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Dentin xenografts to experimental bone defects in rabbit tibia are ankylosed and undergo osseous replacement

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Abstract – *Purpose*: After trauma and losses of teeth, bone augmentation with bone grafts or bone replacement material is sometimes required before implant treatment. The ideal bone replacement material has not yet been characterized. Dentin is known to undergo ankylosis and replacement resorption after replantation of teeth. Dentin has also been shown to contain bone morphogenic protein. These properties may possibly be used making dentin an alternative or supplement to bone grafting to defect areas prior to treatment with osseointegrated implants. The aim of this study was to investigate if dentin is ankylosed and replaced by newly formed bone when transplanted to bone defects. *Materials and methods*: Ten New Zealand rabbits were used for the experiment. The rabbits were subjected to surgical exposure and preparation of bone cavities in the tibia bilaterally. Dentin blocks from human premolars extracted for orthodontic reasons were used as grafts. Dentin blocks were inserted in the cavities penetrating into the marrow space in 16 tibias. Four tibias were prepared with the same cavities, but without being subjected to dentin grafting and served as controls. Five rabbits were sacrificed after 3 months and five rabbits after 6 months. Histological processing and evaluation were performed and tissue conditions evaluated. The area of ankylosis was estimated. *Results*: All dentin blocks healed with ankylosis in contact with bone without inflammatory reactions. In the cortical regions of the tibia, fusion of bone with dentin was seen in 86% of the dentin surface after 3 months and 98% after 6 months. On the dentin blocks inserted into the marrow space, bone was formed on the dentin block on average covering 51% of the dentin after 3 months and covering 77% after 6 months. Resorption of the dentin was seen to a larger extent after 6 months with osseous replacement in the resorption cavities. *Conclusion*: Dentin xenografts have a potential to be incorporated in bone without inflammation and gradually resorbed and replaced by new bone.

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Treatment with osseointegrated titanium implants for lost or missing teeth has been extensively documented and is today a well recognized treatment method performed worldwide after tooth losses (1–7). In some patients, it is impossible to install implants due to bone deficiency caused by bone resorption or by the trauma itself. For this reason, bone augmentation with bone grafting has been used to augment the bone. Small bone grafts can be harvested from intraoral sites such as lateral mandible and chin (8). 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, usually harvested from the iliac crest. However, iliac crest grafting was associated with higher morbidity such as gait disturbances, pain and numbness (9). Similar morbidity was reported when taking large intra oral bone grafts from the chin region (8, 10, 11). For this reason, xenogenic bone replacement materials are today in use for either replacing the bone graft or used in combination with smaller amounts of intraorally harvested autogenous bone (12–15).

From clinical and experimental studies, we know that a replanted tooth without a viable periodontal membrane was ankylosed (16–21). The dentin was fused with the bone (16, 17). Such a replanted tooth was gradually replaced by bone by osseous replacement, also called replacement resorption (16–21). Human dentin possesses osteoconductive and osteoinductive properties probably related to its content of bone morphogenic protein (BMP) (22–26).

In a recent experimental study, it was shown that the rabbit tibia was a model well suitable for implantation studies of dentin (27). In that study, it was reported that bone was fused to human dentin implanted in cortex wall of rabbit tibia after 3 months.

The aim of this study was to investigate if transplanted human dentin fused to bone and was replaced by new bone over a longer, 6-month period.

Materials and methods

Animals and anaesthesia

Ten 3–4 month-old New Zealand white rabbits were used in the experiments. The rabbits were bred in the Animal Research Laboratory, Health Sciences Center, Kuwait University. The rabbits were sedated with Xylazine HCl (Rompun, Bayer, Leverkusen, Germany) 5 mg kg^{-1} by intramusvular injection and antibiotics Pen-Hista-Strep (Vetoquinol SA, Lure Cedex, France) 50 mg kg^{-1} by intramuscular injection were given 30 min before surgery. The rabbits were anaesthetized by Ketamine (Tekan; Hikma, Amman, Jordan) HCl 35 mg kg^{-1} by intravenous injection. A veterinarian was responsible for administering the sedation and anaesthesia. All experiments were carried out according to the animal experiment protocol of the Animal Research laboratory to assure a high ethical standard. The methodology has been described in an earlier study (27).

