J Clin Periodontol 2005; 32: 435–440 doi: 10.1111/j.1600-051X.2005.00692.x

Journal of Clinical Periodontology

Healing of extraction sockets and surgically produced – augmented and non-augmented – defects in the alveolar ridge. An experimental study in the dog

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Cardaropoli G, Araújo M, Hayacibara R, Sukekava F, Lindhe J. Healing of extraction sockets and surgically produced – augmented and non-augmented – defects in the alveolar ridge. An experimental study in the dog. J Clin Periodontol 2005; 32: 435–440. doi: 10.1111/j.1600-051X.2005.00692.x. © Blackwell Munksgaard 2005.

Abstract

Objectives: The current experiments had three aims (i) to determine whether the absence of the periodontal ligament (PDL) may alter features of the healing of an extraction socket, (ii) to examine if there were differences in the proportion of different tissues in resolved extraction sockets and surgically produced defects after 3 months of healing, (iii) to study the influence of different biomaterials on the healing of surgically produced bone defects.

Material and Methods: *Extraction sites*: In five dogs, the 4th mandibular pre-molars were hemi-sected and the distal roots were removed. The extraction socket of one of the pre-molars was instrumented to eliminate all remnants of the PDL tissue. The socket of the contra-lateral pre-molar was left without instrumentation. The dogs were sacrificed after 3 months of healing.

Defect sites: In five dogs, the pre-molars and 1st molars on both sides of the mandible were first removed and 3 months of healing allowed. After this interval three standardized cylindrical defects were prepared in each side of the mandible. The defects were 3.5 mm in diameter and 8 mm deep.

In each quadrant one defect was grafted with Bio-Oss® Collagen, one with Collagen Sponge and one defect was left non-grafted. The dogs were sacrificed 3 months after the grafting procedure.

Results: *Extraction sites*: The two categories of extraction sockets did not differ with respect to gross morphological features. The tissue of the extraction sites, apical of a newly formed bone bridge, was dominated by bone marrow. Few trabeculae of lamellar bone were also present.

Defect sites: The non-augmented defect was sealed by a hard-tissue bridge. In the central and apical portions of the defect bone marrow made up about 61%, and mineralized bone 39% of the tissues. The invagination of the surface of this crestal bone was 0.8 ± 0.3 mm.

The defect augmented with Collagen Sponge was covered by a hard-tissue bridge 38% of the tissue within the defect was made up of bone marrow while the remaining 62% was occupied by mineralized bone. The invagination of the hard-tissue bridge was on the average 0.6 ± 0.1 mm.

In defects augmented with Bio-Oss® Collagen the biomaterial occupied a substantial portion of the tissue volume. Eighty-five percent of the periphery of the Bio-Oss® particles were found to be in direct contact with newly formed mineralized bone. Woven bone and bone marrow made up 47% and 26% of the newly formed

Key words: biomaterial; bone defect; bone formation; extraction socket; grafting; wound healing

Accepted for publication 23 August 2004

tissue. The invagination of the most coronal part of the bone defect was 0.1 ± 0.1 mm. **Conclusion:** Sockets that following tooth removal had their PDL tissue removed exhibited similar features of healing after 3 months as sockets which had the PDL retained. The tissues present in an extraction site appeared to be more mature than those present in a surgically produced defect of similar dimension. The Bio-Oss® Collagen augmented defect exhibited less wound shrinkage than the non-augmented defect.

Healing of an extraction socket includes a series of events including the formation and maturation of a coagulum that subsequently will become replaced by a provisional matrix and woven bone (e.g. Amler 1969, Kuboki et al. 1988, Lin et al. 1994). Further, the socket walls will be resorbed, gradually remodelled and the distinct outline of the extraction socket disappeared (Araùjo & Lindhe 2005). When during healing a cortical ridge was established in the entrance of the socket, the immature woven bone was remodelled and replaced by lamellar bone and marrow (Cardaropoli et al. 2003). It was suggested that mesenchymal cells from the periodontal ligament (PDL) may participate in the healing of the socket wound (e.g. Evian et al. 1982, Lekic et al. 2001, Cardaropoli et al. 2003) and that such PDL cells could differentiate into osteoblasts and produce bone (McCulloch & Melcher 1983). Limited information is available regarding socket healing in the absence of a PDL.

