Integration of Dental Implants in Conjunction with Grafted Dentin. An Experimental Study in the Rabbit Maxilla

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Abstract

Objectives: The present study was undertaken to evaluate the healing pattern of xenogenic non-demineralised dentin granules and blocks grafted to maxillary bone of rabbits and integration of titanium micro-implants installed in grafted areas.

Material and methods: Fifteen 6-months old New Zealand male rabbits were used in the experiments. Dentin blocks and dentin granules from human premolars were implanted in cavities prepared on either side of the maxilla (n=15x2). After a healing period of 6 months, one micro implant (5 mm long, 2 mm in diameter) was installed in each surgical site.

Results: Three rabbits died during the healing period and 6 surgical sited were encapsulated by soft tissue. Hence a total of 18 implants were installed. All rabbits were sacrificed 24 weeks after the second surgery and histological examination was carried out. Measurements of bone-to-implant contact (BIC) and the bone fill area (BA) within the threads were calculated. The dentin specimens revealed a mean BIC of 17.8% and the native bone resulted in a BIC of 24.4% (p=0.188). The mean values for BA reported were 31.6% and 42.6% (P=0.360) respectively. Only fragmentary areas of direct contact between the dentin and the titanium surface could be noted.

Conclusion: The result of this experimental and study showed only limited or no bone contact between micro-implants and xenogenic dentin grafts. Furthermore, it was indicated that the granulae were encapsulated by means of a fibrous connective tissue in the majority of cases, whereas most dentin blocks were fused with the bone.

Key Words: Grafted dentin, Dental implants, Experimental study

Introduction

Autogenous bone grafts have been the gold standard to reconstruct bone deficiency situations for many years [1]. Their range of advantages includes early revascularization, resistance to infections and evidence of immune activation [2,3]. However, a disadvantage is that this technique requires a second surgical site to harvest the bone graft. Moreover, there are drawbacks such as donor site morbidity, limitations in the quantity of available bone, prolongation of surgery and treatment cost [4,5]. This has encouraged research to find an acceptable bone substitute. The ideal bone substitute should be readily available, well tolerated by the host, possess both osteoinductive and osteoconductive properties and be able to be resorbed gradually with the regeneration of new osseous tissue and healing of the bone defect. Availabe bone substitutes on the market are either synthetically derived or of xenogenic origin and may be associated with additional cost for the patient. Guided bone regeneration (GBR) is another option in bone formation where use of biocompatible membranes or scaffolds form an obstacle for ingrowth of non-osteogenic tissue to the site of bone formation [6].

Dentoalveolar ankylosis after replantation of avulsed teeth is sometimes followed by long-term resorption of roots and replacement by bone [7-9]. This condition indicates that dentin has the potential to be used as a suitable osteoconductive material in repair and regeneration of bone. It has been shown that xenogenic dentin implanted in rabbit tibia ankylosed and was replaced by bone [10].

Limited data exists regarding the interaction between dentin as a bone substitute material and placement of dental implants in the same location. In vivo studies has demonstrated that successful implant integration can be obtained in the presence of intentionally retained root fragments [11]. These findings mainly comprise of the establishment of newly formed rootcementum and establishment of a periodontal ligament in the contact areas [11-14]. All these studies have in common that they involve a root with the presence of a viable root cementum, periodontal ligament and dentin with vascular support from the pulp. Data of the use of dentin originating from other species, for bone augmentation in conjunction with implant installation is limited. Hence, the aim of this study was primarily to evaluate the healing pattern of xenogenic non-demineralised dentin granules and dentin blocks grafted to maxillary bone of rabbits and secondarily to study integration of titanium micro-implants installed in grafted areas.

Material and Methods

Animals and anaesthesia

Fifteen 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 by intramuscular injection. Animals were anaesthetised by intravenous injection of 35 mg/kg of ketamine HCl (Tekan, Hikma, Amman, Jordan). The protocol for animal experimentation by the Animal Research Centre of the Health Sciences Center, Kuwait was strictly adhered to. A veterinarian was responsible for administering

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the sedation, anesthesia and for the intra- and postoperative care of the animals. The animals were kept in separate cages and fed pellets and water *ad libitum* throughout the duration of the study.

