Socket grafting with the use of autologous bone: an experimental study in the dog

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Abstract
Background: Studies in humans and animals have shown that following tooth removal (loss), the alveolar ridge becomes markedly reduced. Attempts made to counteract such ridge diminution by installing implants in the fresh extraction sockets were not successful, while socket grafting with anorganic bovine bone mineral prevented ridge contraction.

Aim: To examine whether grafting of the alveolar socket with the use of chips of autologous bone may allow ridge preservation following tooth extraction.

Methods: In five beagle dogs, the distal roots of the third and fourth mandibular premolars were removed. The sockets in the right or the left jaw quadrant were grafted with either anorganic bovine bone or with chips of autologous bone harvested from the buccal bone plate. After 3 months of healing, biopsies of the experimental sites were sampled, prepared for buccal–lingual ground sections and examined with respect to size and composition.

Results: It was observed that the majority of the autologous bone chips during healing had been resorbed and that the graft apparently did not interfere with socket healing or processes that resulted in ridge resorption.

Conclusion: Autologous bone chips placed in the fresh extraction socket will (i) neither stimulate nor retard new bone formation and (ii) not prevent ridge resorption that occurs during healing following tooth extraction.

Following tooth extraction (loss), the alveolar ridge undergoes a marked change [Pietrokowski & Massler 1967; Schropp et al. 2003; Pietrokowski et al. 2007]. During the first year after tooth extraction, about 50% of the bucco-lingual ridge dimension will be lost [Schropp et al. 2003]. Furthermore, ridge reduction will become more pronounced from a buccal than from a lingual/palatal aspect [Pietrokowski & Massler 1967]. It was suggested that the ridge dimensions could be preserved if implants were placed in the fresh extraction socket [Denissen et al. 2000; Paolantonio et al. 2001]. The validity of this hypothesis was refuted by Botticelli et al. [2004] and Sanz et al. [2010], who in prospective studies involving large numbers of subjects and sites, clearly documented that also following immediate implant placement (Type 1; Hämmerle et al. 2004), the edentulous site underwent substantial diminution.

Processes of hard tissue modeling and remodeling following tooth extraction were studied in the dog model [Cardaropoli et al. 2003; Araújo & Lindhe 2005]. It was demonstrated that the socket was first occupied by a coagulum that was replaced with granulation tissue, provisional connective tissue and woven bone. This immature...
Material and methods

The research protocol was approved [State University of Maringá, Brazil]. Five beagle dogs, about 1 year old, were used. The dogs were anesthetized with intravenously administered ketamine 10% (8 mg/kg, Aéger União, São Paulo, Brazil).

Sulcus incisions were made in the posterior premolar region and small buccolingual full-thickness flaps were elevated. The third and fourth mandibular premolars [\(P_3\), \(P_4\)] were hemi-sectioned. The canal of the mesial root was reamed and filled with gutta-percha.

In one quadrant, bone chips harvested from the buccal surface of the mandible using a sharp chisel were soaked in blood and packed into the fresh sockets [autograft]. In the contra-lateral quadrant, a xenograft [a blend of granules of denaturated bovine bone (90%) and porcine collagen fibers (10%)]; Bio-Oss \(^{\text{®}}\) collagen, Geistlich \(^{\text{®}}\), Wolhusen, Switzerland] was placed [xenograft] in a similar manner.

Three months after the grafting procedure, the dogs were euthanized with an overdose of ketamine and perfused, through the carotid arteries, with a fixative containing a mixture of 5% glutaraldehyde and 4% formaldehyde [Karnovsky 1965].

The premolar sites, including the mesial root and the distal socket area, were dissected [Exact \(^{\text{®}}\) Apparatebeau, Norderstedt, Hamburg, Germany]. The tissue samples were processed according to the methods described by Donath & Breuner [1982] and Donath [1988], dehydrated in ethanol, infiltrated with Technovit \(^{\text{®}}\) 7200 VLC resin [Kulzer, Friedrichsdorf, Germany], polymerized and sectioned [Exact \(^{\text{®}}\) Apparatebeau]. From each premolar site, four sections were prepared, two sections from the mesial root and two sections from the healed socket. The sections were cut in the buccal–lingual plane and were sampled from the central area of either the root or the socket. The sections were, by micro grinding and polishing, reduced to a thickness of about 25–30 \(\mu\)m and stained in Ladewig’s fibrin stain.

Examinations

The histological examinations were performed in a Leitz DM-RBE \(^{\text{®}}\) microscope [Leica, Wetzlar, Germany] equipped with an image system [Q-500 MC \(^{\text{®}}\), Leica].

