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Studies on dentin grafts to bone defects in rabbit tibia and mandible; development of an experimental model

Lars Andersson[1], Abbas Ramzi[2], Bobby Joseph[3]

[1]Oral & Maxillofacial Surgery, Department of Surgical Sciences, Faculty of Dentistry, Health Sciences Center, Kuwait University; [2]Oral & Maxillofacial Surgery, Bneid Al Gar Specialist Center, Ministry of Health; [3]Oral Pathology, Department of Diagnostic Sciences, Faculty of Dentistry, Health Sciences Center, Kuwait University, Kuwait

Correspondence to: Lars Andersson, Oral & Maxillofacial Surgery, Department of Surgical Sciences, Faculty of Dentistry, Health Sciences Center, Kuwait University, P.O. Box 24923, Safat 13110, Kuwait
Tel.: +965 498 6695
Fax: +965 532 6049
e-mail: lars.andersson@hsc.edu.kw

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Abstract – *Background and Aim:* Dentin contains bone morphogenic protein which is important in bone induction and dentin can act as a slow releasing carrier. This property may possibly be used as an alternative or supplement to bone grafting to defective areas after trauma prior to treatment with osseointegrated implants. Hence, the objective of this study was to investigate if dentin can be used as a graft in bone defects in an experimental rabbit model. *Materials and Methods:* Eight New Zealand White Rabbits were used to prepare bone cavities either in the angle of the mandible or tibia. Six of the eight tibial and six of the eight mandibular bone defects were filled with dentin blocks from human premolars which were extracted for orthodontic treatment. Two mandibular and two tibial bone cavities were used as controls and all the rabbits were sacrificed after 3 months. Radiographic and histological examinations were performed. *Results:* There was a difference in healing pattern between the mandibular and tibial defects. In the mandible, the dentin blocks were resorbed to a larger extent and more often surrounded by fibrous tissue, probably due to the fact that the dentin blocks were mobile because of the thin mandibles and muscular activity in that area. Only some dentin blocks were ankylosed with the mandibular bone. In the tibia however, all dentin blocks were fused to bone over a large area. Osseous replacement resorption was seen. In control cavities, bone formation was seen but was never complete. No signs of inflammatory changes were seen in any fused grafts. *Conclusions:* Dentin grafts have a potential to be incorporated in bone without inflammation and can be used as bone inducer and later replaced by bone. Thus, rabbit tibia served as a better model for further studies of this phenomenon when compared to the mandible.

In patients subjected to dental trauma, bone may be lost due to direct trauma or indirectly after alveolar bone resorption following tooth avulsion. Bone deficiency can be a major drawback when reconstruction with titanium implants is planned (1). For this reason, different ways to augment the bone prior to implant treatment have been tried. The best way for bone augmentation is to use bone graft harvested from one area to another in the same patient; autogenous bone graft, and this is considered the golden standard because autogenous bone possesses both osteoconductive and osteoinductive properties, which will give a more predictable outcome (2). Finding sufficient quantities of autogenous bone to harvest from the oral cavity may sometimes be difficult and when larger quantities are required bone must be grafted from extra oral regions, usually the iliac crest. Iliac crest grafting may be associated with high-morbidity such as gait disturbances, pain and numbness even after a long time after surgery (2). Furthermore, general anesthesia and admission at hospital is required especially with bone grafting from extraoral sites. For these reasons, today in many clinics priority is given to bone grafts taken from intraoral donor sites such as chin, lateral mandible and infrazygoma areas, however, the quantities are limited. Numbness and pain has been reported at the site of harvest when taking large bone grafts from the chin region (3–5). Bone grafts from the body of the lateral mandible give less postoperative morbidity, however, there are limited bone quantity in the lateral mandibular areas (5). Hence, currently different materials which can substitute bone are developed for replacing the bone graft or combining intra-orally harvested autogenous bone with a bone substitute material to increase the volume of graft material.