Preparation of dentin grafts

Dentin grafts from human premolars, which were extracted for orthodontic reasons, were prepared in the following manner: The coronal part of the tooth was cut and removed with the help of rotary instruments so no enamel remained. The pulp and periodontal ligament were removed with endodontic files and a scalpel blade respectively. With the help of a trephine burr (5 mm diameter) cylinder shaped block was harvested from the premolar and the cylinder was sectioned into 3 mm thick blocks. The rest of the premolar was cut into granules in sizes of 1-3 mm. Granules and blocks were cleaned by being placed in 1% chlorhexidine and stored dry for one month. They were rinsed in saline for one hour before being used as grafts.

Surgical Procedure

As a supplement to general anesthesia and for vasoconstriction purposes, local anesthesia 1 ml lidocainehydrochlorideμg/ml (Xylocain 1% + epinephrine 5 - adrenalin, Astra Zeneca, Luton, UK) was administered in each experimental area.

First surgery - Graft preparation and placement

The bilateral edentulous areas superior to and between incisors and posterior teeth of the maxilla were used as experimental sites. The bone surface was exposed via a 10 mm long incision between buccal and palatal mucosa. A muco-periostal flap was raised. A 5x5 mm wide and 3 mm deep cavity was prepared penetrating through the maxillary cortical bone wall with the use of round burr (3 mm in diameter) under irrigation with saline.

The cavities on the right side were filled with dentin granules and the cavities on the left side with dentin blocks. No membrane or any other type of fixation was used. The incisions were closed with 4/0 Vicryl (Ethicon, Bridgewater, NJ,USA). To compensate for peri-operative and postoperative dehydration 10ml sterile saline solution was injected subcutaneously immediately following surgery according to Alberius et al [15] and antibiotics (Pen-Hista-strep,Vetoquinol SA, Lure Cedex, France) 50mg/kg was administered by intramuscular injection. Antibiotic administration was continued during the first 3 days after surgery. The rabbits were under frequent surveillance during the postoperative period.

Second surgery- Implant placement

After a healing period of 24 wks, rabbits were anesthetized once again as described earlier. Surgical access was accomplished in a similar way and one micro implant (5 mm long, 2 mm in diameter), which were machined from medical grade Ti (grade IV) rods (Elos, Pinol, Gørløse, Denmark) was installed in each surgical site in such a way that the apical half of the implant were placed in native bone, serving as control site, and the coronal part indentin. All rabbits were sacrificed 24 weeks after the second surgery by an overdose of Ketamine and block biopsies were prepared.

Histological and Histomorphometric Analysis

Following surgical removal en bloc, the samples were immersed and fixed in 10% neutral buffered formalin as described elsewhere [16]. In brief the fixated specimen were dehydrated in a graded series of ethanol, infiltrated with plastic resin and polymerized prior to cutting along the long axis of the implant. A central ground section was prepared by cutting and grinding, and was subsequently stained with toluidine blue. Two regions of interest were defined (ROI I and ROI II). ROI I (5 coronal threads) corresponded to the area where dentin blocks or granules were placed. ROI II corresponded to the apical portion of the implants which were installed in the maxillary host bone only (serving as control) *(Figure 1)*. The specimens were observed along their full length. The measurements of bone-to-implant contact (BIC) and the bone fill area (BA) within the threads were calculated on the mesial and distal aspect of each specimen. A mean value was then calculated for each specimen (ROI I and II respectively). The dentin and the bone-to-implant contact and the relative amount of bone and dentin within the threads, were determined using light microscopy (Nicon Eclipse E600) at 10 times magnification. The specimens were assessed using NIS Elements Microscope Imaging Software, Nikon.

