

# Long-term Clinical Outcome after Reconstruction of Periodontal Defects using a Bovine-Derived Xenograft: a Retrospective Cohort Study

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The present retrospective study evaluates the long-term results of reconstructive periodontal surgery using a bovine-derived bone mineral with or without a bio-resorbable collagen membrane. Teeth (241) in 54 patients were treated. Treatment was performed regardless of the patients' genetic predisposition, medical status or social habits. The patients had to follow a strict oral hygiene programme prior to periodontal surgery. One year after treatment, an overall reduction of the mean pocket probing depth (PPD) of 2.69 mm on average (from  $6.88 \pm 1.89$  mm to  $4.19 \pm 1.40$  mm) and an increase in clinical attachment level (CAL) of 3.14 mm on average (from  $8.56 \pm 2.39$  mm to  $5.42 \pm 1.50$  mm) could be shown. At two years from baseline, no statistical differences in PPD reduction were found compared to the one year's data.

Within the limits of the present study, it can be concluded that periodontal surgical therapy by the use of bovine-derived bone mineral with or without a bio-resorbable collagen membrane in combination with a strict oral hygiene program results in significantly high PPD reduction and CAL gain. Thus this treatment appears to be a suitable treatment for severe periodontal defects, leading to predictable and stable results.

**Key words:** Alveolar infrabony defects, deproteinised bovine-derived xenograft, collagen membrane, periodontal regeneration, clinical attachment level gain, pocket probing depth reduction

## INTRODUCTION

Guided tissue regeneration (GTR) is thought to function as the controlled stimulation of new bone and connective tissue attachment formation by the means of mechanical barriers in periodontal defect sites. It is aimed to provide the functional and aesthetic characteristics (Dahlin et al, 1988; Karring et al, 1993).

For a long time the gold standard in GTR of severe periodontal defects has been a two-step procedure using non-resorbable barrier membranes. A

first step surgery involving complete root debridement and defect coverage was followed by membrane removal after wound healing in a surgical re-entry procedure (Murphy and Gunsolley, 2003; Needleman et al, 2005; Trombelli, 2005).

In recent years, GTR has been given support by the technological development of bone substitutes and resorbable membranes, avoiding a second surgical intervention and providing space for periodontal regeneration underneath the membrane (Bunyaratavej and Wang, 2001). Clinical studies have shown that the treatment of periodontal os-



seous defects using deproteinised bovine-derived xenograft (BDX), either in combination with a GTR/membrane technique or without, leads to significant clinical advantages when compared with open flap debridement alone. It can yield to a reduction of clinical pocket probing depth (PPD), to an increase of the clinical attachment level (CAL) and therefore to a higher probability of a successful regeneration of the periodontium. However, most studies included only small patient pools with regeneratively treated defects (Camargo et al, 2000; Clergeau et al, 1996; Dietrich et al, 2003; Houser et al, 2001; Murphy and Gunsolley, 2003; Needleman et al, 2005; Paolantonio, 2002; Scabbia and Trombelli, 2004; Sculean et al, 2005; Sculean et al, 2004a, 2004b; Tonetti et al, 2004; Trombelli, 2005; Yamada et al, 2002).

The data collected in clinical trials have been underlined by a limited number of histological evaluations of the GTR-treated defect sites. Human histologies revealed that combination therapy using deproteinised BDX and a collagen membrane can result in periodontal regeneration, including new formation of cementum, periodontal ligament and alveolar bone (Camelo et al, 1998; Camelo et al, 2001; Hartman et al, 2004; Mellonig, 2000; Sculean et al, 2004a, 2004b).

In general, BDX has shown to be osteoconductive and can be well integrated into the natural bone remodelling (Artzi et al, 2003). BDX is only slowly resorbed and therefore space maintaining (Scarano et al, 2004; Schlegel and Donath, 1998), resulting in long-term stabilisation of periodontal regeneration (Stavropoulos and Karring, 2005). To the authors' knowledge, this is so far the only study investigating the long-term stability of BDX in combination with GTR, using resorbable copolymer membranes for regenerative procedures.

The combination therapy using BDX covered by a collagen membrane can lead to higher CAL gain than surgical treatment with a collagen membrane alone in defects of unfavourable architecture (Camelo et al, 2001; Paolantonio, 2002). So far, little is known about the periodontal regenerative outcome if no membrane, but only BDX is used (Hanna et al, 2004).

The objective of this retrospective study was to evaluate the clinical outcomes following treatment of infrabony defects either with BDX alone or in combination with a collagen barrier membrane.

