin can serve for immediate or future grafting procedures.

Results:

Clinical Evaluation

During the period of 2 years, more than 100 dentists are employing the present procedure for preparing autogenous dentin particulate from extracted teeth for immediate grafting in same patient. It should be noted that teeth that were root canal treated are not indicated for processing them for grafting. Also, no demineralization of the dentin particles is needed. If intact teeth are processed the enamel and cementum can be included. Any restoration or carious lesions should be cut off by tungsten burr drill and irrigating water. Here, we present typical case presentations where teeth were extracted and processed into bacteria-free particulate autogenous tooth dentin for immediate grafting in same patient.

From a series of 16 wisdom teeth that included partially impacted, horizontally impacted and others that their crown was destroyed by caries were processed by our procedure and immediately grafted in the extraction sockets. We present here a horizontal impacted tooth 48 that was in close proximity to distal root surface of 47 (Figure 4b), creating a deep void. The surgically extracted tooth 48, exposed the distal root surface of 47 almost denuded from bone tissue. Usually, in such cases an immediate graft should be placed in order to generate new bone formation to restore the distal bone support of the tooth 47. Here, we processed immediately the tooth 48 into particulate graft which filled totally the extraction site (Figure 4c). The healing and recovery after surgical procedure and grafting was without complications. A follow up after 4 month revealed a normal pattern of marginal gingiva around the tooth 47. Probing was normal 1-2mm in depth. On x-ray distal to tooth 47, new bone and particulate dentin was integrated into bone restoring completely the site of extraction and a distal bone support of tooth 47 (Figure 4d).

Another series of extracted teeth because of poor periodontal attachment, bone loss and mobility is presented here by a patient, 56 years old male with localized advanced periodontal condition in the posterior part of mandible. Teeth 47 and 48 were extracted and the granulation tissue was removed exposing bone tissue walls. Tooth 47 had a root canal filling and therefore was discarded. Tooth 48 processed into particulate dentin by the SDG device and prepared for immediate grafting in the extraction sites. The grafting of one tooth produced volume of particulate dentin that was adequate to overfill the extraction site sockets. A PRF membrane from patients blood was prepared according to Choukrouns PRF technique[14] to cover the graft. Mucoperiosteum was sutured to PRF, avoiding tension of tissues. An improved healing is

achieved because of the PRF membrane. 2 month later two implants were placed, followed by a cemented bridge of 47-48 crowns. After two years, clinical and x-ray follow up revealed a very radiopaque bone integrated into implants, most possibly consisting of bone formed onto particulate dentin producing a very solid support for implants (Figure 5).A similar procedure was performed in same patients in left side of lower jaw. X-ray showing bone loss around teeth 36,37 and 38(Figure 5g). 2 month after grafting with particulate dentin of tooth 38 three implants were inserted (Figure 5h), and one year later observe the bone density and bone level with no signs of bone resorption at the crest after restoration (Figure 5j).

Autogenous dentin particulate can serve as superior grafting matrix for augmenting bone in maxillary sinuses, as is presented in the next case. Here, tooth 26 in the upper jaw is part of a bridge. Alveolar bone loss can be seen on x-ray with infrabony pockets that extended into the maxillary sinus of tooth 26 (Figure 6). The bridge was removed and tooth 26 was extracted, cleaned and processed into a bacteria free particulate dentin. An immediate grafting of the extraction socket was performed and the tract into the sinus was occluded by the particulate dentin. Closure of the wound and sutures of mucoperiosteum flap was performed. Healing was normal and three month later an alveolar ridge of minimum 8.3 mm height was achieved, allowing insertion of 3 implants. It should be noted that one molar of tooth 26 produced at least 2cc of particulate dentin which allowed augmenting the extraction socket and part of sinus. Moreover, we found that autogenous dentin grafting enables to insert implants after 3 month in the upper jaw, because the new bone that was integrated with particulate dentin produced a solid support for implants. Loading of implants followed. During preparation of a slot for implant insertion, a core of bone was recovered from the grafted socket site. The histology revealed new bone integrated with grafted dentin producing a bone-dentin interface connectivity (Figure 7a and 7b).

