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New bone formation around xenogenic dentin grafts to rabbit tibia marrow

Adel Al-Asfour[1], Lars Andersson[1], Mohammad Kamal[1], Bobby Joseph[2]

[1]Department of Surgical Sciences, Faculty of Dentistry, Health Sciences Center, Kuwait University, Kuwait; [2]Department of Diagnostic Sciences, Faculty of Dentistry, Health Sciences Center, Kuwait University, Kuwait

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Correspondence to: Lars Andersson, Department of Surgical Sciences, Faculty of Dentistry, Health Sciences Center, Kuwait University, P.O.Box 24923, Safat 13110, Kuwait
Tel.: +965 2498 6695
Fax: +965 2498 6732
e-mail: lars.andersson@hsc.edu.kw

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Abstract – *Purpose*: From traumatology, it is well known that dentoalveolar ankylosis results in osseous replacement and formation of new bone. This principle is used after decoronation for preservation of the height and width of the alveolar bone crest after trauma. Dentin possesses bone-forming properties and may possibly also be used as a bone augmentation material prior to implant placement. The aim of this study was to investigate whether xenogenic dentin particles inserted into the marrow space of rabbit tibia, a space where there is no solid bone tissue initially, would contribute to new bone formation. *Materials and Methods*: Dentin chips from human teeth were inserted into tibias of ten New Zealand rabbits. The tibial bones were processed for histology after 6 months, and new bone formation was quantified. *Results*: Bone formation was ranging from 0 to 86% on the dentin fragments, and there was minor inflammation. Bone formation was seen to a larger extent on dentin grafts located close to the native tibial bone wall. There was a significant correlation ($r = -0.579$, $P < 0.001$) between the amount of bone formation around the dentin graft and distance to the tibial cortical wall. *Conclusion*: Dentin promotes new bone formation when located close to native cortical bone and may have a potential as a bone augmentation material.

It has been demonstrated in many experimental and clinical studies in traumatology that teeth replanted after delay with a non-viable periodontal membrane will ankylose with the bone (1–5). The dentin of such teeth will gradually be replaced by bone, also called replacement resorption or osseous replacement (1–5). This is considered to be mainly a bone remodeling process (2–4). In adults, such teeth can be left to be replaced by bone, hence preserving the height and width of the alveolar bone crest. In growing patients, such ankylosed teeth are today recommended to be decoronated and maintained in the alveolar process. The root dentin is left for replacement resorption to preserve the width and height of the alveolar bone crest and to avoid inhibition of growth of the alveolar process (6–8). In recent experimental studies, it was shown that dentin xenografts in rabbits had a potential to be incorporated in bone without inflammation and were gradually resorbed and replaced by new bone (9, 10).

Augmentation of bone crest deficiencies with grafting is often carried out prior to treatment with osseointegrated titanium implants (11). Autogenous bone graft has been considered best because of its osteoinductive and osteoconductive properties and immunogenic compatibility (11). Small autogenous bone grafts can be harvested from intra-oral sites such as lateral mandible and chin (12). However, finding sufficient volumes of autogenous bone to harvest within the oral cavity may sometimes be difficult. In some situations, larger volumes of bone are required making bone grafting from extra-oral sites unavoidable. Autogenous bone can be harvested from the iliac crest but is associated with higher morbidity such as gait disturbances, pain and numbness (13). Some morbidity has been reported when taking large intra-oral autogenous bone grafts from the chin region (12, 14, 15). Moreover, autogenous bone grafts are prone to bone resorption, which sometimes can be substantial (11, 16). For this reason, xenogenic bone replacement materials are today sometimes used as osteoconductive scaffolds such as hydroxyapatite, calcium triphosphate and deproteinized bovine bone either replacing the bone graft or used in combination with smaller volumes of intra-orally harvested autogenous bone (16, 17). Although osteoconductive, there is no osteoinductivity in these materials. For this reason, osteoinductive growth factors like bone morphogenic protein (BMP), mainly BMP-2 and BMP-7, have been in use to further promote bone healing (18, 19). However, the ideal carrier for BMP has not yet been found (20).

Human dentin possesses osteoinductive properties possibly related to its content of bone morphogenic protein (BMP) (21–25) and is therefore a carrier of BMP. However, we do not know whether dentin possesses the properties to form bone in spaces where there is no bone initially such as in the marrow space of rabbit tibia, which could be of clinical value for bone augmentation prior to implant placement, or whether the bone formation is mainly related only to the osteoconductive properties seen when dentin is

close to bone. Our hypothesis was that dentin implanted centrally in the bone marrow will form less new bone than dentin implanted close to the native cortical bone.