Size of the cross-section area of the edentulous ridge

The profile of the ridge as well as the size of the cross-section area of the edentulous portion of the \(P_3\) and \(P_4\) sites were determined in accordance with a method described by Araújo et al. [2008]. In brief, the image of the profile of the alveolar process that harbored the mesial root of an experimental tooth site was divided into three equally high portions. The size of each of the apical, middle and marginal portions was determined with a cursor and expressed in mm\(^2\). The corresponding measurements were then performed at the edentulous, healed distal socket site. The relative alteration of the size of the alveolar process that had occurred in each dog after tooth extraction was estimated by subtracting the value obtained at the extraction site from the corresponding value at the mesial root site (for further details, see Araújo et al. 2008).

Morphometric measurements

The composition of the alveolar process as well as the newly formed tissue in the edentulous distal socket site was determined using a point-counting procedure. A lattice comprising 100 light points (modified from Schroeder & Münzel-Pedrazzoli [1973]) was superimposed over the target area and the percentage area occupied by lamellar bone, newly formed bone (mainly woven bone), BMUs [basal multicellular units], bone marrow, provisional connective tissue and graft particles was determined [magnification \(\times 100\)].

The mean values and standard deviations of the different variables were calculated using the dog as the statistical unit.

Results

In all experimental sites, healing was uneventful. After 3 months of healing, a keratinized mucosa was observed to cover the entrance of the edentulous part of all premolar sites.

Gross histological findings

Sites with an autograft

A large portion of the central area of extraction sites was occupied by bone marrow in which an island of newly formed, mineralized bone could be observed [Fig. 1]. Remnants of non-vital bone chips [Fig. 2a and b] were few in numbers, but occurred in the entire part of the newly formed bone. The graft particles had the nature of lamellar bone and were frequently separated...
from the newly formed hard tissue by reversal lines. A varying thick layer of mainly woven bone was present in the entrance region of the socket site. The old buccal bone crest was located about 2 mm apical of its lingual counterpart in all specimens.

Sites with a xenograft
The newly formed bone and provisional connective tissue in socket sites that had been filled with Bio-Oss® collagen harbored large numbers of graft particles [Fig. 3]. The marginal and middle portions of the site were comprised of newly formed woven and parallel-fibered bone. Bone marrow was observed mainly in the apical portion of the site. Bio-Oss® particles present within the bony housing were consistently surrounded by parallel-fibered bone or were in direct contact with multinucleated cells [Fig. 4a and b]. A fibrous capsule surrounded particles of the biomaterial that were located in the mucosa adjacent to the bone compartment of the ridge. The old buccal bone crest was consistently located about 1–2 mm apical of the lingual crest.

Fig. 1. Microphotograph of a buccal–lingual section representing an experimental site after 3 months of healing. The fresh extraction socket was grafted with autologous bone chips. The lingual bone wall is wider than the buccal wall. Note the area of mineralized bone residing in the bone marrow. The socket entrance is occupied by a bridge of mineralized bone residing in the bone marrow. B, buccal bone wall; L, lingual bone wall; BM, bone marrow. Ladewig fibrin stain; original magnification ×16.

Fig. 2. Higher magnification of newly formed bone. Note the presence of non-vital autologous bone chips (asterisk) that are delineated by a dark-stained reversal line [a]. The same detail presented in polarized light [b]. The autologous bone chips are made of lamellar bone and are in direct contact with woven and parallel-fibered bone [b]. Ladewig fibrin stain; original magnification ×100.

Fig. 3. Microphotograph of a buccal–lingual section representing an experimental site after 3 months of healing. The fresh extraction socket was grafted with Bio-Oss® collagen. The xenograft particles (blue stain) are embedded in newly formed bone and some provisional connective tissue. Note that the biomaterial occupies a large portion of the socket entrance. Bio-Oss® particles can also be seen in the mucosa of the ridge. B, buccal bone wall; L, lingual bone wall; BM, bone marrow. Ladewig fibrin stain; original magnification ×16.

Fig. 4. Microphotograph (a) and polarized microphotograph (b) of newly formed bone. Note the presence of non-vital autologous bone chips (asterisk) that are delineated by a dark-stained reversal line (a). The same detail presented in polarized light (b). The autologous bone chips are made of lamellar bone and are in direct contact with woven and parallel-fibered bone (b). Ladewig fibrin stain; original magnification ×100.

Composition of the alveolar process and the healed socket

Alveolar process (Table 2)
The alveolar process that harbored the autologous bone graft was comprised of 49.1 ± 6.6% old lamellar bone, 24.5 ± 3% newly formed bone, 20.7 ± 8.1% bone marrow, 4.4 ± 1.2% BMUs and 1.4 ± 1.6% graft material.