Bovine xenogenic bone has been used as graft material combined with autogenous bone (6–8). However, there is a minor risk for transmitting infectious diseases from animals to humans. Ideally, it is therefore desirable to use bone substitute materials where there is no risk for transmitting diseases from animals to human.

Human dentin has both osteoconductive and osteoinductive properties (9). From clinical and experimental studies, it is well established that a tooth without a viable periodontal membrane will become ankylosed if

replanted (10–15). Such an ankylosed tooth will be gradually replaced by osseous replacement, also called replacement resorption (10–13, 15–18). In our series of studies, we would like to study the properties of dentin as a bone replacement material. Our working hypothesis for our preliminary study was that dentin implanted in rabbit mandible or tibia will fuse with bone to form ankylosis and replacement resorption. Hence, the purpose of the present study was to develop an experimental model in rabbits with experimentally created standardized bone defects to which dentin could be grafted. In this study, the objective was to develop an efficient surgical method and standardised histological methods to evaluate the suitability of rabbit mandible and tibia as dentin grafts in bone.

Materials and methods

Animals

Eight 3-months-old New Zealand white female rabbits were obtained from the Animal Research Laboratory, Health Science Center, Kuwait University. Thirty minutes prior to the experimental surgery, the rabbits were sedated with Xylazine HCl (Rompun, Bayer, Leverkusen, Germany) 5 mg kg^{-1} body weight and Benzylpenicillin + dihydrostreptomycin and dexametasone (Pen Hista Strep, Vetoquinol SA, Lure Cedex, France) 50 mg kg^{-1} body weight was administered by intramuscular injection. Animals were anesthetized by an intramuscular injection of Ketamine HCl (Tekan, Hikma, Amman, Jordan, 35 mg kg^{-1}). In four of the rabbits the experiments were carried out in the mandible, in the other four rabbits the experiments were carried out in the tibia. All experiments were carried according to the animal experiment protocol of the Animal Research Laboratory to assure a high ethical standard.

Dentin grafts

Dentin blocks from human premolars extracted for orthodontic reasons were prepared by removing the crown, cutting the root into two halves and removing the pulp and periodontal ligament mechanically. Blocks sized 5–6 mm in diameter with a thickness of 3 mm were prepared and cleaned by being placed in 1% chorhexidine for 10 min.

Surgical procedure

The surgical area was shaved and washed with chlorhexidine 0.2% solution. All instruments used in the surgery were autoclaved before surgery. Local anaesthesia 1 ml (Xylocaine-Adrenaline 2% 1:80 000; AstraZeneca, Södertälje, Sweden) was administered in the soft tissue adjacent to the experimental areas bilaterally.

In the *mandible group*, an incision was made through the skin over the inferior border of the mandible bilaterally (Fig. 1). In the *tibia group*, an incision was made over the tibia bilaterally. In both groups bone was exposed after blunt dissection to the bone and standardised bone defects were created in the bone using a bur. (Fig. 2a,b). All defects were 6 mm in diameter penetrating through the cortical bone until cancellous bone was engaged. The area was continuously irrigated with sterile saline to reduce thermal damage to the bone. Six defects in each group were

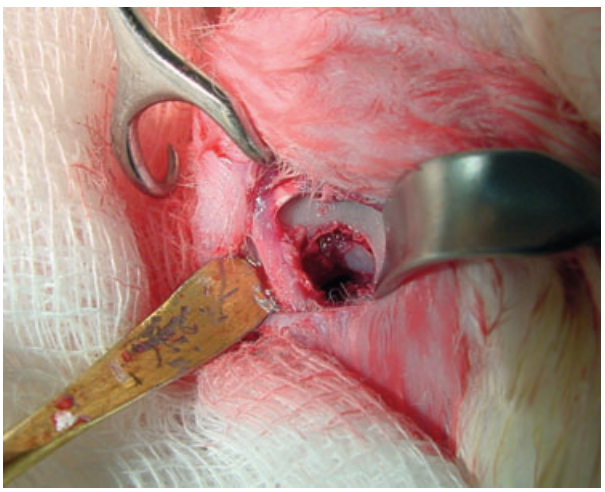

Fig. 1. Surgical exposure and preparation of bone cavity in the inferior part of mandibular ramus.