Surgical procedure

The surgical area was shaved and washed with chlorhexidine 0.2% solution and the animal, surgeons and assistants were prepared for surgery under aseptic conditions. The instruments were autoclaved before surgery and surgeons were wearing sterile gloves and masks. As a supplement to the general anaesthesia and for vasoconstriction purposes, local anaesthesia 1 ml lidocainehydrochloride 1% + epinephrine 5 μg ml^{-1} (Xylocain-Adrenalin; Astra Zeneca, Södertälje, Sweden) was administered over each experimental area. Incision was made through the skin over the superior anterior tibia and the bone was exposed. Bilateral tibia of ten rabbits was used in the experiment. In each tibia, a defect was created in cortex on approximately equal distance from the joints using a round bur. The defects were made in standardized sizes (6 mm diameter) engaging the full thickness of cortical bone until spongious bone was reached. During bone cutting, the bone was continuously irrigated with sterile saline to reduce thermal damage.

Human teeth, extracted for orthodontic reasons, were prepared by removal of enamel, periodontal ligament and pulp from the teeth and each tooth was sectioned and standardized dentin blocks were prepared. The block sizes were 6 mm in diameter with a thickness of 3 mm. The blocks were stored dry before animal experiments were carried out, but were placed in 1% chlorhexidine for 10 min prior to insertion in the prepared bone defects. The dentin blocks were inserted in the cavities penetrating into the marrow space in sixteen tibias (Fig. 1). Four tibias were prepared with the same cavities, but without being subjected to dentin grafting and served as controls.

The incisions were closed in two layers by sutures Vicryl 4-0. To compensate for perioperative and postoperative dehydration 10 ml sterile saline solution was injected subcutaneously immediately following surgery according to Alberius et al. (28). Antibiotic administration was continued during the first 3 days after surgery. The rabbits recovered in a cage with one animal per cage.

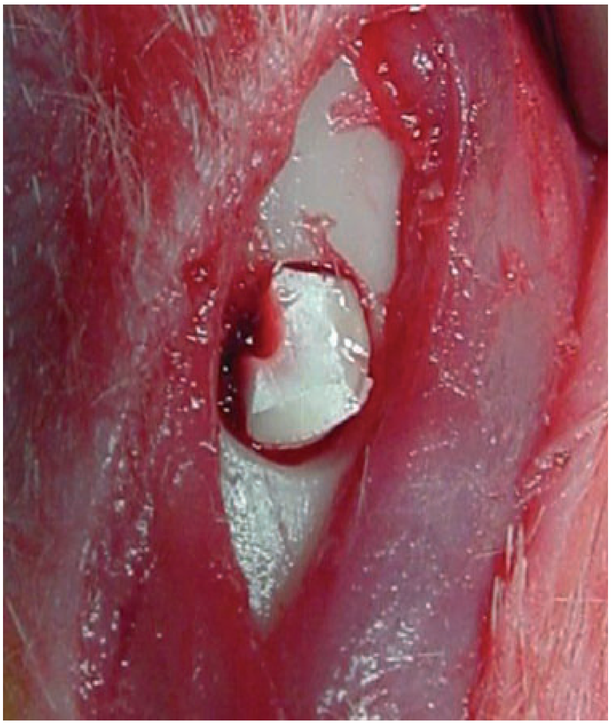

Fig. 1. Dentin block placed into a surgically created bone defect.

The rabbits were under frequent surveillance during the postoperative period. Five rabbits were sacrificed after 12 weeks and five rabbits were sacrificed after 24 weeks by an overdose of Ketamine. The tibia bones were dissected free from soft tissue and radiographs were taken before histological procedures were carried out.