The first aim of the current experiment was to determine, therefore, if the absence of PDL tissue may alter the relative distribution of woven/lamellar bone and marrow in the extraction socket.

Surgically produced defects in the edentulous ridge were often used in model experiments to study de novo bone formation (e.g. Dahlin et al. 1990, Schenk 1994, Lundgren et al. 1995, Carmagnola et al. 2003). It was suggested that the process that resulted in the resolution of such defects had many features in common with the healing of fractures in the long bone and also with the extraction sockets (Schenk 1994, Hollinger & Wong 1996).

The second aim of the present experiment was to examine if differences exist in the proportion of various tissues in the resolved extraction socket and in a surgically produced defect of similar dimension following 3 months of healing.

Biomaterials/biological agents such as autogenous bone, bioactive glass, coralline calcium carbonate, decalcified freeze dried bone, deproteinized bovine bone, hydroxyapatite etc. are frequently used to augment compromised regions of the ridge and to make the edentulous site available for implant installation (for a review, see Becker 2003). It was demonstrated that several of the biomaterials were (i) incorporated in newly formed bone tissue, (ii) maintained as inactive fillers and (iii) resorbed when the host tissue was undergoing remodelling (Araùjo et al. 2001).

The third aim of the present investigation was to study the influence of different biomaterials on the healing of surgically produced bone defects.

Material and Methods

The research protocol was approved by the Regional Ethics Committee for Animal Research, Maringa State University, Brazil.

Ten mongrel dogs, (about 12 months old and weighting about 10 kg each), were used for the experiment. During surgical procedures, the animals were anaesthetized with intra-venously administered Pentothal Natrium® (30 mg/ml; Abbot Laboratories, Chicago, IL, USA). The dogs were placed on a plaque control program, which called for tooth cleaning with toothbrush and dentifrice three times a week.

Extraction sites

In five dogs, the 4th mandibular premolars ($_4$ P $_4$) were hemi-sected, the pulp of mesial roots was removed and the canals were filled with guttapercha. The distal roots of the hemi-sected premolars were subsequently removed with the use of small elevators.

The extraction socket of one of the pre-molars, $_4$P or P_4, was instrumented in an attempt to eliminate all remnants of the PDL tissue that adhered to the socket walls (Non-PDL site). Thus, with the use of a small scaler (Golman Fox® No. 3; Hu-Friedy, Chicago, IL, USA) the internal surfaces of the extraction socket was curetted until the hard-tissue walls were smooth and considered free of soft tissue.

The extraction socket of the distal root of the contralateral 4th pre-molar was left without instrumentation (PDL site).

The buccal and lingual gingivae at the extraction sites were stabilized with interrupted sutures. The sutures were removed after 10 days.

The dogs were sacrificed after 3 months of healing with an overdose of Pentothal Natrium® and perfused with a fixative containing glutaraldehyde and formaldehyde (Karnovsky 1965). The mandibles were removed and placed in the fixative. The extraction sites were dissected into blocks using a diamond saw (Exact® Apparatebeau, Norderstedt, Hamburg, Germany). The blocks were decalcified in EDTA, dehydrated in ethanol and embedded in paraffin. Sections were prepared in the mesiodistal plane and parallel with the long axis of the extraction socket. The microtome was set at 7 µm. Three sections, about 20 µm apart, representing the central part of the socket were stained in haematoxylin and eosin and used in the histological examination.

Defect sites

In five dogs, a sulcus–crestal incision was placed in the pre-molar/molar regions on both sides of the mandible. Buccal–lingual full thickness flaps were elevated. The pre-molars and 1st molars on both sides of the mandible were carefully extracted. The flaps were closed with interrupted sutures. The sutures were removed after 10 days.

After 3 months of healing following the removal of the teeth, crestal incisions were placed in the edentulous ridge and buccal–lingual full thickness flaps were elevated. By the use of ITI® implant preparation drills for solid screw implants (Straumann AG, Waldenburg, Switzerland), three standardized cylindrical defects were prepared in each side of the mandible. The defects were 3.5 mm in diameter and 8 mm deep.