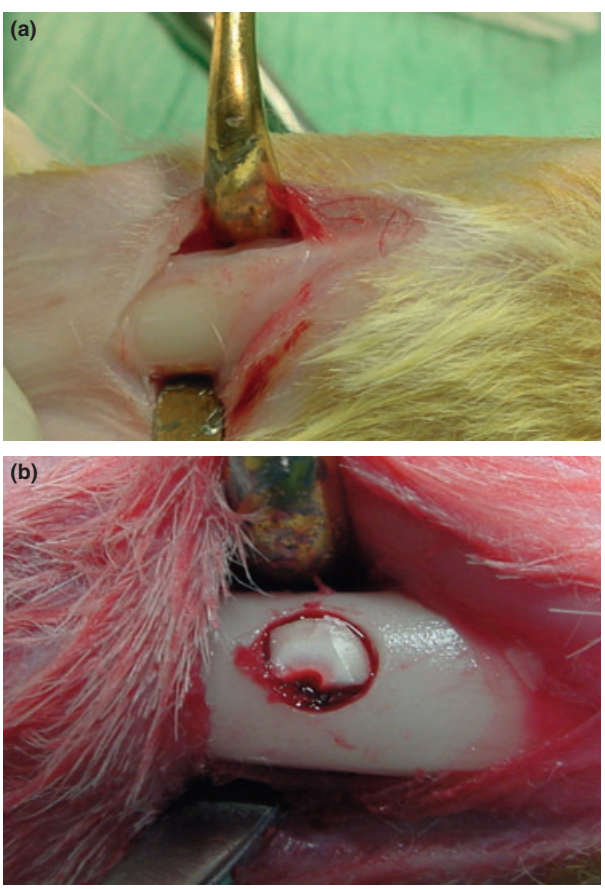

Fig. 2. Surgical exposure and preparation of bone cavity in the tibia. (a) Surgical preparation of defect in tibia, (b) Defect filled with a block of dentin.

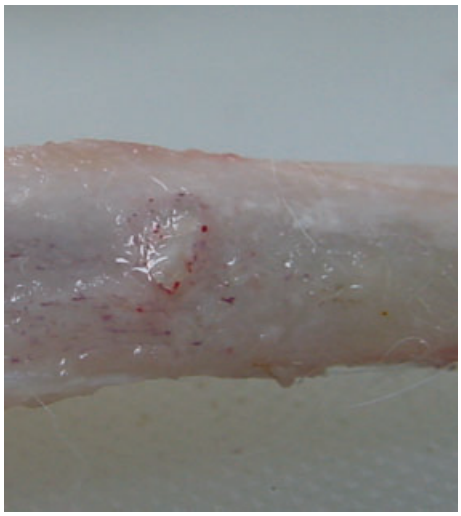

Fig. 3. Rabbit tibia 12 weeks after implantation of dentin block. The dentin block is fused to the bone.

filled with dentin block which was placed in the cavity by carefully hammering the dentin block into position. No other fixation was used (Fig. 2b). Two defects were not filled but served as controls. The incisions were closed in two layers by sutures Vicryl 4-0.

Post surgical period

To compensate for perioperative and postoperative dehydration, 10 ml sterile saline solution was injected subcutaneously immediately following surgery according to Alberius et al. (19). Antibiotic administration was continued during the first 3 days after surgery. The animals tolerated the experiments well with full recovery and each animal was put in one cage. The rabbits were under frequent surveillance during the healing period. After 12 weeks, the animals were sacrificed by an overdose of Ketamine. The bone was dissected free from soft tissue (Fig. 3) and radiographs were taken before histologic procedures were carried out (Figs 4 and 5).