Hence, the aim of this study was to further investigate whether transplanted xenogenic dentin chips inserted into the marrow space of rabbit tibia, a space where there is no solid bone initially, would contribute to bone formation and to study the pattern of such bone formation.

Materials and methods

Animals and anesthesia

Ten 6-month-old New Zealand male white rabbits were used in the experiments. The experiments were carried out at the Animal Research Centre, Health Sciences Centre, Kuwait University. Thirty minutes prior to the experimental surgery, the rabbits were sedated with xylazine HCl (Rompun, Bayer, Leverkusen, Germany) 5 mg kg^{-1} by intramuscular injection. Animals were anesthetized by intravenous injection of 35 mg kg^{-1} of ketamine HCl (Tekan, Hikma, Amman, Jordan). The animals were kept in separate cages and fed pellets and water *ad libitum* throughout the duration of the study. The protocol for animal experimentation by the Animal Research Centre of the Health Sciences Center, Kuwait, was strictly adhered. Moreover, to assure a high standard, a veterinarian was administering sedation, anesthesia, and caretaking of the animals following an already used methodology (9, 10).

Surgical procedure

Preparation of dentin chips for grafting

Human teeth, extracted for orthodontic reasons and then stored dry, were prepared by first removing the crowns by cutting horizontally. Then, the roots were sectioned vertically, so the pulp and root canal were exposed. The periodontal ligament and pulp were removed mechanically, and dentin chips 2–3 mm in diameter were prepared by crushing the remaining dentin in a mortar and passing it through a 2-mm grid to maximize the size of the chips. The chips were cleaned from smaller dentin particles by soaking them in 1% chlorhexidine for 5 min and then stored dry. The dentin chips were later inserted in the recipient tibia marrow space.

Preparation of recipient sites in tibia

The surgical areas were shaved and washed with iodine 7.5% solution, and the animals were prepared for surgery. As a supplement to general anesthesia and for vasoconstriction purposes, local anesthesia 1 ml lidocaine hydrochloride 1% with epinephrine 5 μg ml^{-1} (Xylocain-adrenalin, Astra Zeneca, Södertälje, Sweden) was administered in each experimental area. Incisions were made through the skin over the superior anterior tibia bilaterally, and the bone was exposed (9, 10). Bilateral tibias of ten rabbits were used in the experiment. In each tibia, a cortical bone defect was made, in which cortical bone was removed in a standardized way using a round bur and hand piece rotating 2000 rpm (9, 10). The defects were made in standardized sizes (6 mm diameter) engaging the full thickness of cortical bone until the marrow space was reached (9, 10). The bone was continuously irrigated with sterile saline during cutting to reduce thermal damage. Dentin chips were inserted through the defect into the bone marrow space until the defect was completely filled with chips.

The incisions were closed in two layers by resorbable sutures Vicryl 4-0. Antibiotics were applied locally in the wound when suturing. Moreover, antibiotics were administered intramuscularly once daily during the first three days after surgery. The rabbits recovered in a cage with one animal per cage. The rabbits were under frequent surveillance during the postoperative period. The animals tolerated the experiments well and all survived the healing period except for one rabbit which did not survive for reasons not related to the experiment. The rabbits were sacrificed after 6 months by an overdose of ketamine. The tibia bones were dissected free from soft tissue, radiographs were taken, and histological procedures were carried out.

Radiographic procedure

Radiographs occlusal films (Kodak Insight; Eastman Kodak, Rochester, NY, USA) were taken of each tibia and were analyzed using a light viewer to localize the site in the tibia where dentin had been placed.

Histological procedure

Following surgical dissection, the samples were immersed and fixed for 48 h in 10% neutral buffered formalin. The samples were then decalcified in neutral EDTA for 3 months, dehydrated in alcohol and embedded in paraffin under vacuum using standard histological methods. Serial sections were cut at 5 μm thickness and were mounted on polylysine-coated slides and then stained with hematoxylin and eosin and examined using light microscopy. The best sections, comprising both cortex and marrow areas, were selected and evaluated. Sections were evaluated with regard to tissue morphology, signs of inflammation, and formation of new bone.

Quantification of bone formation

Dentin fragments were evaluated with regard to bone formation, and the bone–dentin contact area was evaluated for each dentin fragment. The circumference area where bone was in contact with dentin was measured using a software program (Leica application suite v3.1, Leica Microsystems, Switzerland).The total circumference was then measured in micrometer around each dentin fragment. Bone formation for each dentin graft was expressed as a quotient between total bone contact area and total circumference area and expressed as a percentage. Using the same software application, the distance from each dentin fragment to the most adjacent cortical bone wall was measured.