Discussion and conclusion:

More than 40 years ago, autogenous teeth were transplanted into extraction sockets routinely when possible. It is evident that transplanted teeth that are ankylosed in jaw bone undergo replacement resorption of root dentin by bone, during 5-8 years[15]. In addition, it is well documented that avulsed teeth that are implanted back into their sockets undergo firm reattachment to the socket because new bone is formed directly on root dentin or cementum, leading to ankylosis[16]. An ankylosed root is continuously resorbed and replaced by bone, eventually resorbing the entire root. Although the condition is progressive, it is slow in adult people, while, the alveolar process is preserved during this period and later. In a recent review Malmgren[17] stressed that ankylosed teeth that are treated by decoronation, the alveolar ridge is maintained in the buccal/palatinal direction, while vertical height will even increase. Although, a continuous resorption of the root and replacement of tooth substance by bone without pathologic changes happens, root remnants may still be present in some cases but made no obstacle for insertion of implants and the healing process[18]. These reports indicate that teeth and jawbone have a high level of affinity for each other, having similar chemical structure and composition.

Tooth extraction is one of the most widely performed procedures in dentistry today and it has been historically well documented that this may induce significant dimensional changes of the alveolar ridge[1]. The dilemma that clinicians face is how to manage tooth extractions to provide for the future placement of a dental implant or to maximize ridge dimensions for the fabrication of a fixed or removable prosthesis. If performed inadequately, the resulting deformity can be a considerable obstacle to the esthetic, phonetic, and functional results. In a recent review, Horowitz et al.[1] stated that less ridge resorption is occurring when alveolar ridge preservation procedures were used versus the placement of no graft material in fresh alveolar sockets. In dentistry allogeneic bone and synthetic mineral materials are the main source for grating in bone. However, fresh autogenous bone graft is still considered gold standard in spite of the need for harvesting of grafts and possible morbidity resulting from it. Although, it is known that tooth dentin has the same properties and consistence as that of cortical bone it was not employed routinely as a bone substitute, in humans. We and others propose that non-functional teeth or periodontally involved teeth that are diagnosed to be extracted should be considered as native patient-own tooth derived dentin graft for socket preservation and bone augmentation. In fact, Korea Tooth Bank (KTB) was established in Seoul 2009 for a unique service of tooth-derived graft materials. This service system in Japan and Korea is based on the preparation and delivery of autogenic demineralized dentin matrix graft as block-type or granular-type[12,13,19] . This 'Tooth Bank' system is delaying the grafting procedure from several hours to several days and therefore needs an additional surgical session. In contrast, the modified novel procedure that is described in the present paper allows the preparation of bacteria-free particulate dentin from freshly extracted autologous teeth, ready to be employed as autogenous graft immediately, in the same session. Dentin particles have the advantage, being cell free mineralized matrix to maintain its mechanical stability, allowing early loading after grafting in fresh sockets and bone defects. Although demineralized dentin exposes matrix derived growth and differentiation factors for effective osteogenesis, the newly formed bone and the residual demineralized dentin are weak to support implant anchorage, delaying further implant insertion and loading. On contrary, in spite of delayed inductive properties of the calcified dentin and bone[20,21] , the mineralized dentin is firmly integrated with newly formed bone, creating a solid site for anchorage of dental implants (Figure 7). In a rabbit study, completely demineralized dentin matrix induced bone in the muscle at 4 weeks, while calcified dentin induced bone at 8-12 weeks after implantation[20]. These results indicate that highly calcified tissues such as cortical bone and calcified dentin are slowly undergoing remodeling, thus releasing osteogenic factors during long period of time than spongy bone, DBM, and demineralized dentin matrix (DDM) [20,23,24] . In fact, our clinical data indicate that implant insertion and loading can be performed in lower and upper jaws 2-3 month after grafting of dentin. Since the mineralized dentin is very slowly remodeled in comparison to cortical bone or most biomaterials the esthetic and structure pattern of the alveolar crest and mucoperiosteum is maintained for years.