In each quadrant one defect was grafted with Bio-Oss® Collagen (Bio-Oss®

Collagen is comprised of Bio-Oss® spongiosa granules with the addition of 10% highly purified porcine Collagen Type I; the collagen in this device acts, according to the manufacturer, as a cohesive for the Bio-Oss® particles) (Geistlich Pharma AG., Wolhusen, Switzerland), one with Collagen Sponge (The Collagen Sponge consists of highly purified and cross-linked porcine Collagen Type I and III; the sponge in this device is, according to the manufacturer, optimized regarding its mechanical stability, hydrophilicity and porous structure) (Geistlich Pharma AG) and one defect was left non-grafted.

The flaps were replaced and secured with interrupted sutures. The sutures were removed after 10 days. The dogs were sacrificed 3 months after the grafting procedure. Biopsies were sampled and prepared for histological examination in the manner described above. The final blocks were cut in the mesio-distal plane and with the microtome set at 7 µm.

Three sections representing the central part of each defect, about 20 µm apart, were selected for histological analyses.

Histological Examination

Extraction sites

The overall characteristics of the various tissues of the healed extraction sockets were described following examinations performed in a Leitz® DM-RBE microscope (Leica, Wetzlar, Germany) equipped with an image system (Q-500 MC®; Leica).

Morphometric measurements were performed by a trained and blinded examiner and according to a method originally described by Schroeder & Münzel-Pedrazzoli (1973). The measurements were confined to the central and apical portions of the extraction socket, i.e. the newly formed cortical ride that sealed the socket was not included in the measurements. A lattice comprising 100 light points were superimposed over the identified portions of the socket. The relative volumes occupied by blood clot, granulation tissue, provisional matrix (connective tissue including mesenchymal cells embedded in a fibrous matrix), woven bone, lamellar bone and bone marrow (adipocytes and large vessels) were determined.

Defect sites

The overall characteristics of the various tissues of the healed defects were first examined and described. The invagination of the hard-tissue bridge that had formed in the marginal portion of the defects was determined in the following way: a line was draw to connect the crest of the old bone at the mesial and distal edges of the defect. The vertical distance between this line and the most ''marginal'' level of the invagination of the bridge was assessed and expressed in millimeter.

Morphometric measurements were performed in the manner described above. The newly formed hard tissue that bridged all the healed defects was not included in the measurements. The relative volumes occupied by blood clot, granulation tissue, provisional matrix, woven bone, lamellar bone, bone marrow and biomaterial in the central and ''apical'' portions of the defects were determined.

In addition the proportions of the periphery of the Bio-Oss® particles that were in direct contact with mineralized bone was assessed using the point counting procedure.

Data Analysis

Mean values and standard deviations were calculated for each variable, extraction site/defect and group of dogs using descriptive statistics.

Results

Extraction sites

After 3 months of healing, the two categories of extraction sockets, i.e. PDL-sites and Non-PDL sites did not differ with respect to gross morphological characteristics. Both types of extraction sockets (Figs 1 and 2) were characterized by the presence of a hard-tissue bridge that was continuous with the old bone in the mesial and distal borders of the socket. This bridge of bone ''sealed'' the marginal entrance of the socket. The hard-tissue wall was composed of woven bone that exhibited signs of remodelling.

The tissue of the extraction sites, apical of the newly formed bone bridge, was dominated by bone marrow rich in adipocytes and blood vessels (Table 1). Few and mostly thin trabeculae of mineralized bone were also present within the bone marrow. These trabeculae were comprised of lamellar bone with secondary osteons, although in some portions of the trabeculae, woven bone with primary osteons could be observed.