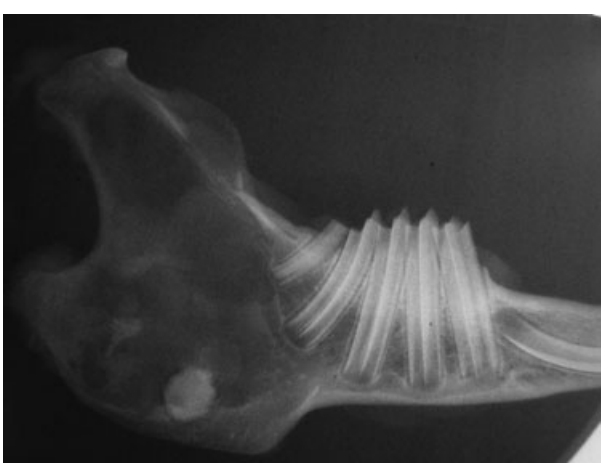

Fig. 4. Rabbit mandible 12 weeks after implantation. A partially resorbed dentin block can be seen in the radiolucent area in the inferior angular area.

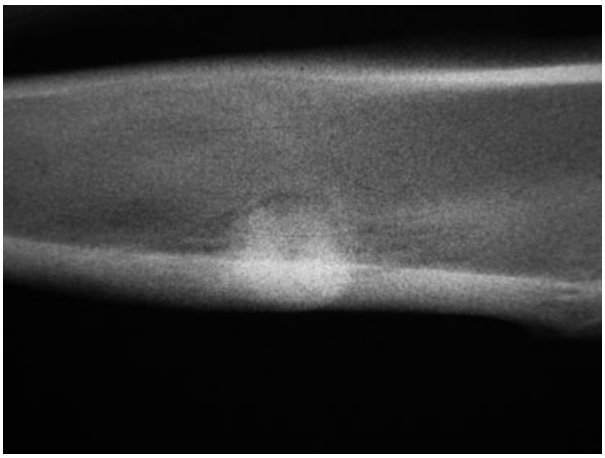

Fig. 5. Radiograph of rabbit tibia 12 weeks after implantation. A dentin block filling the experimental cavity is seen.

Radiography

Occlusal radiographs (Kodak Insight; Eastman Kodak Co, Rochester, NY, USA) were taken of each side of the mandible and tibia and analyzed using an X-ray viewer.

Tissue preparation for histology

Following surgical removal, mandibles/tibias were immersion fixed for 48 h in 10% neutral buffered formalin. The mandibles/tibias were then decalcified 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

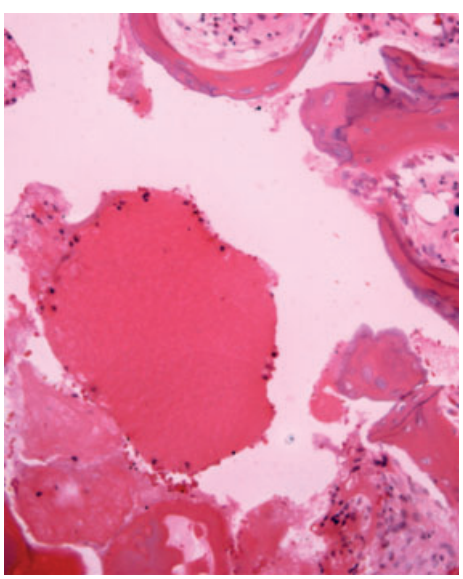

Fig. 6. Histological section (×5) through dentin and bone 3 months after implantation of dentin in experimental cavity in the mandible. Implanted dentin in the mandibular experimental cavity. Dentin has not been fused to bone. Some inflammatory cells are seen.