In a very recent publication Kim et al.,[23] compared the safety and efficiency of autogenous dentin bone graft material to grafting biomaterials that are in clinical use in dentistry. They

concluded that autogenous tooth bone graft materials have structures and physicochemical characteristics that are most similar to those of autogenous cortical bones. In the present study we found that during 2-3 years of using this modified procedure of immediate grafting of bacteria free particulate of mineralized dentin in more than 100 cases, no adverse effects were observed. The dentin bone structure (Figure 7), made possible to insert implants into very dense tissue 2-3 month grafting of dentin in upper and lower jaw, allows to proceed to the prosthetic phase shortly after that. Moreover, because of very slow replacement resorption of dentin, the preservation of the grafted site is predicted for years. It seems that autogenous mineralized dentin particulate that is grafted immediately after extractions should be considered as the gold standard for socket preservation, bone augmentation in sinuses or filling bone defects.

FOOTNOTES: 'Smart Dentin Grinder'™ is distributed by KometaBio ltd., Holon,Israel

CONFLICT OF INTEREST:

We certify that IB and LR have shares in KometaBio company and helped to develop the Smart Dentin Grinder™.GH, CN and AY have no conflict of interest

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Figures:

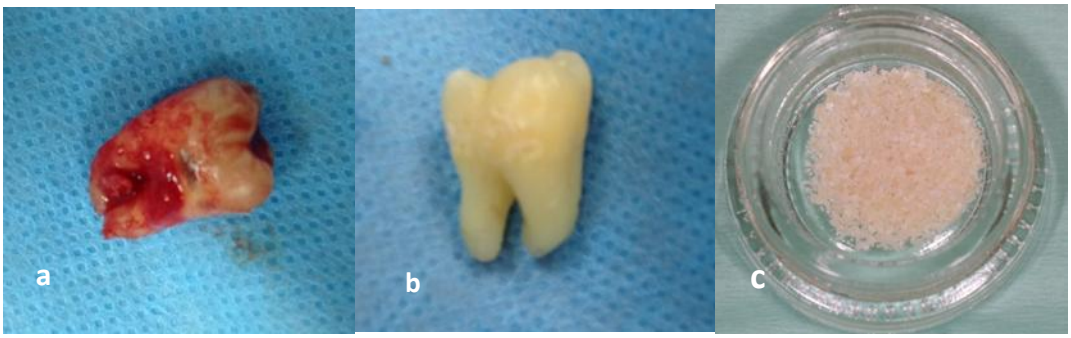


Figure 1: From extraction to clean particulate: (a) Tooth after extraction, debris and calculus; (b) same tooth after reducing debris with tungsten carbide burr. (c) Particulate dentin after grinding and sorting. The particulate dentin size is of 300-1200um.