## MATERIALS AND METHOD

In our private periodontal office, 54 patients underwent regenerative procedures of infrabony alveolar defects using a deproteinised BDX (Bio-Oss®, Geistlich, Wolhusen, Switzerland) between the years 2000 and 2003 (Table 1). In total, 241 teeth ( $4.48 \pm 3.68$  teeth per patient) were treated either with BDX alone or in combination with a collagen membrane (Bio-Gide®, Geistlich, Wolhusen, Switzerland). There were 11 patients showing a single defect each. No exclusion of any kind of patients had been decided prior to the therapeutic procedure (Table 1).

After successful initial periodontal therapy, PPD and CAL were measured before periodontal surgery (PCP11, Hu-Friedy, Leimen, Germany) as well as at 12 and 24 months from baseline (Table 2). PPD was defined as the distance from the gingival margin to the deepest sounding of the selected site of the tooth. Two periapical X-ray sets taken during initial periodontal therapy and at one year post-operatively served for CAL measurement. CAL was defined as the radiographic distance from the cemento-enamel junction (CEJ) to the bottom of the infrabony defect. The data have been clinically verified during surgery after debridement of the site.

### Pre-surgical Procedure

After initial periodontal examination stating periodontal treatment needs, all patients had to undergo a preoperative initial therapy composed of three sessions by a professional dental hygienist, including oral hygiene instructions, scaling and root planing (SRP) and patient's motivation. During that time the baseline data was recorded by the dental hygienist and re-evaluated by the periodontist: PPD, CAL, bleeding on probing (BOP), plaque index (PI), recessions, furcation involvement of molars and upper 1st premolars, and tooth mobility. Based on these clinical data and on the patient's compliance to the given oral hygiene instructions, the decision for performing a regenerative treatment was made. Depending on the severity of attachment loss and clinical appearance, microbial DNA-testing (microdent®, Probe Sampling PCR-Test, Hain Diagnostics, Nehren, Germany) was done. In accordance to the test results, antibiotic drugs were systemically administered to the patients in 57% of cases (Table 1).



**Table 1** Patient and defect characteristics at baseline (n = 241).

Variable	Patient	Defect
Number of subjects	54	241
Age	47.7 ± 11.4	NA
Gender (number (%) of females)	31 (57.4%)	116 (48.7%)
Smokers (%) (> 10 cigarettes/day)	20 (37.0%)	113 (47.1%)
Antibiotic treatment	31 (57.4%)	142 (63.3%)
Defect type		
– Predominantly one wall		62 (25.9%)
– Predominantly two wall		161 (67.4%)
– Predominantly three wall		16 (6.7%)

NA, not applicable.

### Surgical Procedure

The surgical procedures were performed by one of the authors each (Bröseler and Tietmann, 2004; Bröseler and Tietmann, 2005; Pini Prato et al, 2004). The post-operative data were taken by our office dental hygienist.

The surgical protocol was as following:

- Elevation of a full-thickness mucoperiosteal access flap or papilla preservation flap
- Complete excision of granulation tissue from alveolar bone defect with hand instruments and rotating burs
- SRP with ultrasonic (Cavitron/Slimline, DENTSPLY, Konstanz, Germany) and hand instruments
- Filling of the bone defect with BDx
- Coverage of the filled defect by adaptation with a collagen membrane or only by suturing of the tissues for primary closure
- Primary closure, suturing with modified mattress sutures using 4-0 synthetic polyfilament (Ethibond® Excel DA, Ethicon, Germany) and 6-0 monofilament single sutures (Premilene® USP6/0 – DS13, B.Braun, Tuttlingen, Germany; Seralene® USP6/0 – DS12, Serag Wiessner, Germany; Seralene® USP6/0 – DS15, Serag Wiessner) for papilla adaptation and vertical releasing incisions
- No periodontal dressing was used

### Post-surgical Protocol

Post-operative care included administration of 0.2% chlorhexidine mouth-rinse (Curasept ADS 220, Curaden AG, Kriens, Switzerland) three

times a day over a four-week period, or until complete wound healing had been stated. The patient was advised to leave out brushing of the regeneratively treated sites. Teeth with nonphysiological mobility were stabilised using a removable acrylic splint or semi-permanent retainers. Sutures were removed 7 to 10 days post-operatively depending on the progress of wound healing. During the first post-operative period, no membrane exposure was observed that needed further corrective treatment.

The patients were kept on a short recall basis (6–8 week intervals) for professional oral hygiene for the first 6 months post-operatively. Afterwards the recall intervals were re-adjusted according to the patient's individual needs. Clinical data, as recorded at baseline, were taken at 1 and 2 years after surgery, again by the professional dental hygienist.

