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A comparative study on the use of a HA/collagen/chondroitin sulphate biomaterial (Biostite®) and a bovine-derived HA xenograft (Bio-Oss®) in the treatment of deep intra-osseous defects

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Abstract

Objectives: This parallel-group, randomized, clinical trial was designed to evaluate the clinical outcome of deep intra-osseous defects following reconstructive surgery with the use of a synthetic hydroxyapatite/equine Type I collagen/chondroitin sulphate biomaterial (Biostite®), as compared to a bovine-derived hydroxyapatite xenograft (Bio-Oss®).

Material and methods: Twenty-four systemically healthy subjects with moderate to advanced periodontitis, 11 females and 13 males, aged 30–64 years, seven smokers, were selected. Patients presented with one interproximal deep intra-osseous defect (intra-osseous component $\geqslant 4$ mm) as clinically and radiographically evaluated. Immediately before surgery and 12 months after surgery, pocket probing depth (PPD), clinical attachment level (CAL) and radiographic depth of the defect (DEPTH) were evaluated.

Results: Thirteen defects were treated with Biostite® (test) and 11 defects with Bio-Oss® (control). In the test group, PPD amounted to 7.8 ± 1.3 mm before surgery, and decreased significantly to 3.6 ± 1.6 mm 12 months following surgery, while in the control group PPD significantly decreased from 7.5 ± 2.0 mm pre-surgery to 3.1 ± 1.0 mm post-surgery. At 1 year, CAL gain and DEPTH gain were 2.9 ± 1.9 and 2.5 ± 1.4 mm, respectively, in the test group, and 4.0 ± 2.4 mm and 3.1 ± 1.8 mm, respectively, in the control group. No statistically significant differences for PPD reduction, CAL gain and DEPTH gain were detected between the groups.

Conclusions: The results of the present study indicate that both Biostite® and Bio-Oss® grafting biomaterials have determined a clinically and statistically significant improvement in terms of CAL gain, PPD reduction and radiographic DEPTH gain when used for the treatment of deep intra-osseous defects.

Key words: bone substitutes; collagen Type I; grafts; hydroxyapatites; intra-osseous defects; periodontal diseases; periodontal regeneration; periodontal therapy; proteochondroitin sulfates; surgery; therapy

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Deep intra-osseous defects represent a major challenge for the clinician. Sites with intra-osseous lesions have been shown to be at higher risk of disease progression in subjects who had not received systematic periodontal therapy (Papapanou & Wennström 1991). Moreover, bony lesions may not be readily accessible to periodontal debridement, often requiring access flap surgery alone or in association with bone-resective techniques or regenerative procedures.

Among treatment modalities, grafting of biomaterials/bone substitutes have been used with varying success to

accomplish the reconstruction of lost attachment apparatus in deep intra-osseous defects. A systematic review on grafting biomaterials and biological agents in the treatment of deep intra-osseous defects showed that an additional clinical benefit may be obtained from various bone substitutes used as an adjunct to open flap debridement (OFD) procedure. Overall, the results indicated that the implantation of bone substitutes produced a more favorable CAL gain, PPD reduction and increased defect fill when compared to OFD alone (Trombelli et al. 2002a).

Hydroxyapatites (HAs) represents a family of bone grafting materials which have come under consideration in the last decades. HAs are complex calcium phosphates which resemble bone mineral in their chemical composition [$Ca_{10}(PO_4)_6(OH)_2$]. Naturally derived forms of apatite are made from crushed coral, or are represented by the mineral fraction of allogenic/xenogenic bone. The synthetic forms, on the other hand, are bioceramics which exhibit a denser structure and a very low chemical solubility compared to natural HAs (de Groot 1983). HAs present remarkable biocompatibility with little inflammatory response when implanted within connective and bone tissues (Beckham et al. 1971, Jarcho et al. 1977, de Putter et al. 1983). Moreover, deposition of bone mineral crystals may occur directly onto the surface of implanted apatite particles (Jarcho et al. 1977, Ogiso 1998). A meta-analysis of results from randomized controlled clinical trials evaluating the use of various forms of HA in the treatment of deep intra-osseous defects has shown significantly greater attachment gain and probing depth reduction when compared to conventional OFD (Trombelli et al. 2002a).