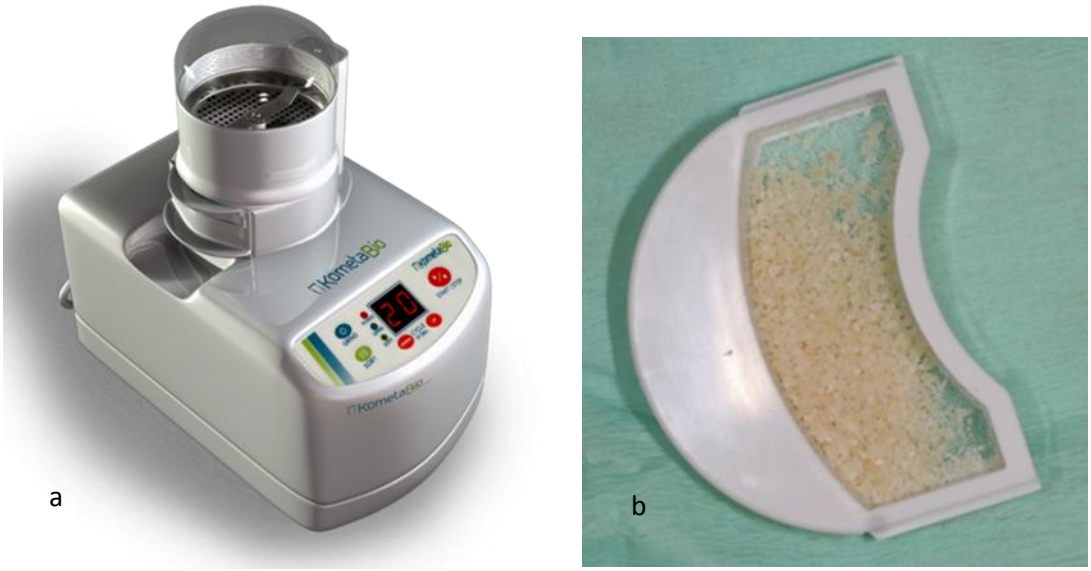


Figure 2: Smart Dentin Grinder and drawer with particulate dentin of 300-1200um size ready for cleanser treatment.(a) Smart dentin grinder and sorter.(b) drawer that collects particulate dentin after grinding and sorting. The size of particles in this drawer are of 300-1200um.

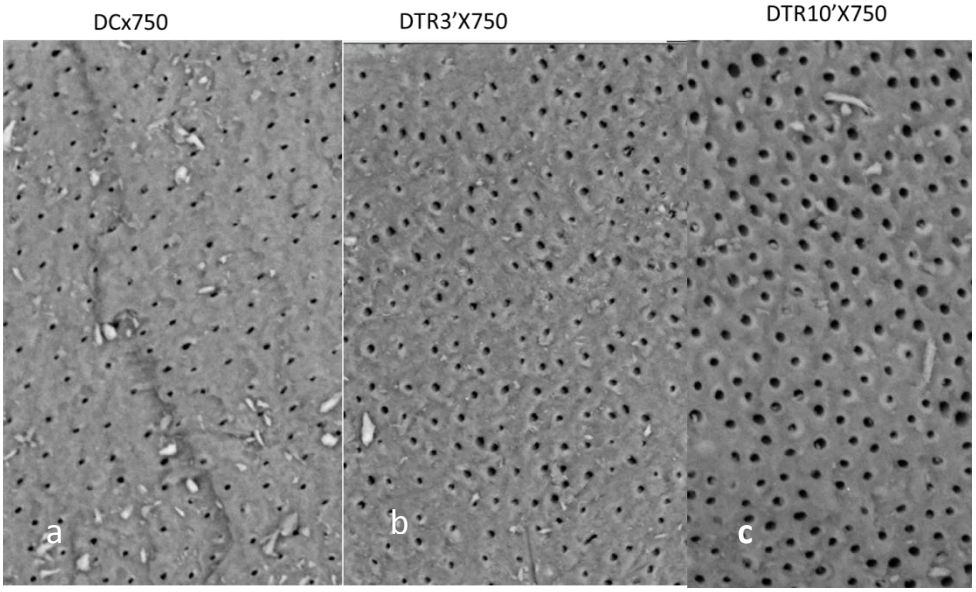


Figure 3: SEM (X750) of particulate dentin at 0 minutes (a), 3minutes (b) and 10 minutes (c) after treatment with cleanser. See the wide open tubuli openings after treatment with cleanser for10 minutes. Bacteriological test revealed no bacteria growth after 10 minutes of treatment with cleanser.