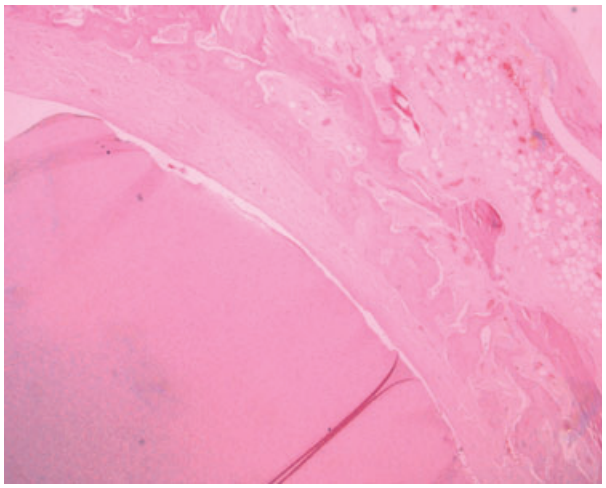

Fig. 7. Histological section (×5) through dentin and bone 3 months after implantation of dentin in experimental cavity in the mandible. Dentin has not been fused to bone but is surrounded by connective tissue. No inflammation is seen.

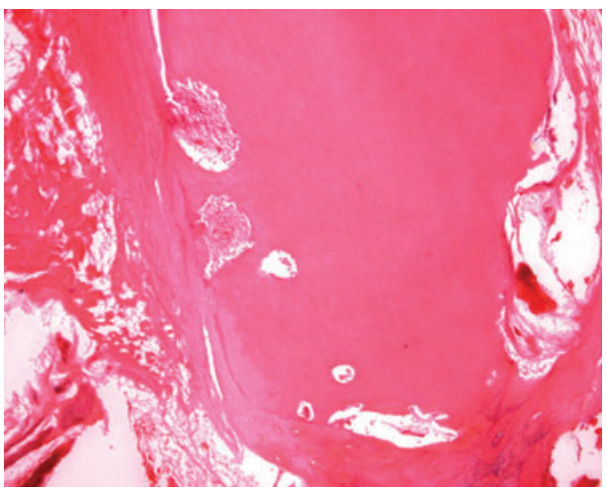

Fig. 8. Histological section (×2.5) through dentin and bone 3 months after implantation of dentin in experimental cavity in the mandible. Fusion between dentin and bone is seen (ankylosis) and dentin has been resorbed and replaced by bone (replacement resorption). No inflammation is seen.

sections were cut at a thickness of 5 μm, 100 μm apart until the mandible/tibia was completely sectioned. The sections were mounted on polylysine coated slides and were stained with hematoxylin and eosin, and examined using light microscopy. Sections were evaluated for tissue morphology, signs of inflammation, repair and histological measurements.

Results

Radiographic analysis

Radiographic examination showed dentin grafts in place in all the cavities after 3 months. Radiolucent areas in general surrounded the dentin blocks in the mandible

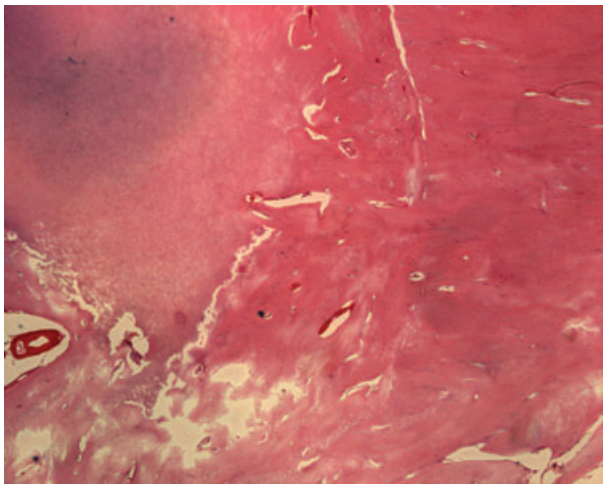

Fig. 9. Histological section (×5) through dentin and bone 3 month after implantation of dentin in experimental cavity in the tibia. Fusion between dentin and bone is seen (ankylosis) and dentin has been resorbed and replaced by bone (replacement resorption). No inflammation is seen.

(Fig. 4) while the dentin in the tibia were fused with bone (Fig. 5).