Clinical (Scopp et al. 1966, Older 1967) and histological (Arrocha et al. 1968, Nielsen et al. 1980, 1981) studies promoted the use of anorganic bovine bone for grafting in intra-osseous defects. In this context, an anorganic xenograft (Bio-Oss®, Geistlich Pharma AG, Wolhusen, Switzerland) was developed for bone regeneration procedures. This grafting material is a natural, porous, cancellous bone mineral derived from bovine bone from which all native organic material has been removed by a chemical low-heat (300°C) extraction process, maintaining the physical architecture of bone intact. Chemically, Bio-Oss® is a low crystalline apatite (crystallite size of approximately $100 \times 200 \times 500$ Å) with a 7% content of carbonate (Benke et al. 2001). The infra-red spectra and X-ray diffraction patterns show a calcium content of $37.1 \pm 0.7\%$ and a phosphorous content of $17.8 \pm 0.5\%$, corresponding to a Ca–P ratio of 2.1 ± 0.1 (Jensen et al. 1996). Histologically, bone formation in humans with bovine bone mineral has been demonstrated in sinus elevation procedures (Valentini et al. 1998, 2000, Piattelli et al. 1999, Hallman et al. 2001), in alveolar ridge preservation/augmentation procedures (Callan & Rohrer 1993, Artzi & Nemcovsky 1998, Zitzmann et al. 2001), around endosseous implants (Berglundh & Lindhe 1997, Skoglund et al. 1997, Zitzmann et al. 1997, Hämmerle et al. 1998, Schlegel & Donath 1998), and in periodontal bone defects (Camelo et al. 1998, Mellonig 2000). Clinically, the effectiveness of Bio-Oss® xenograft in the reconstructive treatment of intra-osseous defects in humans has been evaluated when used alone (Hutchens 1999, Richardson et al. 1999, Scheyer et al. 2002), in association with resorbable membranes (Hutchens 1999, Camargo et al. 2000, Paolantonio et al. 2001, Pietruska 2001) and in combination with enamel matrix protein derivative (Lekovic et al. 2000, Scheyer et al. 2002, Velasquez-Plata et al. 2002).

Recently, a new biomaterial has been introduced to periodontics. This material (Biostite®, Vebas s.r.l., S. Giuliano M., Milan, Italy) is a mixture of synthetic HA (88.0%, granulometry of 160–200 μm, total porosity of 60%), equine Type I collagen (9.5%), and chondroitin sulphate (2.5%). Chemically, Biostite® particles demonstrated a major phase represented by polycrystalline synthetic HA ($\geqslant 99\%$), little presence of β-tricalcium phosphate and CaO ($\leqslant 1\%$), and a Ca–P ratio ranging from 1.665 to 1.697. Biostite® has been shown by transmission electron microscopy to induce in vitro formation of a calcified collagenous matrix when cultured with human osteoblast-like cells (Serre et al. 1993), and by light microscopy to be highly biocompatible and osteoconductive in animals (Benqué et al. 1992, Brunel et al. 1992) and in humans (Benqué et al. 1985, Scabbia et al. 2003). Furthermore, active resorption of implanted granules has been demonstrated in animals (Penaud et al. 1992) and in humans (Parodi et al. 1996, Santarelli et al. 1996, Scabbia et al. 2003). Previous reports have evaluated the use of Biostite® graft in combination with a collagen membrane in animals (Benqué et al. 1992, Brunel et al. 1992) and in humans (Parodi et al. 1996, Santarelli et al. 1996, Benqué et al. 1997). However, no clinical studies are available on healing of human intra-osseous defects treated with Biostite® alone in addition to an OFD procedure.

Therefore, the purpose of the present parallel-group, randomized, clinical trial was to evaluate the clinical and radiographic outcomes of reconstructive surgery in human deep intra-osseous defects with the use of a synthetic HA/equine Type I collagen/chondroitin sulphate biomaterial (Biostite®), as compared to a bovine-derived HA xenograft (Bio-Oss®).

Material and Methods

Experimental design

A parallel-group, randomized, clinical trial was designed to test the effectiveness of a synthetic HA/equine Type I collagen/ chondroitin sulphate biomaterial (Biostite®, Vebas s.r.l., S. Giuliano M., Milan, Italy) following reconstructive surgery in deep intra-osseous defects, as compared to a bovine-derived HA xenograft (Bio-Oss®, Geistlich Pharma AG, Wolhusen, Switzerland). Treatment outcome was evaluated clinically at 6 and 12 months following surgery, and radiographically after 12 months of healing.