### Data Analysis

The patient was regarded as the statistical unit. Data were expressed as mean ± SD. Significances of mean differences between pre- and post-surgery were analysed using Student's *t*-test for paired observations. The level of significance was set at  $\alpha = 0.05$  (5%).

Of the 241 treated teeth, 220 could be investigated (91%) in order to evaluate the success of the described periodontal treatment. Four patients (21 teeth) were lost to follow-up due to unrelated reasons. For three patients (8 teeth), no one-year follow-up X-ray was recorded, therefore no CAL data was obtained. Three teeth (1.24%) had been removed: two teeth (0.83%) had been lost after ther-



Fig 1a to h Regenerative procedure of maxillary first left bicuspid.



Fig 1a Preoperative situation: note the recession (2.5 mm) and the edematous swelling of the gingiva; pathologic tooth mobility grade III.



Fig 1b Situation during surgery: horizontal and severe vertical attachment loss; furcation involvement.



Fig 1c Measurement of 9 mm CAL after debridement.



Fig 1d Filling of the periodontal lesion with BDX (BioOss Collagen®).

apy due to severe tooth mobility (1) or root caries (1); and one tooth had been kept only until implant placement (1).

## RESULTS

### Patient Retention and Missing Data

A total of 54 patients, combining 241 teeth, were evaluated for this retrospective study. No criteria had been stated to select the patients or to keep out any patient from investigation. All regenerative-treated patients have been followed up, regardless of their smoking habits, genetic predisposi-

tion, diabetic condition, gender and kind of infection (Table 1). During the one-year evaluation period, four patients were lost to follow-up due to unrelated reasons (21 teeth). Furthermore, three teeth were removed due to root caries, severe tooth mobility and prior to implant placement. Complete observations after one year were available for 49 (90.7%) patients and 217 teeth (90.0%), and for 46 patients (86.8%) and 209 teeth (86.7%) CAL data is available.

### Baseline and Defect Characteristics at Baseline

The retrospective study included 54 patients with one-, two-, and three-wall intrabony defects (Table



**Fig 1e** Coverage of the augmented lesion with a resorbable collagen membrane (BioGide®).



**Fig 1f** Primary closure with 4-0 and 6-0 sutures. Coronally positioned mucoperiosteal flap after flap mobilization by periosteal splitting.



**Fig 1g** Situation 5 days after surgery.



**Fig 1h** Situation 10 months after surgery. Gingival appearance without any inflammation signs, PPD 3 mm, Recession 3.0 mm. Pathologic tooth mobility grade I.

1). The most severe defect site of each tooth was entered into the statistical evaluation. The investigated defects were associated with a wide range of PPDs (3–12 mm) (average  $6.88 \pm 1.89$  mm), and with a CAL of 4–15 mm (average  $8.56 \pm 2.39$  mm).

Of the 49 patients, 46 (93.9 %) who were followed up for the present study showed a successful clinical outcome one year after treatment, meaning that the regeneratively treated teeth could be kept in place, stabilised and revealed ameliorated hard and soft tissue clinical appearance (Table 1).

### Pocket Probing Depth

The clinical results one year after treatment are presented in Table 2. PPD improved with high significance compared with baseline ( $p < 0.001$ ).

One year after therapy, the 220 regeneratively treated periodontal sites investigated showed an average reduction in PPD of  $2.69 \pm 1.88$  mm (from  $6.88 \pm 1.89$  mm to  $4.19 \pm 1.40$  mm). PPD measurements after two years revealed an average reduction of 2.32 mm, while data from one and two years were consistent, revealing no statistically significant change (Table 2).



Fig 2a to c Radiographic examination of maxillary first left bicuspid.



Fig 2a Situation at initial examination. Attachment loss > 70%, circular crater, furcation involvement.



Fig 2b Post-operative control after augmentation with BDX. Second molar had to be removed due to furcation involvement grade III.



Fig 2c Radiographic control one year post-operatively.

	Baseline (241 teeth PPD; 220 teeth CAL)	1 year (217 teeth PPD; 209 teeth CAL)	2 years (83 teeth PPD)
CAL	8.56 ± 2.39	5.42 ± 1.50*	–
PPD	6.88 ± 1.89	4.19 ± 1.40*	4.56 ± 1.65*

Table 2 Defect characteristics at baseline/1 year/2 years.

\* $p < 0.05$  with respect to baseline.

### Clinical Attachment Level

The results of the clinical attachment level change one year post-operatively are presented in Table 2. CAL improved significantly compared with baseline ( $p < 0.001$ ). One year after therapy, the 209 regeneratively treated teeth for which one-year follow-up data was available showed an average CAL gain of  $3.14 \pm 1.93$  mm (from  $8.56 \pm 2.39$  mm to  $5.42 \pm 1.50$  mm).