Radiographic procedure

Radiographs (Kodak Insight; Eastman Kodak, Rochester, NY, USA) were taken of each tibia and were analysed using a light viewer.

Histological procedure and evaluation

Following surgical removal, the tibias were immersed and fixed for 48 h in 10% neutral buffered formalin. The tibias were then calcified with Rapid Decalcifier (Apex Engineering Corporation, Aurora, IL, USA) for 2 days with two changes, dehydrated in alcohol and embedded in paraffin under vacuum using standard histological methods. Serial sections were cut at 5, 100 μm apart. The sections were mounted on polylysine-coated slides and were stained with haematoxylin and eosin, and examined using light microscopy. Sections including both the dentin block and the surrounding bone were selected and evaluated for tissue morphology, signs of inflammation, ankylosis and replacement resorption. The total circumference of dentin in a section was measured and the part of this total circumference where bone was fused with dentin was expressed as a percentage of the total circumference.

Statistics

Descriptive statistics was used to present the data. Wilcoxon signed ranks test was used for comparisons between the 3 and 6 months groups and between surfaces facing marrow and surfaces facing cortex. A *P*-value < 0.05 was chosen as level of significance.

Results

All animals survived the postoperative months apparently in good health. After sacrifice and exposure of the tibias, clinical inspection revealed that all dentin blocks were fused in the dentin wall. The dentin blocks had been fused in the dentin wall. A reduction in size of dentin could be seen (Fig. 2).

Radiographic evaluation

Radiographic examination showed dentin grafts fused to the bone in all experimental areas.

Histological evaluation

All dentin blocks healed with ankylosis between dentin and bone. No inflammatory reactions were seen. On the surface of the dentin blocks facing cortical bone, fusion of bone with dentin was seen in 86% of the dentin surface after 3 months and 98% of the dentin surface after 6 months.

On the surface of the dentin blocks facing the marrow space, bone was formed on the dentin surface on average covering 51% of the dentin surface after 3 months and covering 77% of the dentin surface after 6 months (Table 1).

There was significantly (0.005) more ankylosis at six compared with 3 months, both on surfaces facing the marrow (0.005) and on surfaces facing cortex (0.005).

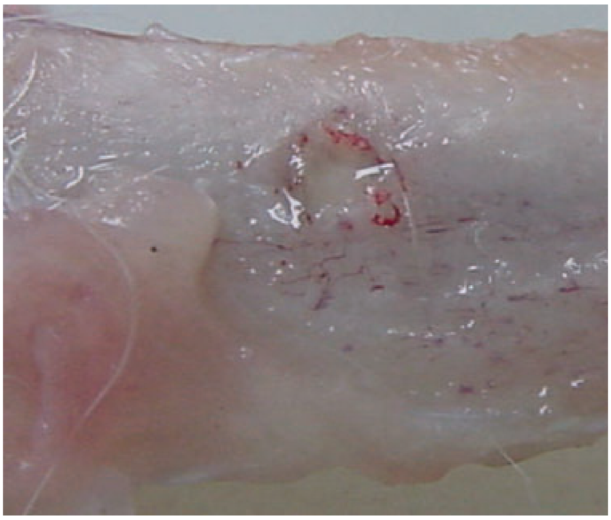

Fig. 2. Dentin block grafted to rabbit tibia. The dentin block is fused to the tibial bone. Status 6 months after grafting. Peripheral resorption with replacement of bone has resulted in a reduced size of the dentin block.