Subject population

Patients were recruited among those seeking care for moderate to severe chronic and aggressive periodontitis at Research Centre for the Study of Periodontal Diseases, University of Ferrara, Italy.

Inclusion criteria were as follows:

(1) presence of one interproximal deep intra-osseous defect ($\geqslant 4$ mm). Depth of the intra-osseous component of the defect was clinically and radiographically evaluated during the screening phase but had to be confirmed during surgery;
(2) patients previously treated only by oral hygiene instruction and scaling and root planing;
(3) both smokers and non-smokers subjects were included. In smokers, daily

cigarette consumption was noted, as well as years of smoking habit.

Exclusion criteria were as follows:

(1) subjects with a history of severe acute or chronic systemic disease, women pregnant or lactating, subjects taking medications known to affect the gingival status;
(2) subjects younger than 18 years

Third molars, teeth affected by endodontic lesions and/or inadequate endodontic treatments, teeth showing restorations with overhanging margins, teeth with degree 3 mobility, and, in general, all teeth with a hopeless prognosis at the combined clinical and radiographic evaluation were not included. Defects extending into a furcation and defects involving the mesial aspect of mesially inclined molars were not considered.

Pre-surgical phase

Prior to surgery, patients received a complete periodontal examination, oral hygiene instructions, and multiple scaling and root planing sessions. At least 4 weeks elapsed from the completion of the non-surgical therapy until re-examination (baseline) and surgery.

Surgical procedure

The surgical procedure with supracrestal soft tissue preservation was described in details in a previous report (Trombelli et al. 2002b). Briefly, flap design in the interdental area consisted of one of the following alternatives: (1) sulcular incisions with the split of buccal and lingual papilla; (2) incision with the preservation of the buccal papilla, according to the simplified papilla preservation technique (Cortellini et al. 1999); (3) incision with the preservation of the buccal papilla, according to the modified papilla preservation technique (Cortellini et al. 1995); (4) incision with the preservation of the lingual/palatal papilla, according to Takei papilla preservation procedure (Takei et al. 1985). Vertical releasing incisions were performed when needed for better access and/or primary closure of the surgical wound.

Full-thickness mucoperiosteal flaps were raised, in order to gain complete access to the defect, and thorough debridement and root planing of the exposed root surfaces was performed by a combination of ultrasonic and hand instrumentation. In no cases was osteoplasty/ostectomy carried out. Defects were consecutively assigned to one of the two treatment procedures using a randomization list generated by toss of a coin. In the test sites, a synthetic HA/equine Type I collagen/chondroitin sulphate biomaterial (Biostite®, Vebas s.r.l., S. Giuliano M., Milan, Italy) was applied to fill the defect, while in the control defects a bovine-derived HA xenograft (Bio-Oss®, Geistlich AG, Wolhusen, Switzerland) was used. In relation to flap design, flaps were positioned at the pre-surgery level or slightly coronal in order to achieve primary closure of the interdental area without any tension. When needed, partial-thickness flaps were raised to permit their coronal displacement and ensure passive adaptation without tension. Flaps were held in place by means of non-resorbable e-PTFE sutures (Gore-Tex™ CV-5 or CV-6 Suture Material, W.L. Gore and Associates, Flagstaff, AZ, USA) to completely cover the implanted material. Selection of the suturing technique was based on flap design. No surgical dressing was used.

Post-surgical infection control

A 0.12% chlorhexidine mouthwash, twice daily, was prescribed for at least 4 weeks post-surgery. No antibiotic therapy was administered post-surgery. In order to minimize traumatic injury to the marginal tissues, patients were instructed to avoid mechanical oral hygiene procedures in the treated area for at least 4 weeks. Sutures were removed after 14 days. At that time, presence of post-surgery infections or pulpal complications, and flap sloughing were recorded.

The patients were recalled at 1, 2 and 4 weeks after surgery, then at 2, 3, 4, 5, 6, 9 and 12 months. Supportive care program included professional supragingival polishing and scaling and oral hygiene reinforcement. No periodontal probing and/or subgingival re-instrumentation of the surgically treated sites were performed prior to 6 months of healing.