Histological analysis

Mandible

Defects filled with dentin. The thin mandibles were difficult to cut and some dentin blocks were detached in the cavities. Some dentin blocks and were surrounded by fibrous tissue (Figs 6 and 7) and few areas in direct bone to dentin fusion were seen (Fig. 8). Most of the dentin blocks were confined to the connective tissue without any bone contact (Fig. 6). There was no inflammatory infiltrates in the sections examined.

Control defects. These defects were partially filled with new bone and partially with connective tissue. There was no evidence of an inflammatory infiltrate in the sections examined.

Tibia

Defects filled with dentin. The dentin blocks were generally fused with bone over large areas (Fig. 9). Some superficial resorption of dentin with replacement of the dentin by bone was seen in the resorption cavities. There was no inflammatory infiltrate in the sections examined.

Control defects. The defects were partially filled with new bone and partially with connective tissue. There was no inflammatory infiltrate in the sections examined.

Discussion

The present study aims at developing an experimental rabbit model in which it is possible to implant dentin blocks and achieve dentin-bone ankylosis after 3 months with replacement resorption in all specimens. In the rabbit tibia, it was possible to achieve dentin-bone ankylosis in all specimens and replacement resorption was seen. In the rabbit mandible however, it was more

difficult to achieve dentin bone ankylosis in all specimens.

The difference between the results in the mandible and tibia is probably due to the thin mandibles and that the soft tissue around the mandible comprises muscles and many mobile structures in a very active area for chewing and swallowing. This may increase the risk of the dentin block being moved after some initial resorption. The frequent findings of fibrous tissue around dentin blocks lying free from mandibular bone support this theory. In contrast, the dentin blocks implanted in tibial cortex were all fused directly with bone over large areas with little or no connective tissue. In the tibial bones, the dentin blocks were not as subjected to soft tissue movements as compared to the thin mandibles. Hence we concluded that tibia is more suitable than mandible for our future studies of ankylosis and replacement resorption of dentin grafts to bone defects.

The defects of 6 mm in diameter were chosen because it was a reasonable size for efficient dentin grafting. However, it has been shown that 6 mm is not enough for a critical size defect (20), which was verified by the partial bone formation seen in these defects. However, larger defects will increase the risk for fracture of the tibia and cannot therefore be used. For this reason, the model is less suitable for making comparisons between various bone replacement materials with control cavities. However, for evaluating the fusion of dentin to bone and development and progress of replacement of dentin by bone this will be a suitable model and the control cavities in this study verified that bone also was formed in empty cavities although not filling the cavity completely.

The choice of experimental time was based on experience from other animal studies of osseous replacement resorption (10, 11, 14, 16, 17). Dento-alveolar ankylosis is a long term process over several years in humans and in animals having a higher bone turnover rate a shorter period is necessary otherwise all dentin will probably be replaced by bone. Using 3 months showed to be an adequate time in that both ankylotic fusion and initial osseous replacement resorption could be seen. To be able to study further progress longer observation periods will be necessary in future studies.

In the fused blocks, no inflammation was seen although this was actually a xenogenic transplant, human dentin to rabbit bone. This is probably due to that dentin does not seem to possess much antigenic properties. Antigenic properties are mainly related to the soft tissue of the periodontal membrane (PDM) and pulp. As these tissues were removed from the root prior to implantation the grafts did not possess much antigenic properties, in spite of the fact that dentin xenografts were used in this study.