Table 1. Ankylosed proportions (%) of dentin surfaces Mean (SD)

Dentin block surface	3 months	6 months
Surface facing cortex	86.2 (7.1)	97.5 (1.1)
Surface facing marrow	50.8 (12.4)	77.3 (11.2)

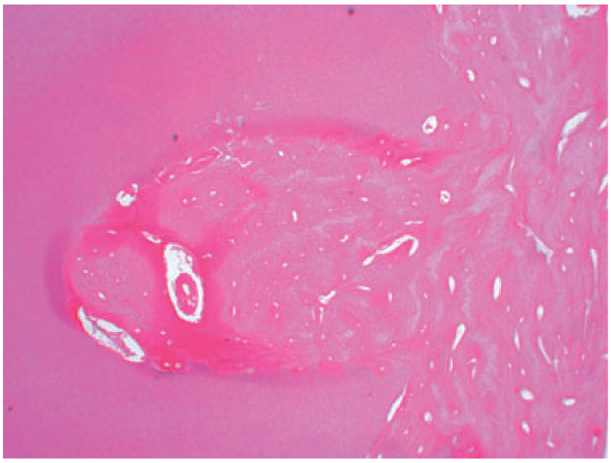

Fig. 3. Histological section 6 months after dentin grafting. Fusion of the dentin and bone (ankylosis) is seen. A deep resorption in the dentin has been filled with new bone.

There was significantly more bone formed on surfaces facing cortex (0.005) than surfaces facing marrow (0.005) in both 3 and 6 month groups.

Deep resorption cavities in the dentin, where the dentin had been replaced by newly formed bone could be seen in the 6-month group (Fig. 3). Newly formed bone was seen on the marrow side of the dentin.

In the control cavities, partial bone healing of the defect was seen with a somewhat thinner cortex in the defect areas as compared with the adjacent cortex.

Discussion

In this study, it was shown that xenografts of dentin are ankylosed to the bone and subjected to resorption with osseous replacement over a 6-month period. Significantly more ankylosis area was seen at 6 months compared with 3 months indicating that more time is required to form bone on the dentin surface. Replacement of the dentin by bone also took place with increased time.

Xenografts, human dentin to rabbit, were used in the present experiments. There was no inflammation around any dentin indicating that immunogenic response of the dentin is not a major factor. The immunogenic properties of a tooth are related to the soft tissue, the periodontal ligament and pulp, which were both removed before the dentin was used for grafting in the present experiments. If no immunogenic reactions were seen from xenografts in the present study, it also opens up a potential for successful auto and allografting of dentin. Dentin can be harvested from sources like extracted third molars or premolars extracted teeth for orthodontic reasons.

The choice of experimental time was based on other experimental animal studies on osseous replacement resorption (16–19). In an earlier study with a similar experimental model, it was shown that 3 months is enough to demonstrate fusion between dentin and bone (27). In the present study, we extended the healing time to 6 months to study if the fusion and resorption were progressive which could be verified in this study. This is in accordance with findings from studies on ankylosis after experimental replantation of teeth (16–19). If ankylosis over a large area is seen, the teeth will be gradually resorbed and replaced by bone over time (19, 21). This is considered a remodelling process and not related to infection or inflammation (19–21).

The findings of the present study may be of clinical relevance as dentin can be used as a bone replacement material in bone deficient areas prior to implant treatment and for bone reconstruction. Dentin has bone inducing properties and is fused and gradually replaced by bone when grafted to bone. Most likely this is because dentin possesses osteoinductive properties, possibly by its BMP content. There is currently a high interest in BMP for clinical applications, but the problem has been finding a carrier for the BMP, which is not releasing the BMP too rapidly and the carrier should not itself cause any inflammatory reactions (29, 30). The role of dentin as a BMP carrier for slow release of BMP and osteoinductivity warrants further investigations. The fusion of bone may also be due to osteoconductive properties of dentin, and the role of this should be further evaluated in future experimental research before we can draw conclusions on dentin as a possible bone replacement material in clinical applications. Moreover, the rate of replacement resorption needs to be elucidated as well as studies on later implant installation in an area which has been built up by dentin. Such studies are now in progress in our research centre.

Conclusion

Dentin grafted to rabbit tibia is ankylosed in the cortical bone and gradually resorbed with replacement by new bone. These findings may have a potential for reconstruction of bone defects after trauma or bone resorption.

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