Clinical recordings

All clinical recordings were performed immediately before surgery (baseline), at 6 and 12 months post-surgery. The following indices and clinical measurements were recorded:

(1) Full-mouth plaque score (FMPS), by means of the modified O'Leary plaque control record (O'Leary et al. 1972). After the application of a disclosing erythrosin solution, areas adjacent to the gingival margin exhibiting stain on visual inspection were recorded as having plaque;
(2) Full-mouth bleeding score (FMBS). Sites bleeding within 10 seconds after probe insertion were recorded as positive sites;
(3) Pocket probing depth (PPD), measured from the gingival margin to the tip of the probe;
(4) Clinical attachment level (CAL), measured from the cemento-enamel junction (CEJ) to the bottom of the pocket;
(5) Recession (REC), measured from the CEJ to the gingival margin.

FMPS and FMBS were assessed on all teeth present, excluding third molars, on all six aspects of the tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual/palatal, mid-lingual/palatal, and disto-lingual/palatal). PPD, CAL and REC measurements were recorded (in mm) at the deepest site of the selected interproximal defect by using a standard periodontal probe (UNC 15, HuFriedy, Chicago, IL, USA) with manual pressure of approximately 0.3 N.

Intra-surgery recordings

The following defect measurements were recorded during surgery following debridement of the defect:

(1) distance from the CEJ to the bottom of the defect (CEJ-BD);
(2) distance from the most coronal extension of the interproximal bone crest to the bottom of the defect, i.e. the intra-osseous component of the defect (INFRA).

All intra-surgery recordings were performed at the deepest interproximal point of the defect by using a standard periodontal probe (UNC 15, HuFriedy, Chicago, IL, USA).

Radiographic measurements

Radiographic examination was performed at baseline and 1 year after surgery. Film holders (Rinn centering devices, Dentsply Ltd, Weybridge, UK) with customized bite impressions were used to take standardized reproducible periapical radiographs employing a

long-cone paralleling technique. All radiographs were evaluated under good light conditions to measure the radiographic depth of the defect (DEPTH), calculated as the linear distance (in mm) from the most coronal extension of the radiopaque crest (as perpendicular projection on the long axis of the tooth) to the most apical extension of the defect (i.e. where the periodontal ligament space was considered as having a normal width). Radiographic defect fill (%) was calculated as follows:

$$\frac{\text{baseline DEPTH} - \text{1-year DEPTH}}{\text{baseline DEPTH}} \times 100.$$

Table 1. Patient and defect characteristics for Biostite® and Bio-Oss® groups at baseline

Variable	Biostite®	Bio-Oss®	*p*-value
Subject number	13	11	—
Age (years)	47.5 ± 11.0	47.1 ± 7.4	0.9097
Males/females	7/6	6/5	0.7071
Smokers	5 (38.5%)	2 (18.2%)	0.5263
FMPS (%)	29.1 ± 21.7	44.8 ± 22.7	0.1134
FMBS (%)	12.5 ± 12.5	13.7 ± 9.7	0.8108
Upper/lower teeth	7/6	5/7	0.8430
Anterior/posterior teeth	10/3	6/6	0.3288
PPD (mm)	7.8 ± 1.3	7.5 ± 2.0	0.6988
CAL (mm)	9.0 ± 1.6	9.0 ± 2.0	0.9503
REC (mm)	1.2 ± 1.7	1.5 ± 1.6	0.5795
CEJ-BD (mm)	9.8 ± 1.6	9.9 ± 1.7	0.8924
INFRA (mm)	6.2 ± 2.2	6.6 ± 2.0	0.5790

FMPS, full-mouth plaque score; FMBS, full-mouth bleeding score; PPD, pocket probing depth; CAL, clinical attachment level; REC, recession; CEJ-BD, distance from cemento-enamel junction to the bottom of the defect; INFRA, the intra-osseous component of the defect.

Statistical analysis

Statistical analysis was performed using a software package (MedCalc®, MedCalc Software, Mariakerke, Belgium). The patient was regarded as the statistical unit. Data were expressed as mean ± SD.

Significance of mean differences between pre- and post-surgery scores was analyzed using Student's *t*-test for paired observations. Differences between test and control groups were calculated using the χ^2 test or Student's *t*-test for unpaired observations. The level of significance was set at 5%. Under $\alpha = 0.05$, the study had sufficient statistical power to reveal a true difference in CAL change when the observed difference between test and control treatment modalities was 2.3 mm.