Studies on experimental replantation of teeth has demonstrated that dento-alveolar ankylosis develops if the PDM is injured during the extra alveolar period (11, 13–16, 18, 21). The PDM possesses protectional properties against ankylosis and osseous replacement (22). When these properties are lost ankylosis will occur (10, 11, 13, 15–18, 22). If ankylosis over a large area has occurred the tooth will gradually be resorbed and replaced by bone over time, i.e. replacement resorption (10, 12). The same effect has apparently been the case in our study. The implanted dentin blocks have undergone ankylosis and replacement by bone was seen after 3 months. Dentin may be an interesting implant material in bone defects since it contains bone morphogenic proteins which can induce bone formation (9, 23, 24). When demineralised allogenic lyophilized dentin was used some osteoinduction was seen in rats (9). In a later study by the same research group, no bone formation was seen when dentin was implanted in dogs (25). This may be due to the fact of different experimental animals but may also be due to the fact that demineralised dentin used in these studies may not have long term BMP releasing properties if demineralised. In our study, the grafted dentin was not demineralised, possibly enabling a slow replacement resorption of the dentin over long time, hence the dentin acting as a slow release carrier. Formation of bone has been verified in other studies also using non-mineralised dentin where dentin was mixed with plaster of Paris to cover large bone defects in rats, dogs and humans (26–28). The dose of BMP release may be of importance since BMP-2 stimulated resorption of dentin in high doses while in lower doses hard tissue was induced in a bovine study (29).

There are limitations in our study being only descriptive, nevertheless our findings may have later clinical implications because if dentin can be used for filling bone defects we may use this material instead of using various bone replacement materials from other species e.g. bovine materials. Dentin could possibly be available from teeth in the same patient e.g. wisdom teeth and used as autogenous graft or as allograft from e.g. the high number of premolar extractions carried out in the society before orthodontic treatment and xenografts can be avoided. To investigate this further, a number of studies must first be performed such as quantifying the osseous replacement over time. Another important factor to study in future studies are the size of the dentin blocks which is of importance when grafting (28, 30) and the effects of using dentin alone as compared with dentin in combination with autogenous bone grafts.

We conclude that within the limitations of this descriptive study, it was possible to implant dentin blocks in rabbit tibia and achieve dentin-bone ankylosis after 3 months with osseous replacement resorption and with no signs of inflammation. The rabbit mandible however does not seem to be a predictable model for achieving ankylosis with dentin blocks. It will now be interesting to use the rabbit tibia model for studying dentin grafts and its property to induce ankylosis and replacement by bone over longer periods of time.