Statistical analysis

The analysis was performed using SPSS 17.0 (SPSS, Chicago, IL, USA) for data analysis and graphical presentation. The variables, distance to closest cortical bone and percent bone–dentin fusion, were examined for normality of data with Kolmogorov–Smirnov test, and descriptive statistics presented as mean ± standard error (SE) or median with interquartile (IQ) range. The percent bone–dentin was categorized into three groups: 0, 1–50 and >50% to find any significant difference in the mean distance for respective amount of bone formation, applying Kruskal–Wallis test. The relationship between the degree of bone formation and the distance to most adjacent cortical bone wall was ascertained with nonparametric Spearman's correlation (rho). The two-tailed probability value $P < 0.05$ was considered statistically significant.

Results

Radiographs taken immediately after sacrifice verified the position of the grafted area and that in all tibias, the dentin grafts had stayed in place and were filling out the marrow spaces all the way through the bone marrow from one cortical bone wall to the to the opposite cortical bone wall at the site of grafting (Fig. 1).

Histological findings

Dentin grafts were visible both in the center of the tibial marrow space as well as toward the periphery, close to the cortical bone (Figs 2 and 3). The tissue morphology was intact to clearly visualize the different cellular components, dentin grafts, and cortical bone. The inflammatory infiltrate was very minimal in the sections examined. In the areas where dentin grafts were visible in the center of the tibial marrow space, there was a lack of bone formation around the dentin grafts (Fig. 2). However, fragments of calcified material consistent with bone were seen adjacent to dentin in certain areas with evidence of osteoclastic activity. The inflammatory component was very minimal in these areas. In areas where the dentin grafts were in close proximity to the cortical bone, new viable bone appeared to encircle the whole dentin chip (Fig. 3). The newly formed bone showed both osteoblastic and osteoclastic activity.

Quantification of bone formation

Forty-four dentin grafts in 16 tibia sections showed sufficient enough quality to be evaluated with regard to bone formation. Bone formation was ranging from 0 to 86% on the dentin fragments. Average bone

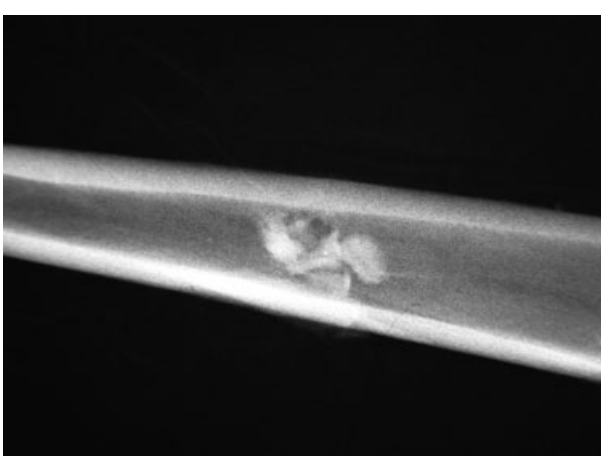

Fig. 1. Radiograph of tibia showing the experimental area 6 months after grafting of dentin to the marrow space of tibia. Dentin grafts can be seen in the marrow space between the cortical walls of tibia.

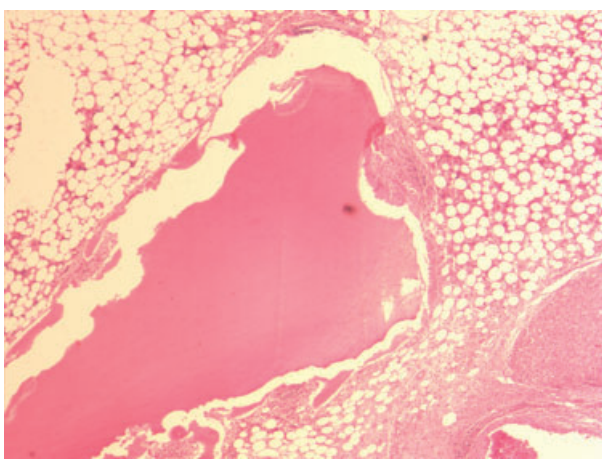

Fig. 2. Histological picture of a dentin graft centrally located in the marrow space 6 months after grafting of dentin to the marrow space. Resorption of the dentin graft can be seen. Minor or no inflammatory reaction is seen around the dentin. No new bone formation can be detected.