Table 2. Pocket probing depth (PPD) and clinical attachment level (CAL) in intra-osseous defects treated with Biostite® and Bio-Oss® grafts as measured (in mm) at baseline, 6 months and 12 months following surgery (mean ± SD)

	PPD			CAL		
	baseline	6 months	12 months	baseline	6 months	12 months
Biostite® ($N = 13$)	7.8 ± 1.3	4.5 ± 2.1*	3.6 ± 1.6*	9.0 ± 1.6	6.7 ± 2.3*	6.1 ± 1.9*
Bio-Oss® ($N = 11$)	7.5 ± 2.0	3.6 ± 1.3*	3.1 ± 1.0*	9.0 ± 2.0	5.8 ± 1.5*	5.0 ± 1.5*
$p^{\dagger}$	0.6988	0.3878	0.7793	0.9503	0.3028	0.2190

*Significantly different from baseline ($p < 0.001$, Student t-test for paired observations).
†Significance of differences between groups (Student's *t*-test for unpaired observations).

Results

The patient and defect characteristics of the test and control groups are summarized in Table 1.

A total of 24 patients (13 test and 11 control) completed the 12-month follow-up period. Mean age was 47.5 years (range: 30–64 years) for the test group, and 47.1 years (range: 35–62 years) for the control group. No statistically significant differences were found between groups for any of the patients characteristics at baseline (Table 1).

A total of 24 defects, one for each patient, were treated. Thirteen defects were treated with Biostite® and 11 defects with Bio-Oss®. No statistically significant differences were found between groups in arch and/or tooth location of defects. Overall, 10 defects were located in central or lateral incisors, six in canines, and eight in premolars or molars. In all patients post-operative healing was uneventful, and no adverse complications were observed.

Pre-surgery FMPS was 29.1 ± 21.7% in subjects treated with Biostite® and 44.8 ± 22.7% in those treated with Bio-Oss® (Table 1). At 12 months post-surgery, FMPS resulted unchanged in test group (29.0 ± 12.2%), and significantly decreased in control group (23.7 ± 12.3%). FMBS shifted from 12.5 ± 12.5% before surgery to 11.3 ± 5.4% 12 months after surgery in test group, and from 13.7 ± 9.7% to 5.4 ± 6.6% in control group. No statistically significant differences in FMPS and FMBS changes were found between groups.

CEJ-BD distance was 9.8 ± 1.6 mm (range: 6–12 mm) for test defects, and 9.9 ± 1.7 mm (range: 8–12.5 mm) for control defects. In defects treated with Biostite®, the INFRA component was 6.2 ± 2.2 mm (range: 4–10 mm), while in those treated with Bio-Oss® was 6.6 ± 2.0 mm (range: 4–10 mm). No significant differences were observed for any of the intra-surgery defect characteristics between test and control sites at baseline (Table 1).

Table 2 shows pre- and post-surgery PPD and CAL recordings, as assessed in both group treated with Biostite® and Bio-Oss®. Initial PPD and CAL were 7.8 ± 1.3 and 9.0 ± 1.6 mm, respectively, for test group, and 7.5 ± 2.0 and 9.0 ± 2.0 mm, respectively, for control group. No significant differences for PPD and CAL measurements were assessed between groups at baseline. At 6 months following surgery, the average gain in CAL was 2.3 ± 1.6 mm for the test sites ($p = 0.0064$) and 3.2 ± 1.6 mm for the control sites ($p = 0.0006$). Twelve months after surgery, CAL gain reached 2.9 ± 1.9 mm and 4.0 ± 2.5 mm, respectively (Table 3). No statistically significant differences were observed between groups at any observation interval (Table 2). Four defects (30.8%) in the test group and five defects (45.5%) in the control group presented CAL gain > 4 mm. No significant differences between groups were observed in frequency distribution of patients according to CAL gain (Table 4).

Table 3. Clinical and radiographic outcomes (in mm, mean ± standard deviation) at 1 year following treatment of intra-osseous defects with Biostite® and Bio-Oss® grafts

Outcome variable	Biostite® ($N = 13$)	Bio-Oss® ($N = 11$)	p-value
CAL gain	2.9 ± 1.9	4.0 ± 2.5	0.2190
PPD reduction	4.2 ± 2.1	4.4 ± 2.3	0.7793
REC increase	1.2 ± 1.9	0.4 ± 1.8	0.2696
DEPTH gain	2.5 ± 1.4	3.1 ± 1.8	0.3940

CAL, clinical attachment level; PPD, pocket probing depth; REC, recession; DEPTH, radiographic depth of the defect.