The corresponding values obtained from measurements in the Bio-Oss® grafted sites were 54 ± 7.5% old lamellar bone, 18.1 ± 7% newly formed bone, 10.5 ± 5.5% bone marrow, 4.1 ± 2.7% BMUs and 8.6 ± 2% graft material. In addition, sites grafted with the biomaterial included 4.7 ± 2.5% provisional connective tissue.

Healed socket sites (Table 3)
The newly formed tissue and the remaining graft material that occupied the post-extraction socket augmented with autologous bone chips was comprised of 57.2 ± 8.6% mineralized bone, 38.3 ± 10.9% bone marrow, 2.9 ± 2.8% BMUs and 1.9 ± 1.9% non-vital bone chips.

The corresponding values for the Bio-Oss® collagen sites were 43.1 ± 10% for mineralized bone, 16 ± 7.6% for bone marrow, 2 ± 2.2% for BMUs, 4.7 ± 2.5% for provisional connective tissue and 24.4 ± 3.7% for Bio-Oss® particles.

Discussion

Sockets grafted with autologous bone exhibited a healing pattern that had many features in common with those described...
for non-grafted post-extraction sites (Car- 
daropoli et al. 2003; Araujo et al. 2005; Araujo et al. 2008). Thus, in the 
current study as well as in the previous 
experiments, the sockets – after 3 months 
of healing – had become filled with similar 
amounts of mineralized bone (mainly wo-
nen bone) and marrow. This indicates that 
the autograft material used in the present 
study apparently failed to enhance healing 
and/or to stimulate hard tissue formation 
in the socket.

The mineralized bone in the grafted sites 
harbored chips of non-vital autologous 
bone. The chips (particles) were never ob-
served in the bone marrow but only within 
the newly formed bone from which they 
were consistently separated by well-de-
defined, often undulating reversal lines. 
This suggests that the autologous bone 
graft during the early, inflammatory phase 
of healing had been exposed to osteoclastic 
activity and that, as a consequence, after 3 
months, comparatively few graft particles 
remained in the socket site.

In the present study, it was furthermore 
noted that in sites grafted with autologous 
bone, there was pronounced resorption of 
the buccal bone wall and also a marked 
reduction (−25%) of the marginal portion 
of the ridge. Similar amounts of buccal wall 
reduction (about 2 mm) and ridge diminu-
tion (−30%) were reported for non-grafted 
sites by Araujo & Lindhe [2005], Araujo 
et al. [2008].

In other words, despite the fact that the 
autogenous bone used in the current 
experiment may have delivered an osteogenic 
as well as an osteoconductive stimulus 
(Burchardt 1987) to the fresh extraction 
socket and although the gold standard for 
bone grafting may include the use of auto-
genous hard tissue from, e.g., intra-oral 
donor sites [for a review, see Hjorting-
Hansen 2002], in the current model, this 
type of graft material failed to prevent ridge 
contraction.

In comparison with sockets that had 
been filled with autologous bone, the Bio-
Oss®-collagen-grafted sites exhibited a de-
layed healing pattern (Table 3). Thus, in 
xenograft-treated sites, there was less 
mineralized bone (43.5 vs. 57.2%), a smal-
ler proportion of bone marrow (16 vs. 
38.3%) and a substantial amount of re-
maining provisional connective tissue (14 
vs. 6%). This conclusion of a delayed 
healing in Bio-Oss®-grafted sites is in 
agreement with observations made pre-
viously (Artzi et al. 2004; Jensen et al. 
2006) and from experiments using the ex-
traction socket model (Araujo et al. 2008, 
2010; Araujo & Lindhe 2009). In a recent 
experiment, Araujo et al. [2010] studied 
the dynamics of Bio-Oss® incorporation in 
extraction wounds and concluded that be-
fore new bone could form in the augmented 
site, the biomaterial had to be exposed to a 
“surface cleaning” that was associated 
with the presence of TRAP-positive multi-
nucleated cells, i.e. osteoclasts. This is in 
agreement with the proposal by Jensen 
et al. [2006] i.e. that the multinucleated 
cells present adjacent to particles of Bio-
Oss® in a healing hard tissue wound “had 
more the function of macrophages, i.e. to 
clean the graft surface and thereby prepare
it for deposition of newly formed bone.” In the studies by e.g. Jensen et al. (2006) and Araujo et al. (2010), the biomaterial was apparently not engaged in the processes of modeling/remodeling. Thus, a large number of Bio-Oss particles remained in the extraction sites. An experimental study in dogs. Journal of Clinical Periodontology 30: 809–818.