### DISCUSSION

Since GTR was introduced as the state of the art in periodontal treatments, many procedures and supportive materials have been put forward (Trombelli, 2005). These materials have been applied with varying success. BDX has been proven to lead to periodontal regeneration when used as augmentative material alone or in combination with resorbable as well as non-resorbable membranes, or with enamel matrix proteins. Previous reports demonstrated that the space maintaining features of BDX can lead to a PPD reduction of 2–5 mm and to an increase in CAL of 2–4 mm

(Needleman et al, 2005; Sculean et al, 2005; Tonetti et al, 2004; Trombelli, 2005).

One year after surgical treatment, the presented regenerative procedure resulted for the re-evaluated teeth in an average gain of CAL of  $3.14 \pm 1.93$  mm (209 teeth) and a reduction in PPD of  $2.69 \pm 1.88$  mm (217 teeth). This is in agreement with the previously published data, keeping in mind that many clinical studies have minimum defect sizes as inclusion criteria (Murphy and Gunsolley, 2003; Needleman et al, 2005; Trombelli, 2005). Furthermore, other clinical protocols published so far excluded patients with systemic diseases or smoking habits.

Tonetti et al (2004) describe a CAL gain of  $3.3 \pm 1.7$  mm and PPD reduction of  $3.7 \pm 1.8$  mm in 124 regeneratively treated sites, excluding patients with uncontrolled systemic diseases and heavy smokers (> 20 cigarettes/day). BDX in combination with a collagen barrier membrane for defect coverage was used in all of the defects. The higher PPD reduction observed by the authors may result from the strict exclusion criteria (Tonetti et al, 2004). In contrast, in the present retrospective study, 51% of the teeth belonged to heavy



smokers and were included in the analysis. In addition, in the present study membranes were used only in 57% of the investigated defect sites.

An average CAL gain of 3.2 mm was reported by Camargo et al (2000). The authors describe a surgical procedure using BDX and coverage by a collagen membrane in a test group including 22 defects. PPD reduction was measured at 4.1 mm on average.

Data from a controlled study presented by Sculean et al (2005), which included 16 paired defect sites, supports the hypothesis that better results can be obtained for larger defects. The clinical outcome of a CAL gain of 4.1 mm and a PPD reduction of 5.4 mm originated from treating patients with a baseline CAL and PPD of 9.4 mm and 8.3 mm respectively.

The higher CAL gain in comparison with the presented data can be explained by a higher severity of the defects at baseline. The data mentioned above compare with 8.6 mm CAL and 6.9 mm PPD in the present study.

A randomised split-mouth double-blinded clinical trial with a patient pool of 13 revealed, in the BDX group (no membrane) in comparison with the present study, a lower PPD reduction of 2.53 mm and a CAL gain of 2.31 mm after 6 months from baseline. However, the inclusion criteria stated that patients with systemic diseases were excluded and only one smoker (7.7%) was included into the study (Hanna et al, 2004).

Our retrospectively evaluated data show highly significant PPD reduction from baseline at one and two years, and no statistically significant change between one and two years. This is in accordance with the recently published five-year post-operative data of Stavropoulos and Karring (2005) after therapy of severe intra-bony defects with GTR in combination with BDX under intensive systemic antibiotic administration. The authors could demonstrate long-term stability of the damaged teeth and the survival rate in total was 86.7% (13 out of 15 teeth).

The results achieved in our retrospective cohort study reveal a high predictability of regenerative procedures with or without the use of collagen membranes covering defects filled with BDX. CAL gain and PPD reduction are in line with the results of previous controlled clinical studies although no exclusion criteria such as smoking habits and systemic diseases were made. Patient's compliance

to participate in a strict pre- and postoperative oral hygiene programme has a major impact on the long-term stability.

Throughout the teeth re-examined in this study, only two teeth (0.83%) were lost due to either root caries and severe tooth mobility, while one tooth had been only kept prior to implant placement. This shows that the treatment procedure gives a high success rate (99%), a predictable outcome and a clear alternative to tooth removal.

## CONCLUSIONS

The presented periodontal regenerative procedure, a single step surgery using bovine-derived bone mineral either in combination with a collagen barrier membrane or without, leads to significant PPD reduction and CAL gain. Predictability, reproducibility and long-term stability are proven by a high number ( $n = 217$ ) of re-evaluated defect sites. Long-term stability data from controlled clinical studies may yet provide more evidence in support of the present data.

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