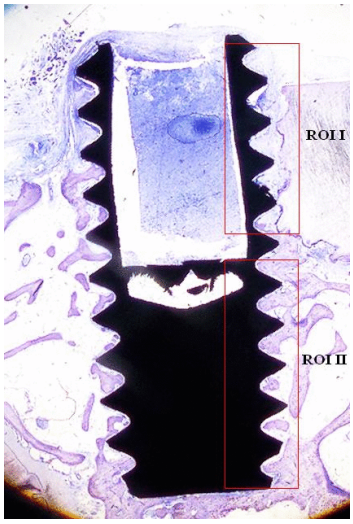

Figure 1. *Shows ROI I & ROI II. ROI I corresponds to the coronal threads embedded in dentin and ROI II corresponds to the apical threads embedded in host bone.*

Statistical Evaluation

Statistical analysis was performed using SPSS Ver 11,5 (SPSS Inc.,Chicago, IL.USA). Student's t-test was used for comparing the groups. P-values of < 0.05 were considered statistically significant.

Results

Three rabbits died during the healing period. The remaining 12 rabbits recovered uneventfully and gained weight. The soft tissue healing in all 12 rabbits was uneventful and there were

no signs of infection. Three sites grafted with dentin granules and 3 sites grafted with dentin blocks were encapsulated by loose connective tissue which did not allow any implant installation due to lack of bone. Hence a total of 18 micro-implants were installed (Block group n=9, granulae group n=9).

Descriptive Histology

In general, the incorporation of the dentin blocks and granulae varied. In the block group, nine out of 12 available blocks were considered enough fused to the surrounding bone and suitable for implant placement. In the granulae group, less fusion to bone was seen. A common feature was that granulae were encapsulated by means of fibrous tissue and only scarce contact between the xenogenic dentin granulae and blocks and the surrounding host bone was found. In general no or limited direct contact between xenogenic dentin and the microimplant surface could be noted. A few osteoclasts could be identified on the surface of the dentin, mostly located adjacent to present native bone tissue *(Figure 2)*. The dentin particles were otherwise surrounded by fibrous tissue with scarce presence of cells. The dentin material per se, did not seem to induce bone apposition on the implant surface. Instead newly formed bone seemed to migrate into the microgap between the dentin and the titanium surface.

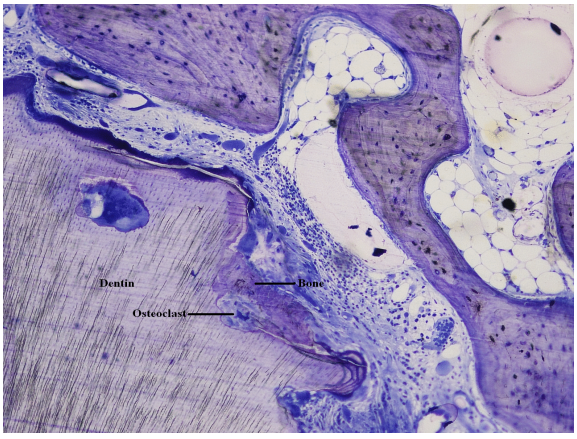

Figure 2. *Osteoclast adjacent to implanted dentin and subsequent bone deposition.*

Histomorphometric Analysis

After exclusion of specimen from the analysis due to the difficulty encountered to show a visible screw during specimen preparation, a total of 18 specimens were available for analysis. ROI I comprised of the first 5 threads and the border between native bone (ROI II) and the dentin area was set at thread 5, where the interface could be assessed.

Since there were no statistical difference between block and particulate groups, they were statistically analysed as one group. The dentin specimens (ROI I) revealed a mean BIC of 17.8% and the native bone (ROI II) resulted in a BIC of 24.4% (p=0.188). The percentages of new bone fill in the area (BA) within the threads (% bone fill) for the dentin specimens were 31.6% and 42.6% (P=0.360) for the native bone *(Table 1)*.