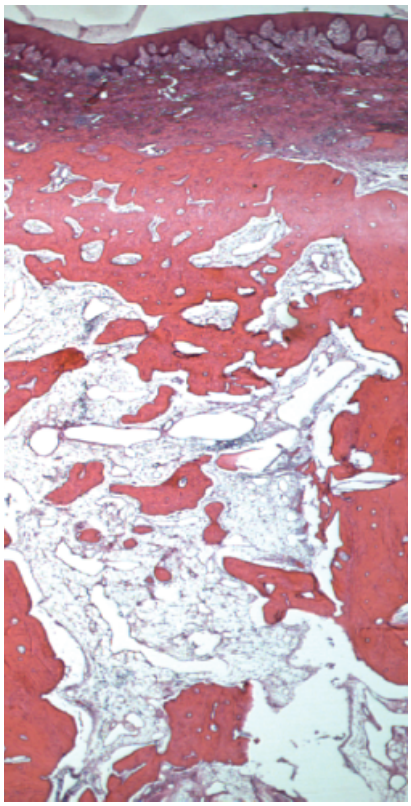

Fig. 1. Extraction site. Mesio-distal section of an extraction site, PDL-site, after 3 months of healing. Note the presence of a hard-tissue bridge that seals the entrance of the site. Apical of this cortical bone layer the tissue is dominated by bone marrow and includes few trabeculae of lamellar bone. Haematoxylin and eosin staining; original magnification × 16. PDL, periodontal ligament.

The morphometric measurements revealed that the tissue apical of the cortical bridge at the Non-PDL sites was comprised of 24.7 ± 11.3% mineralized bone and 75.3 ± 12.0% bone marrow. The corresponding figures representing the PDL sites were 24.4 ± 9.2% and 75.6 ± 9.1%. The proportion of the mineralized bone that was made up of lamellar bone was 78.5 ± 6.1% in the PDL sites and 76.2 ± 7.2% in the Non-PDL sites.

Defect Sites

Non-augmented

The surgically prepared defect was sealed by a hard-tissue bridge that was in direct continuity with the old bone at the mesial and distal borders of the experimental site (Fig. 3). This hard-

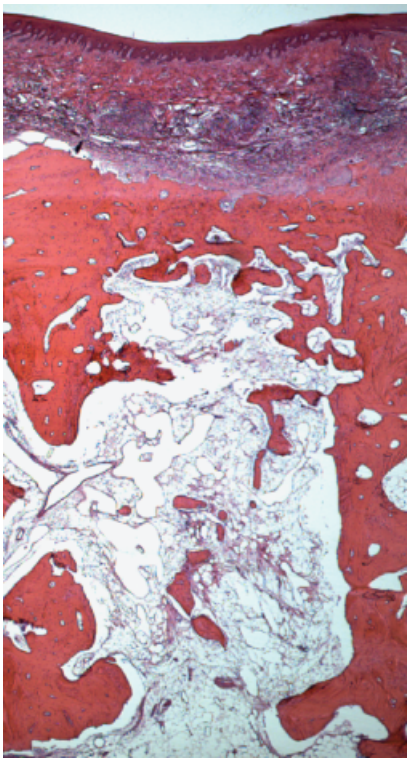

Fig. 2. Extraction site. Mesio-distal section of a Non-PDL-site after 3 months of healing. The tissue composition of this site is in all respects similar to that of the PDL-site presented in Fig. 1. Haematoxylin and eosin staining; original magnification × 16. PDL, periodontal ligament.

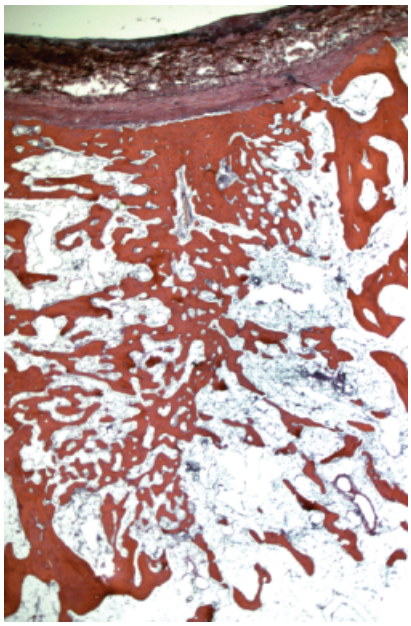

Fig. 3. Defect site. Surgically produced defect that was left non-augmented. Mesio-distal section after 3 months of healing. A hard-tissue bridge had formed to connect the mesial and distal edges of the defect. The defect is dominated by its content of large amounts of woven bone. Haematoxylin and eosin staining; original magnification × 16.

tissue wall was composed of woven bone and some lamellar bone with secondary osteons primarily facing the connective tissue of the mucosa. The invagination of the surface of this crestal bone was on the average 0.8 ± 0.3 mm. In the central portions of the defect, large areas of woven bone including primary osteons were found to reside in the bone marrow. Fractions of the mineralized bone included occasionally lamellar bone with secondary osteons and concentric lamellae. The bone marrow that included densely packed adipocytes made up about 60.7 ± 9.1%, and mineralized bone 39.3 ± 6.2% of the tissues within the defect. Woven bone occupied 72.7 ± 8.3% of the volume of the mineralized bone (Table 2).