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References

1. Andersson L, Emami-Kristiansen Z, Hogstrom J. Single-tooth implant treatment in the anterior region of the maxilla for treatment of tooth loss after trauma: a retrospective clinical and interview study. Dent Traumatol 2003;19:126–31.
2. Sandor GKB, Nish IA, Carmichael RP. Comparison of conventional surgery with motorized trepine in bone harvest from the anterior iliac crest. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;95:150–5.
3. Nkene E, Schultze-Mosgau S, Radespiel-Tröger M, Kloss F, Neukam FW. Morbidity of harvesting chin grafts: a prospective study. Clin Oral Implants Res 2001;12:495–502.
4. Raghoebar GM, Louwerse C, Kalk WI, Vissink A. Morbidity of chin bone harvesting. Clin Oral Implants Res 2001;12:503–7.
5. Andersson L. Patient self-evaluation of intra oral bone grafting treatment to the maxillary region. Dent Traumatol 2008;24: 164–169.
6. Carmagnola D, Berglund T, Lindhe J. The effect of a fibrin glue on the integration of Bio-Oss with bone tissue. An experimental study in labrador dogs. J Clin Periodontol 2002;29:377–83.
7. Hallman M, Cederlund A, Lindskog S, Lundgren S, Sennerby L. A clinical histologic study of bovine hydroxyapatite in combination with autogenous bone and fibrin glue for maxillary sinus floor augmentation. Results after 6–8 months of healing. Clin Oral Implants Res 2001;2:135–43.
8. Hallman M, Sennerby L, Lundgren S. A clinical and histologic evaluation of implant integration in the posterior maxilla after sinus floor augmentation with autogenous bone or bovine hydroxyapatite, or with 20:80 mixture. Int J Oral Maxillofac Implants 2002;17:635–43.
9. Pinholt EM, Bang G, Haanaes HR. Alveolar ridge augmentation by osteoinduction in rats. Scand J Dent Res 1990;98:434–41.
10. Andreasen JO. Analysis f pathogenesis and topography of replacement root resorption (ankylosis) after replantation of mature permanent incisors in monkeys. Swed Dent J 1980; 4:231–40.
11. Andreasen JO. Effect of extra-alveolar period and storage media upon periodontal and pulpal healing after replantation of mature permanent incisors in monkeys. Int J Oral Surg 1981;10:43–53.
12. Andersson L, Bodin I, Sörensen S. Progression of root resorption following replantation of human teeth after extended extra-oral storage. Endod Dent Traumatol 1989;5:38–47.
13. Trope M. Clinical management of the avulsed tooth: present strategies and future directions. Dent Traumatol 2002;18:1–11.
14. Panzarini SR, Perri de Carvalho AC, Poi WR, Sonoda CK. Use of vitamin C in delayed tooth replantation. Braz Dent J 2005;16:17–22.
15. Andreasen JO, Andreasen FM, Andersson L (editors). Textbook and Color Atlas of Traumatic injuries of the teeth, 4th edn. UK: Oxford, Blackwell Munksgaard; 2007.
16. Andersson L, Blomlöf L, Lindskog S, Feiglin B, Hammarström L. Tooth ankylosis. Clinical, radiographic and histological assessments. Int J Oral Surg 1984;13:423–31.
17. Andersson L, Lindskog S, Blomlöf L, Hedström K-G, Hammarström L. Effect of masticatory stimulation on dentoalveolar ankylosis after experimental tooth replantation. Endod Dent Traumatol 1985;1:13–6.
18. Andersson L. Dentoalveolar ankylosis and associated root resorption in replanted teeth. Experimental and clinical studies in monkeys and man. Swed Dent J Suppl 1988;56:1–75.
19. Alberius P, Klinge B, Isaksson S. Management of craniotomy in young rabbits. Lab Anim 1989;23:70–2.
20. Aaboe M, Pinholt EM, Hjorting-Hansen E. Unicortical critical size defect of rabbit tibia is larger than 8 mm. J Craniofac Surg 1994;5:201–3.
21. Barrett EJ, Kenny DJ. Avulsed permanent teeth: a review of literature and treatment guidelines. Endod Dent Traumatol 1997;13:153–63.
22. Lindskog S, Blomlöf L, Hammarström L. Evidence for a role of osontogenic epithelium in maintaining the periodontal space. J Clin Periodontol 1988;15:371–3.
23. Mizutani H, Mera K, Ueda M, Iwata H. A study of the bone morphogenic protein derived from bovine demineralized dentin matrix. Nagoya J Med Sci 1996;59:37–47.
24. Ike M, Urist MR. Recycled dentin root matrix for a carrier of recombinant human bone morphogenic protein. J Oral Implantol 1998;24:124–32.
25. Pinholt EM, Haanaes HR, Donath K, Bang G. Titanium implant insertion into dog alveolar ridges augmented by allogenic material. Clin Oral Implants Res 1994;5:213–9.
26. Kim SG, Yeo HH, Kim YK. Grafting of large defects of the jaws with a particulate dentin-plaster of Paris combination. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999;88:22–5.
27. Kim SG, Chung CH, Kim YK, Park JC, Lim SC. Use of particulate dentin-plaster of Paris combination with/without platelet-rich plasma in the treatment of bone defects around implants. Int J Oral Maxillofac Implants 2002;17:86–94.
28. Su-Gwan K, Hak Kyun K, Sung-Chul L. Combined implantation of particulate dentine, plaster of Paris, and a bone xenograft (Bio-Oss). For bone regeneration in rats. J Craniomaxillofac Surg 2001;29:282–8.
29. Miyaji H, Sugaya T, Kato K, Kawamura N, Tsuji H, Kawanami M. Dentin resorption and cementum-like tissue formation by bone morphogeneticic protein application. J Periodontal Res 2006;41:311–5.
30. Xu H, Shimizu Y, Asai S, Ooya K. Experimental sinus grafting with the use of deproteinized bone particles of different sizes. Clin Oral Implants Res 2003;14:548–55.