Table 4. Distribution of subjects in Biostite® and Bio-Oss® groups according to CAL gain, as assessed at 12 months following surgery

	CAL gain		
	0–2 mm	>2 to 4 mm	>4 mm
Biostite® ($N = 13$)	6	3	4
Bio-Oss® ($N = 11$)	4	2	5

CAL, clinical attachment level.

At 12 months post-surgery, PPD reduction was 4.2 ± 2.1 mm in the test sites ($p = 0.0000$) and 4.4 ± 2.3 mm in the control sites ($p = 0.0001$), without significant differences between groups. The 1-year gingival margin recession from baseline position amounted to 1.2 ± 1.9 mm in test group ($p = 0.0395$) and to 0.4 ± 1.8 mm in control group ($p = 0.5189$), the difference being not statistically significant (Table 3).

Radiographic DEPTH gain after 12 months of healing was 2.5 ± 1.4 mm for defects treated with Biostite® ($p = 0.0000$), and 3.1 ± 1.8 for defects treated with Bio-Oss® ($p = 0.0002$), which represented, respectively, a radiographic defect fill of 45.5% and 50.8% (Table 3). No significant difference in DEPTH gain was evident between groups.

Discussion

The aim of the present study was to evaluate the clinical outcome of reconstructive surgery in human deep intra-osseous defects grafted with a synthetic HA/equine Type I collagen/ chondroitin sulphate biomaterial (Biostite®), as compared to a bovine-derived HA xenograft (Bio-Oss®). A total of 24 patients were treated, 13 with Biostite® and 11 with Bio-Oss®. The results demonstrate that both treatment modalities provide clinically and statistically significant improvements in PPD and CAL measurements.

Intra-osseous defects treated with Bio-Oss® did show 1-year CAL gain and PPD reduction of 4.0 ± 2.5 and 4.4 ± 2.3 mm, respectively. These results exceeded those obtained in previous studies evaluating the same xenograft used alone in the treatment of deep intra-osseous defects (Richardson et al. 1999; Scheyer et al. 2002) where 6-month CAL gain varied from 1.7 ± 0.5 mm to 3.7 ± 1.5 mm, and PPD reduction ranged from 3.0 ± 1.7 to 3.9 ± 1.3 mm. To the best of our knowledge, this is the first report on the use of Biostite® in periodontal reconstructive surgery. Defects grafted with Biostite® demonstrated a 1-year CAL gain averaging 2.9 ± 1.9 mm, and a PPD reduction of 4.2 ± 2.1 mm. In a previous study evaluating Biostite® graft in association with guided tissue regeneration using collagen membrane, similar CAL gain and PPD reduction were observed in deep intra-osseous defects (Benqué et al. 1997).

Reconstructive properties of HA-based biomaterials in the treatment of intra-osseous defects have been recently reviewed (Trombelli et al. 2002a). Studies have shown that the addition of collagen to HA may increase *in vitro* fibroblast chemotactic activity (Postlethwaite et al. 1978), provides for the formation of space for a rapid bone ingrowth, and promotes periodontal fiber regeneration and new cementum formation (Yaffe et al. 1984; Blumenthal et al. 1986; Minabe et al. 1988; Sugaya et al. 1989). Moreover, chondroitin sulphate has been shown to promote mineralization of three-dimensional collagen matrices when seeded either with bone-derived cells (Bouvier et al. 1990a; Serre et al. 1993) or dental-pulp cells (Bouvier et al. 1990b). Adding glycosaminoglycans to Type I collagen-reconstituted matrices modifies cell proliferation and migration and may induce the expression of a differentiated phenotype cell (Docherty et al. 1989).

From a surgical standpoint, the addition of collagen to the anorganic HA component in Biostite® gives the biomaterial favorable handling characteristics, which include: (i) ease of delivery to the site, with the possibility of fragmenting or dissecting in blocks; (ii) ease of adaptation to different defect morphology; (iii) ability of the material to adhere to the defect walls, stabilizing the graft into the defect; (iv) increase of inter-particle binding, preventing excessive particle dispersion; (v) capability to favor clot formation and stabilization due to collagen hemostatic properties.

Although statistical analysis revealed no significant differences between treatments, a trend towards a greater improvement in treatment outcome was observed in patients treated with Bio-Oss®. This result may be partly due to better plaque and bleeding scores, although not statistically significant, as observed in Bio-Oss® group compared to Biostite® group after 12 months of healing. Differences in regenerative/ osteoconductive properties between the two biomaterials, which, in turn, may relate to the differences in the physico-chemical and structural characteristics, can also be considered.