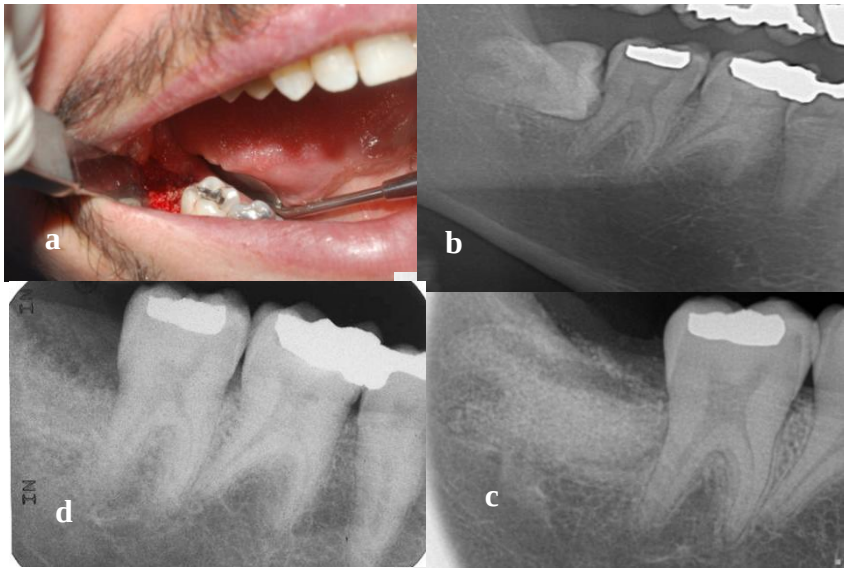
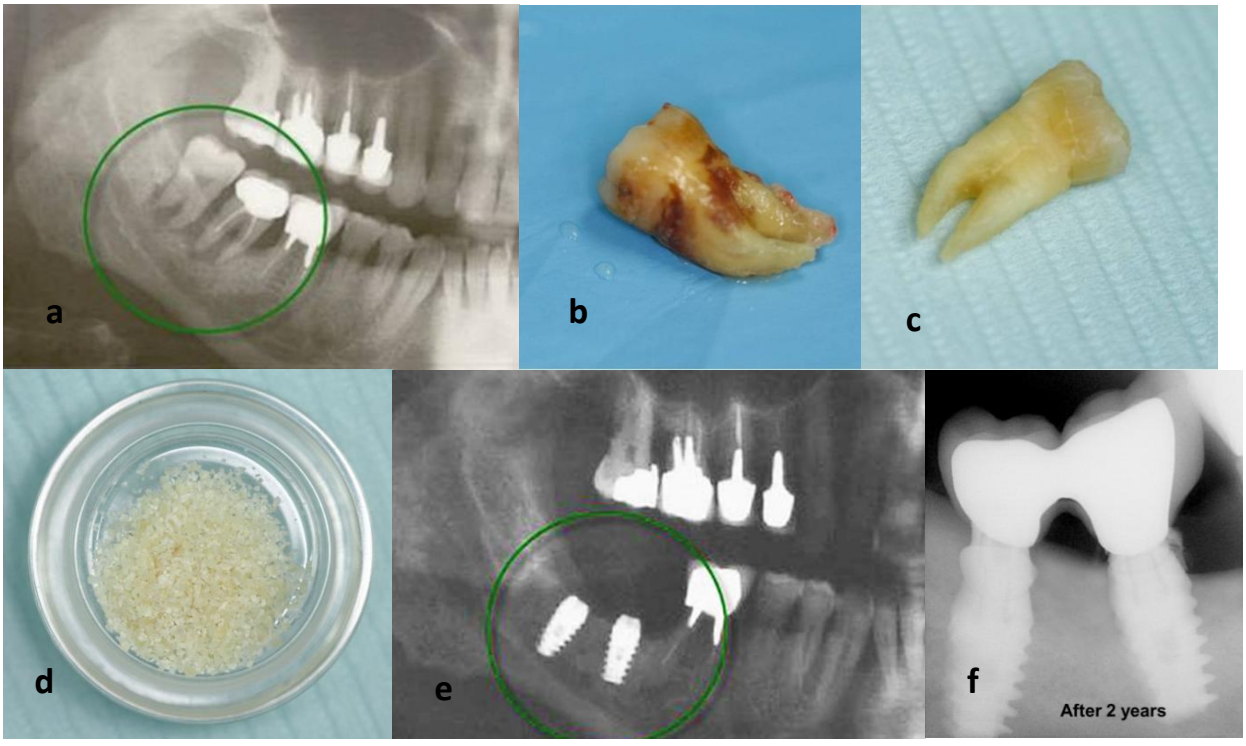
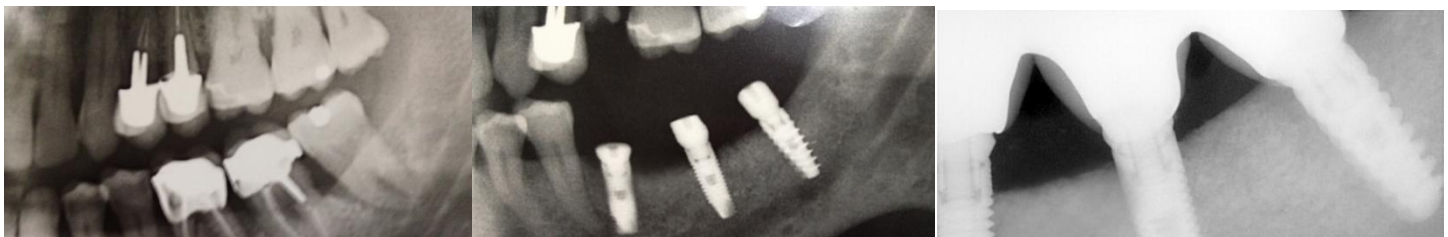