Collagen sponge

The bridge of mineralized bone that enclosed the defect was continuous with a wide zone of woven bone that extended deep into the newly formed tissue (Fig. 4). The invagination of the surface of the hard-tissue bridge was on the average 0.6 ± 0.1 mm. Small amounts of lamellar bone with concentric lamellae could be observed within areas of the woven bone compartments. 38.3 ± 8.3% of the tissue within the defect was made up of bone marrow while the remaining 61.8 ± 7.0 was occupied by mineralized bone. 80.3 ± 7.2% of the mineralized bone was comprised of immature woven bone.

Table 1. Proportion (%) of different tissues in the extraction sockets and the bone defects

	Socket		Defect		
	PDL	non-PDL	non-grafted	collagen sponge	Bio-Oss® collagen
Mineralized bone	24.4 (9.2)	24.7 (11.3)	39.3 (6.2)	61.8 (7.0)	46.7 (7.4)
Bone marrow	75.6 (9.1)	75.3 (12.0)	60.7 (9.1)	38.3 (8.3)	26.1 (8.0)
Bio-Oss® particles	–	–	–	–	27.2 (7.1)

Mean (SD). PDL, periodontal ligament.

Bio-Oss® Collagen: In this category of defects the biomaterial occupied a substantial portion of the tissue volume (Fig. 5). The biomaterial was present not only in the central portions of the defect but was also included in the immature bone of the cortical ridge that sealed the marginal entrance of the defect. The invagination of the surface of this bridge was on the average 0.1 ± 0.1 mm. Woven bone and lamellar bone of varying dimension had also formed in direct contact with most of the Bio-Oss® particles in the lateral and apical portions of the defect. In the central segments, however, a small portion of the biomaterial was surrounded by provisional matrix. 85 ± 9.1% of the periphery of the Bio-Oss® particles were found to be in direct contact with newly formed mineralized bone. Mineralized bone and bone marrow made up 46.7 ± 7.4% and 26.1 ± 8.0%, respectively, of the newly formed tissue while 27 ± 7.2% of the tissue was comprised of the biomaterial. 79.4 ± 7.1% of the newly formed mineralized bone was made up of woven bone (Table 2).

Discussion

The findings from the present experiment in the dog disclosed that 3 months following the removal of a tooth with an intact periodontium, the two types of extraction sockets examined – PDL sites and Non-PDL sites – exhibited close to identical wound healing characteristics. The marginal entrance of both sites was covered with a hard-tissue bridge of varying dimension, while areas apical of the bridge harbored similar proportions of lamellar bone as well as mature bone marrow. This finding must not be interpreted to indicate that when present the PDL cells in a fresh extraction socket are unimportant in the early phases of healing. On the contrary, Cardaropoli et al. (2003) reported that during the first week of healing following tooth extraction, the PDL adjacent to the bundle bone of the socket walls maintained its vitality and that PDL cells in this period apparently migrated into the provisional matrix residing in the socket. This observation is in agreement with findings by Lin et al. (1994) who studied the fate of PDL fibroblasts during socket healing after tooth extraction in the rat. They demonstrated that PDL fibroblasts after tooth extraction ''actively proliferate, migrate into the

Table 2. Proportion (%) of woven and lamellar bone present in the newly formed mineralized bone, in the various types of sites (sockets and defects) included in the study

	Socket		Defect		
	PDL	non-PDL	non-grafted	collagen sponge	Bio-Oss® collagen
Woven bone	21.5 (5.0)	23.8 (6.2)	72.7 (8)	80.3 (7)	79.4 (7)
Lamellar bone	78.5 (6.1)	76.2 (7.2)	27.3 (6)	19.7 (5)	20.6 (5)

Mean (SD). PDL, periodontal ligament.