Variables related to substrate material, porosity, surface geometry and surface chemistry play a determinant role in osteoconductive capacities of a graft (Bauer & Muschler 2000). In particular, macroporosity (Daculsi & Passuti 1990) and relative surface area size (Kurioka et al. 1999) are important factors that could additionally favor bone formation and stability of HA bone substitutes. Structurally, when evaluating parameters such as the inner surface area (97 m^2/g), total porosity (70–75%), and pore size (300–1500 μm), Bio-Oss® most closely resembles human cancellous bone as compared to demineralized freeze-dried bone allograft and synthetic HA (Peetz 1997). The interconnected porous system of Bio-Oss® with a larger internal surface and an higher degree of porosity may be more favorable to initial blood clot stability and

vessel ingrowth, and could enhance osteoblastic migration thus promoting new bone formation (Jensen et al. 1996, Clergeau et al. 1996).

It is noteworthy that the observed difference in CAL gain between Bio-Oss® and Biostite® parallels the difference in post-operative REC levels between groups. One possible explanation for varying post-surgical shrinkage of supracrestal soft tissues may involve differences in particle size between biomaterials. Particles of Biostite® are spherical in shape with a granulometry varying from 0.16 to 0.20 mm, and are arranged to form inter-granular micropores of 200–300 μm (total porosity of 60%) (DePuy Bioland Laboratories, Toulouse, France; unpublished data). Size of cancellous HA particles of Bio-Oss® is higher than Biostite®, ranging from 0.25 to 1.0 mm. Large-sized particles may have provided a more solid physical scaffold, thus acting as a structural space filler and limiting post-operative collapse of supracrestal gingival tissues into the defect. It could also be hypothesized that the presence of residual, large-sized Bio-Oss® particles in supracrestal soft tissues may have resulted in a stronger mechanical resistance to probe penetration.

At 12 months post-surgery, radiographic DEPTH gain was similar between the 2 groups. In our material, radiographic depth of the interproximal defects was calculated as the vertical distance from the most coronal extension of the *radiopaque* crest to the most apical extension of the defect. Question arises whether the radiographically assessed defect fill following implantation of HA-based biomaterials could represent new bone formation, permanence of dense HA particles, or both. Although both biomaterials have been reported as resorbable, the presence of residual particles of grafted biomaterials intertwined with newly formed bone at 12 months cannot be excluded. Earlier histologic studies have documented resorption of both Bio-Oss® (Jensen et al. 1996, Berglundh & Lindhe 1997, Hämmerle 1998) and Biostite® (Parodi et al. 1996, Santarelli et al. 1996, Scabbia et al. 2003), by reporting the presence of osteoclastic-like cells around the implanted particles. However, several investigators have demonstrated the very slow resorption process of Bio-Oss® and the permanency of particles up to 6 years after implantation (Skoglund et al. 1997, Schlegel & Donath 1998, Piattelli et al. 1999, Hallman et al. 2001). The incorporation of collagen in Biostite® has been shown to enhance the resorption process in animals (Penaud et al. 1992) and humans (Parodi et al. 1996, Santarelli et al. 1996). Nevertheless, human biopsies taken at 6–9 months post-implantation of Biostite® have clearly demonstrated the presence of graft particles in close proximity to newly formed trabecular bone (Scabbia et al. 2003).

When considering clinical outcomes in relation to observation intervals, results indicate that further CAL gain and PPD reduction occurred from 6 to 12 months post-surgery for both treatment groups. This observation may be indicative of the biological duration of wound healing processes, thus suggesting the need for longer-term clinical evaluations following periodontal reconstructive surgery with graft biomaterials. Unfortunately, only limited evidence exist with respect to outcome assessment of grafting procedure after 12-month observation interval (Trombelli et al. 2002a). Time-related improvements in clinical parameters may be partly ascribed to the efficacy of the maintenance program to prevent bacterial re-infection, as confirmed by low levels of FMBS assessed in our patients throughout the healing period.

In conclusion, results from the present study indicate that surgical reconstructive treatment of deep intra-osseous defects with Biostite® and Bio-Oss® grafts resulted in clinically and statistically significant improvement in terms of CAL gain, PPD reduction and radiographic DEPTH gain.

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