Overall the BIC and percentage of new bone fill of the block specimens were higher than the same parameters for the particulate graft. Only fragmentary areas of direct contact between the dentin and the titanium surface could be noted *(Figure 3a & b)*.

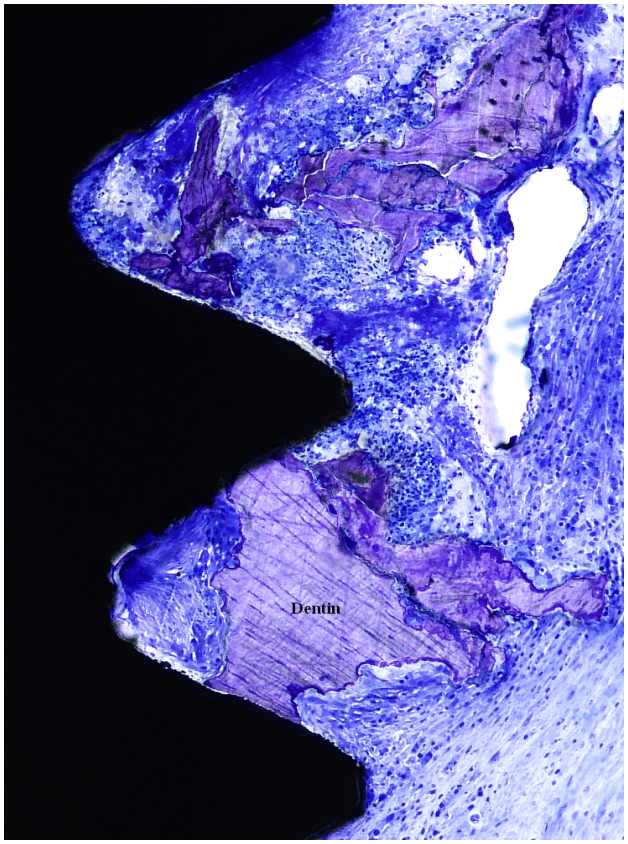

Figure 3a. *Fragmentary contact between dentin and titanium surface.*

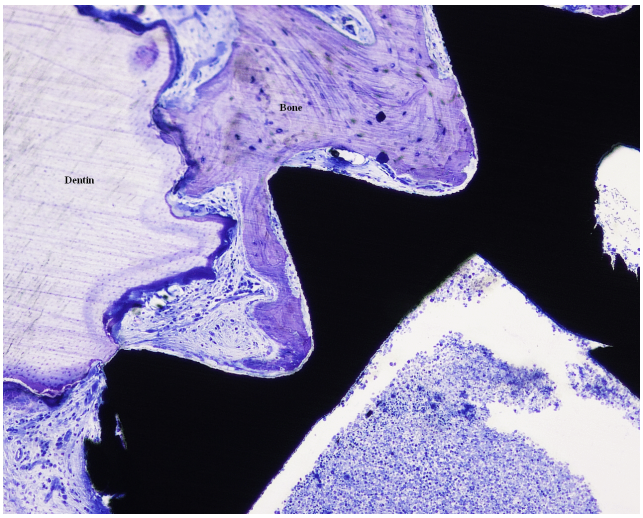

Figure 3b. *Migration of newly formed bone in the space between the dentin fragment and the titanium surface.*

Table 1. *Descriptive statistics showing range of BIC and BA in dentin and host bone. T-test pairs= Dentin with host bone. BIC= Bone implant contact, BA= Bone implant area.*

	n	Minimum	Maximum	Mean	Std. Deviation	*P* value
Dentin (BIC)	18	0	38.7	17.767	13.1881	0.188 n.s
Host bone (BIC)	18	7.5	56.4	24.356	13.48547	
Dentin (BA)	18	0	61,40	31.556	19.59268	0.36 n.s
Host bone (BA)	18	13.3	63.1	42.644	14.04586	

Discussion

The result of this experimental and descriptive study showed only limited or no bone contact between micro-implants and xenogenic dentin grafts. Furthermore, it was indicated that the granulae were encapsulated by means of a fibrous connective tissue in the majority of cases, whereas most dentin blocks were fused with the bone. One may speculate that granules might have been subjected to more mobility in the experimental cavity than a block and that this mobility could have promoted formation of fibrous tissue rather than bone.