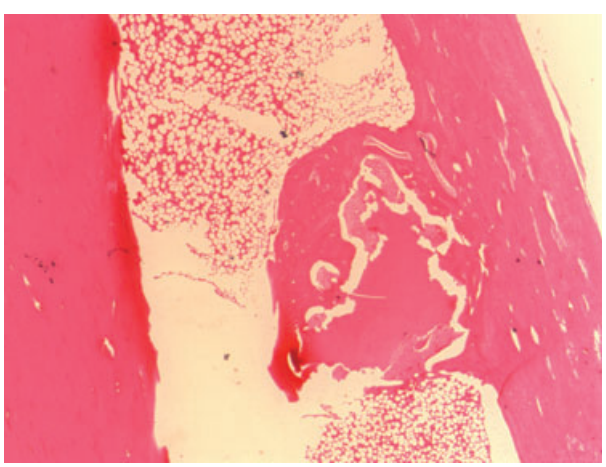

Fig. 3. Dentin graft located peripherally in the tibia marrow space 6 months after grafting. The dentin has been partially resorbed and replaced by new bone formation, which is seen surrounding the dentin graft and the new bone is in contact with the cortical wall of the tibia.

formation was 22.5% ± 4.36 with a median value of 5.5% (IQ 0–42%). Bone formation was seen to a larger extent on dentin grafts located close to the tibia bone wall. Dentin grafts located centrally showed less bone formation or no bone formation at all. The distance from the dentin grafts to the nearest cortical bone wall ranged from 49 μM to 2940 μM (M = 1145, SE121) and median as 1056 (IQ 387–1849). The mean distance was maximum 1630 μM for grafts with no bone formation, while the mean distance was 614 μM when the bone formation was more than 50% ($P < 0.001$) (Table 1). In some of these dentin grafts, bone had been formed around the dentin graft (Fig. 3). Spearman's correlation also revealed a significant negative correlation ($r = -0.579$, $P < 0.001$) between the amount of bone formation on the dentin graft and distance to the tibia cortical wall (Fig. 4).

Discussion

The results of the present study showed that xenogenic dentin possesses properties to form new bone when grafted into the marrow space of tibia, a space where there is no solid bone. The formation of new bone was correlated to the distance of adjacent cortical bone wall of tibia. More bone formation was seen when the dentin graft was located close to the cortical wall of tibia.

Table 1. Bone contact around dentin chips (expressed in %) related to distance (μM) to the nearest native cortical bone wall (expressed in mean, SE, and range)

Bone–dentin (%)	*N*	Mean ± SE	Range
0	20	1630 ± 103	919–2594
1–50	14	833 ± 239*	49–2940
>50	10	614 ± 216*	78–2356
Total	44	1145 ± 121	49–2940

*0 vs (1–50%) and (>50%), $P < 0.001$.

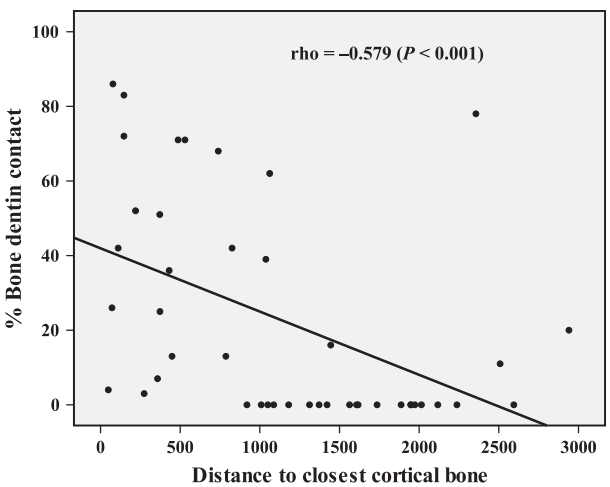

Fig. 4. Relation between the degree of bone formation and the distance to most adjacent cortical bone wall using nonparametric Spearman's correlation (rho), $r = -0.579$, $P < 0.001$.

These findings may have a potential for dentin used as a bone augmentation material, for example, prior to implant placement in bone-deficient areas.