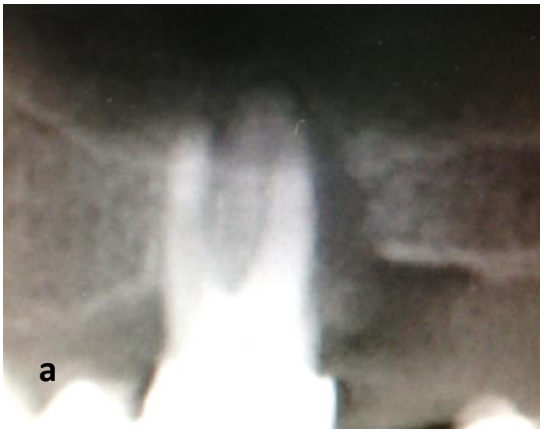
Figure 4: Tooth 48 extraction site filled with particulate dentin prepared from extracted tooth 48 by 'Smart Dentin Grinder' procedure. Clinical view of the extracted site (a) and x-ray of impacted 48 tooth(b); after extraction of tooth 48, particulate of extracted tooth was prepared and placed in extraction site (c); 4 month later the particulate and newly formed bone restored completely the void next to distal root of tooth 47 (d).

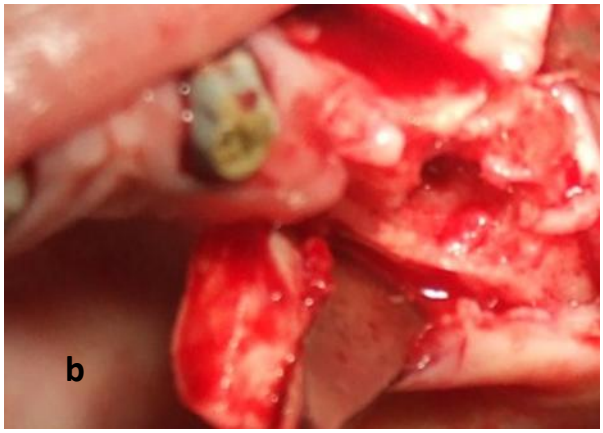


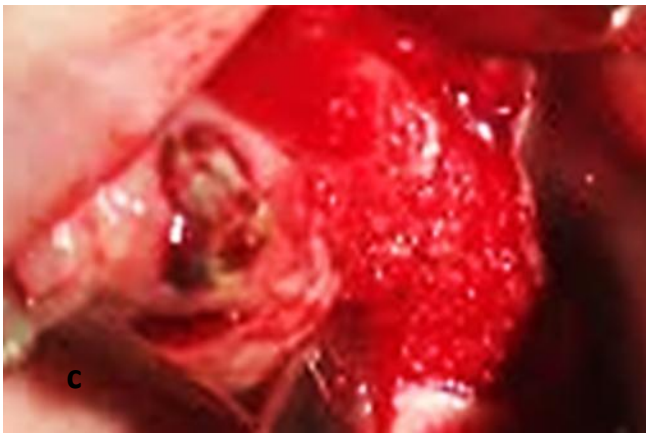


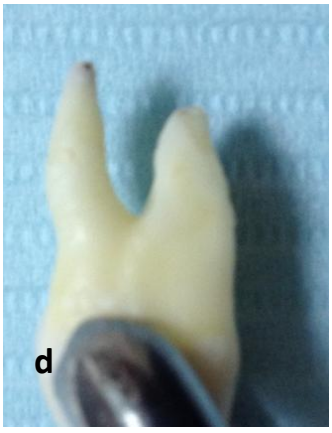
g h j After 1 year

Figure 5: Periodontally involved teeth with extensive alveolar bone loss of teeth,47,48,36,37 and 38. Immediately after extraction of those teeth, only tooth 48 and tooth 38 were employed for particulate dentin prepared by SDG procedure and immediately used to augment the extraction sites. (a) x-ray before extraction of teeth 47 and 48. (b) tooth 48 before mechanical cleaning and (c) after cleaning