It was shown in a previous study [17] that the osteoinductive properties of dentin is very limited despite the fact that dentin contains BMP and for this reason dentin should more or less be regarded as a osteoconductive material in an experimental model similar to this. Hence, we believe that in order to take advantage of the osteoconductive properties of dentin and achieve replacement resorption, stable fixation of the graft is of major importance. Dentin blocks in our study were not fixated to the underlying bone by any means but only fitted passively into the defect. This might have been a contributing factor to why there was limited replacement resorption around dentin blocks. Hence, in the future it would be interesting to study rigidly fixed block grafts.

Preclinical studies provide some evidence that successful implant integration may also be achieved in the presence of intentionally retained root fragments, as demonstrated by deposition of newly formed cementum and establishment of a periodontal ligament in the contact area [12,13]. It has also been previously demonstrated that dental implants can achieve ‘’dentointegration’’ when placed in close vicinity to vital retained roots [11,12]. Dentointegration was a term used to describe the histological features of the contact area between retained roots and dental implants. It was observed that a tubular tertiary dentin mainly originated from the pulp canal further developed into an atubular reparative dentin when exposed to the implant surface. We believe that this phenomenon is highly, result of the dental pulp stem cells exhibiting the ability to differentiate in many cell types [18] in combination with a stable graft. However their impact on hard tissue formation and subsequently integration of dental implants is unknown. It can be hypothesised that the injury caused by implant placement promotes the dentinogenic differentiation potential of pulp stem cells. In our study we used dentin as a non-vital graft and hence we could not expect any differentiation of the stem cells to hard tissue.

In a recent study in rats, it was concluded that demineralisation of dentin blocks in 24% EDTA for 2 or more hours resulted in significantly higher rate of resorption and significantly lower rate of encapsulation [19]. Dentin blocks in our study were not demineralised in any way, because we wanted to use a similar experimental situation like in dentoalveolar ankylosis after trauma to be able to compare our result to studies using the same principle [8-10,17,20-21]. In the future however, it would be interesting to also study the effect of using demineralized dentin.

Dentin grafts in our study were cleaned by being placed in chlorhexidine and stored dry before implantation. In vitro studies has proven chlorhexidine to be toxic to fibroblasts and odontoblast-like cells [22,23]. One might speculate that this fact has affected the integration of our grafts, however the very same processing protocol has been used in previous studies without any adverse effects on healing of the dentin grafts [20,21].

Despite using xenogenic dentin and the fact that the enamel, pulpal tissue and periodontal ligament were removed only by mechanical instruments, we did not see inflammatory cells in the ROIs. This is in accordance with findings in previous studies and indicates that the immunogenic factors are not related to dentin itself, but most likely to the soft tissue like pulpal and periodontal ligament, which was removed before grafting [17,20-21].

Dento-alveolar ankylosis is a long-term process over several years in humans and in animals having a higher bone turnover it is necessary to use a shorter period in the experimental situation. The choice of experimental time was based on experience from our previous study [17] where we used a 3 months healing period and found out no or very little heterotopic bone formation. In this study we extended the healing period to 6 months in order to achieve better fusion of the dentin grafts to the native bone and subsequently better foundation for implant installation.

There are limitations in our study being mostly descriptive, nevertheless our findings will be followed up by more systematic studies and may also have later clinical implications.

Conclusion

The result of this experimental study showed limited or no bone contact between micro-implants and xenogenic dentin grafts. Furthermore, it was indicated that the granulae were encapsulated by means of a fibrous connective tissue in the majority of cases, whereas most dentin blocks were fused with the bone.

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