Fig. 4. Defect site. Mesio-distal section of a defect site that during the surgical preparation was augmented with the Collagen Sponge material. Note the presence of mineralized bone in the marginal and central portions of the site. This mineralized tissue is mainly comprised of immature woven bone that is in the process of remodelling. Haematoxylin and eosin staining; original magnification × 16.

Fig. 5. Defect site. Mesio-distal section of a surgically produced defect in the edentulous ridge. The defect was augmented with Bio-Oss® Collagen. Following a healing period of 3 months the Bio-Oss® particles in most segments of the defect were surrounded by newly formed woven bone. In some central regions the particles appeared to be surrounded by connective tissue – provisional matrix. The biomaterial could also be observed in the hard-tissue wall that bridged the entrance of the defect. Haematoxylin and eosin staining.; original magnification × 16.

coagulum, form dense connective tissue, and differentiate into osteoblasts which form new bone during socket healing''. In this context, it must be realized that PDL cells are not the only source of osteoblasts that occur in the provisional matrix, but bone forming cells may also enter into the wound from the bone marrow lateral to the socket wall (McCulloch & Melcher 1983).

In the present study, comparisons were made regarding the tissue composition after 3 months of healing at extraction sites and at surgically produced non-grafted defects.

In this comparison both similarities and differences were observed. First of all, both types of sites were covered by a bridge of hard tissue that included large amounts of woven bone and occasionally areas of lamellar bone. The content of the wound, apical of the ridge however, was markedly different at the two categories of sites. Hence, while the extraction site included a mature bone tissue, the non-grafted defect sites harbored a large volume of mineralized but immature bone tissue and less bone marrow. Further, in the extraction socket close to 100% of the mineralized bone was made of lamellar bone while in the surgically produced defects, the immature woven bone made up about 70% of the hard-tissue volume. There are reasons to assume, however, that with prolonged healing time, the non-grafted defects will take on a tissue composition similar to that of the extraction wound at 3 months. This hypothesis is supported by observations reported by Frost (1983, 1989a, b) who stated that mechanical injury to bone tissue stimulates cells with bone forming capacity to multiply and form bone, but also that the newly formed woven bone over time will be replaced with lamellar bone and marrow (e.g. Mital & Cohen 1966, Amsel et al. 1969, Araùjo et al. 1999, Stavropoulos et al. 2001, Cardaropoli et al. 2003).

One purpose of placing a graft of biomaterial in a self-contained hard-tissue defect is to offer stability for the coagulum and hence avoid volume reductions and surface invaginations that otherwise will occur when the wound contracts. In the current study these objectives were at least in part satisfied. Thus, the invagination of the hard tissue bridge in the non-grafted defects was 0.8 ± 0.3 mm, while at the Bio-Oss® Collagen grafted sites the corresponding dimension was 0.1 ± 0.1 mm. This finding is in agreement with Carmagnola et al. (2002) who studied bone fill in mechanically produced defects in the mandibular ridge of dogs. They observed that following 3 months of healing the marginal bone of the non-grafted defects had a ''saddle shaped'' appearance while Bio-Oss® grafted sites exhibited no surface invagination.

Another reason for placing a graft in a hard-tissue defect is to provide a scaffold for new bone formation. Also this objective was met in the current experiment. Thus, in comparison with the non-grafted defects, both in the Collagen Sponge and the Bio-Oss® Collagen grafted sites larger amounts of new mineralized bone were observed after 3 months of healing (Table 1). The total relative volume of newly formed mineralized bone amounted to 39.3% in the non-grafted sites, 61.4% in the Collagen Sponge and 46.7% in the Bio-Oss® Collagen grafted defects (Table 1). Most of the newly formed mineralized tissue in the augmented defect sites had the character of woven bone (about 80%), which over time most likely will remodel and be replaced mainly by bone marrow.

In the Bio-Oss® Collagen grafted defects, the Bio-Oss® particles were well integrated and continuous with the newly formed network of woven and lamellar bone. In most respects the current findings are thus in agreement with observations made in clinical and

experimental studies showing that an intimate contact frequently is established between pure Bio-Oss® particles and newly formed mineralized bone (Wetzel et al. 1995, Araùjo et al. 2001, Berglundh & Lindhe 1997, Skoglund et al. 1997, Zitzmann & Schärer 1998).

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