The experimental procedure was designed to study whether dentin possesses properties to form new bone when placed in a place where there is no bone initially. The tibia is a long bone consisting of a substantial marrow space where there is no bone except for the smooth native cortical walls of the tibia bone. There is an advantage in choosing this experimental model because it is easy to evaluate whether there is any bone formation inside the well-defined tibia cortex walls. Furthermore, by placing dentin grafts centrally in the marrow space, we can study properties of osteoinductivity of the dentin, whereas dentin fragments placed close to the cortex may also be subjected to osteoconductivity. Moreover, the dentin grafts are not subjected to any influence of muscular activity as if they would have been placed as onlay grafts outside the bone. Mobility due to muscular activity followed by fibrous encapsulation has been discussed in earlier studies suggested to be a factor for fibrous encapsulation when dentin was implanted in the mandibular ramus (9). This may also be the reason for fibrous encapsulation of dentin grafts reported in some experiments where particulated onlay grafts on the crest underneath the periosteum in goats and dogs (26, 27). In the present study, the dentin grafts were implanted inside the tibia, hence enabling the study of bone formation on the dentin grafts protected from mobility due to muscular activity.

Radiographs were taken immediately after sacrifice with the purpose to determine the position of where the dentin chips had earlier been implanted, hence facilitating finding the correct region for the subsequent histological sectioning. However, the radiographs also gave us information that all dentin chips were seen together in the same area, and hence, no migration had taken place. Although implanted in loose marrow tissue, this tissue was apparently sufficient to keep the chips located in the same region during the experimental period.

In this study, dentin was implanted without any prior demineralization and new bone formation was found around the grafted dentin. This has earlier been reported when dentin has been implanted close to a muscle (28, 29). In those studies, both non-mineralized and mineralized dentin were used (28, 29). One of the studies reported that bone formation was seen 3 months later in non-demineralized dentin compared with demineralized dentin. Hence, it seems that a long time is required before new bone is formed on non-demineralized dentin grafts. In a recent study in rats, it was reported that no new bone was seen after one month in non-demineralized dentin as compared to demineralized dentin where new bone formation was seen after one month (30). In our study, the experimental time was extended to 6 months, and bone formation was seen covering many of the dentin grafts.

In the present model, the new bone formation in the defect itself may also have an influence on the activity of the bone formation. This corresponds well to the

clinical situation when bone or bone replacement material is used. Such materials are in clinical praxis placed adjacent to a surgical site. Furthermore, surgeons often penetrate the native cortical recipient site bone surface by making small holes in the bone in several spots to create bleeding into the grafted area to promote bone healing (31). Nevertheless, more bone was formed on dentin chips and also on the cortical native bone opposite the surgical site. This finding suggests that close relation to native bone is a more important factor than being located close to the surgical defect site.

The grafts did not induce inflammatory response suggesting that immunogenicity of dentin is not an important factor. This was probably due to the fact that all soft tissues were removed from the grafted teeth before dentin particles were cut. The resorption appears to be more of a bone remodeling type similar to the replacement resorption that dentin is subjected to when replanted teeth are ankylosed (1–5). This might be advantageous because in such remodeling process, dentin will be resorbed and gradually replaced by bone, hence acting as a bone replacement material. Whether replacement resorption of dentin is a solely osteoconductive or also an osteoinductive process related to the content of BMP in dentin has not been shown.

Some of the dentin chips showed more bone formation than others, and during the evaluation, we could observe a pattern in that dentin grafts located centrally in the tibia showed less new bone formation than dentin grafts located closer to the cortical bone and we decided to measure not only bone formation but also the distance to the nearest cortical wall of tibia. We found a significant negative correlation between bone formation and distance to the cortical bone wall of tibia. However, one must be aware of that in this study, we based our analysis of single sections and we cannot say anything about the area outside the section. However, in spite of this, significantly more bone was seen when chips were found more closely located to native bone in the sections examined. One reason for this may be that we are dealing mainly with osteoconductivity rather than osteoinductivity, namely that adjacent bone with osteoblasts is a prerequisite for bone formation so cells covering the surface of the adjacent bone more easily can reach dentin fragments located closely. However, if osteoinductivity of the dentin graft would have been a major factor, we would probably have seen more bone formed also around centrally placed dentin graft, which was not the case. There is a need for further studies aiming at assessing the role of osteoinductivity of dentin when implanted in tissue where there is no adjacent bone.

The dentin grafts located close to the cortical wall were sometimes completely surrounded by new bone (Fig. 3). New bone was formed while the dentin was resorbed. This is probably a process similar to replacement resorption also seen after ankylosis of replanted teeth, which is considered a bone remodeling process (1–5). It seems as if we possibly can take advantage of the replacement resorption of the dentin also when augmenting bone.

Conclusion

Dentin promotes new bone formation especially when located close to native cortical bone and may have a potential as a bone augmentation material.

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