with tungsten carbide;(d) particulate dentin after cleanser treatment, ready to graft; (e) 2 month later, 2 implants were inserted in the augmented extraction sites; (f) 2 years later, see the dense bone and lack of bone loss next to implant; (g) x-ray showing bone loss around teeth 36,37 and 38; (h) 2 month after grafting with particulate dentin of tooth 38. Three implants were inserted 2month after grafting; (j) one year later, observe the bone density and bone level with no signs of bone loss next to implants.









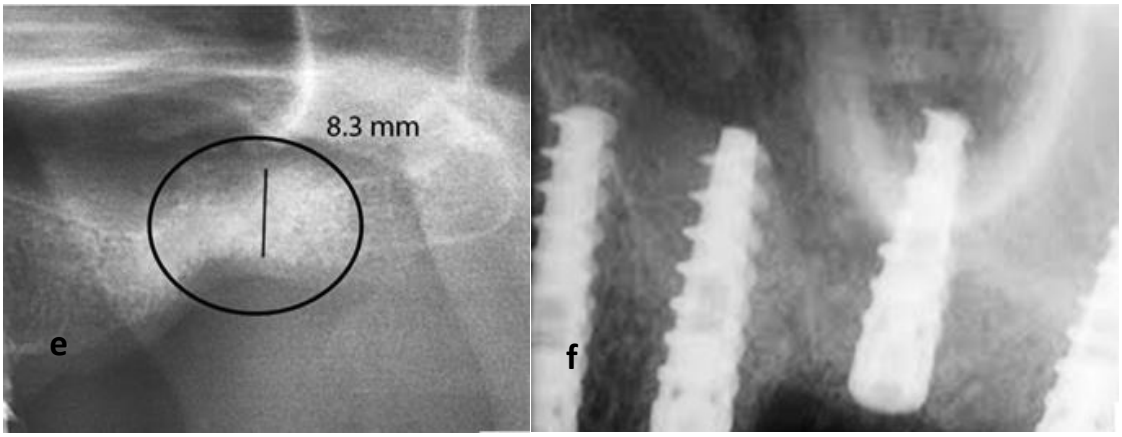


Figure 6: Periodontally involved tooth 26 (a) was extracted and cleaned (d). The alveolar bone after extraction; observe the oro-antral opening (b). After preparation of particulate from tooth 26 the socket was grafted and the oro-antral opening was filled with particulate dentin (c). After 2 month a 8.3mm height of bone was achieved with high density of dentin-bone (e). After 3 month 3 implants were inserted and immediate solid anchorage was achieved.

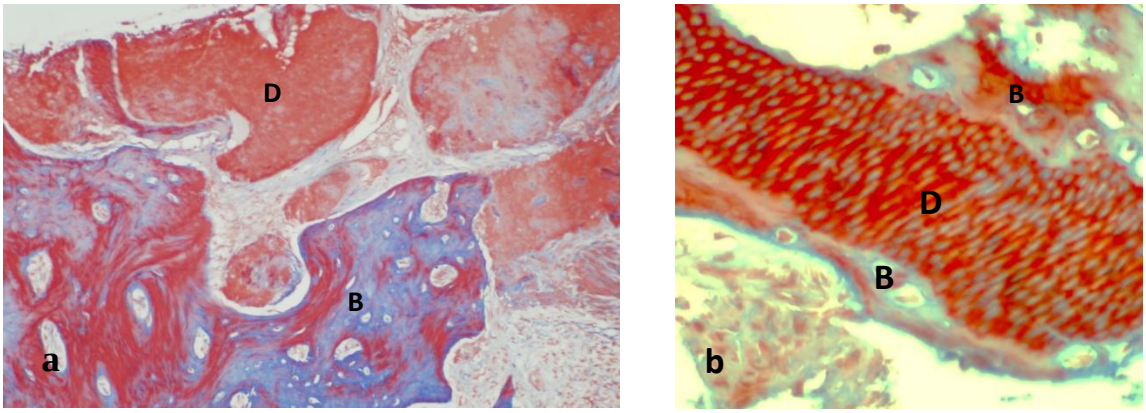


Figure 7: A histology section (Trichrome stain) of a core of bone tissue that was drilled out from upper jaw 3 month after grafting with autogenous dentin (**a**). A higher magnification of dentin-bone interface (**b**).Observe that dentin with its tubules (**D**) is surrounded by newly formed bone matrix (**B**).

Legends to Figures:

Figure 1: From extraction to clean particulate: (a) Tooth after extraction, debris and calculus; (b) same tooth after reducing debris with tungsten carbide burr. (c) Particulate dentin after grinding and sorting. The particulate dentin size is of 300-1200um.

Figure 2: Smart Dentin Grinder and drawer with particulate dentin of 300-1200um size ready for cleanser treatment.(a) Smart dentin grinder and sorter.(b) drawer that collects particulate dentin after grinding and sorting. The size of particles in this drawer are of 300-1200um.

Figure 3: SEM (X750) of particulate dentin at 0 minutes (a), 3minutes (b) and 10 minutes (c) after treatment with cleanser. See the wide open tubuli openings after treatment with cleanser for10 minutes. Bacteriological test revealed no bacteria growth after 10 minutes of treatment with cleanser.

Figure 4: Tooth 48 extraction site filled with particulate dentin prepared from extracted tooth 48 by 'Smart Dentin Grinder' procedure. Clinical view of the extracted site (a) and x-ray of impacted 48 tooth(b); after extraction of tooth 48, particulate of extracted tooth was prepared and placed in extraction site (c); 4 month later the particulate and newly formed bone restored completely the void next to distal root of tooth 47 (d).

Figure 5: Periodontally involved teeth with extensive alveolar bone loss of teeth,47,48,36,37 and 38. Immediately after extraction of those teeth, only tooth 48 and tooth 38 were employed for particulate dentin prepared by SDG procedure and immediately used to augment the extraction sites. (a) x-ray before extraction of teeth 47 and 48. (b) tooth 48 before mechanical cleaning and (c) after cleaning with tungsten carbide;(d) particulate dentin after cleanser treatment, ready to

graft; (e) 2 month later, 2 implants were inserted in the augmented extraction sites; (f) 2 years later, see the dense bone and lack of bone loss next to implant; (g) x-ray showing bone loss around teeth 36,37 and 38; (h) 2 month after grafting with particulate dentin of tooth 38. Three implants were inserted 2month after grafting; (j) one year later, observe the bone density and bone level with no signs of bone loss next to implants.

Figure 6: Periodontally involved tooth 26 (a) was extracted and cleaned (d). The alveolar bone after extraction; observe the oro-antral opening (b). After preparation of particulate from tooth 26 the socket was grafted and the oro-antral opening was filled with particulate dentin (c). After 2 month a 8.3mm height of bone was achieved with high density of dentin-bone (e). After 3 month 3 implants were inserted and immediate solid anchorage was achieved.

Figure 7: A histology section (Trichrome stain) of a core of bone tissue that was drilled out from upper jaw 3 month after grafting with autogenous dentin (a). A higher magnification of dentin-bone interface (b).Observe that dentin with its tubules (D) is surrounded by newly formed bone matrix (B).