Journal of Clinical Periodontology
Official Journal of The European Federation of Periodontology
Founded by the British, Dutch, French, German, Scandinavian, and Swiss Societies of Periodontology

J Clin Periodontol 2008; 35 (Suppl. 8): 117–135 doi: 10.1111/j.1600-051X.2008.01265.x

Journal of Clinical Periodontology

Clinical outcomes with bioactive agents alone or in combination with grafting or guided tissue regeneration

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Trombelli L, Farina R. Clinical outcomes with bioactive agents alone or in combination with grafting or guided tissue regeneration. J Clin Periodontol 2008; 35 (Suppl. 8): 117–135. doi: 10.1111/j.1600-051X.2008.01265.x.

Abstract

Aim: The purpose of the present review was to determine the clinical effect of the use of bioactive agents (BAs) for the treatment of intra-osseous and furcation defects.

Material and Methods: The effectiveness of the BAs was evaluated when used in addition to open flap debridement either alone or in association with grafts and/or guided tissue regeneration (GTR). Among the included agents, recombinant human platelet-derived growth factor-BB (rhPDGF-BB), platelet-rich plasma (PRP), commercially available enamel matrix derivative (cEMD) and peptide P-15 (P-15) have been clinically tested for treating periodontal defects.

Results and Conclusions: The results of the present review indicate that: (1) cEMD either alone or in combination with grafts can be effectively used to treat intra-osseous defects and the clinical results appear to be stable long term; (2) the additional use of a graft seems to enhance the clinical outcome of cEMD; (3) the combined use of rhPDGF-BB and P-15 with a graft biomaterial has shown beneficial effects in intra-osseous defects; (4) contrasting results were reported for PRP and graft combinations; and (5) limited evidence supports the use of BAs either alone or in association with graft/GTR for the treatment of furcation defects.

Key words: bioactive agents; furcation defects; growth factors; intra-osseous defects; morphogens; periodontal disease; periodontitis; regenerative therapy

Accepted for publication 20 May 2008

Reconstructive procedures have been used with varying success during the past decades to accomplish the *restitutio ad integrum* of the lost attachment apparatus in different types of periodontal lesions, including intra-osseous, furcation (inter-radicular) and recession defects. The most investigated reconstructive methods reported in the literature are based on the use of autogenous bone, bone substitutes, membranes for guided tissue regeneration (GTR) or a combination of the above. All these technologies, as used in association with an open flap debridement (OFD) procedure, have shown an additional effect when compared with OFD in terms of bone fill and clinical attachment gain in intra-osseous (Trombelli 2005) and class II furcation (Jepsen et al. 2002) defects. Histologic studies in humans (Dragoo & Sullivan 1973, Hiatt et al. 1978, Stahl et al. 1983, Bowers et al. 1989, Harris 2000, Yukna & Mellonig 2000) have demonstrated that GTR as well as some of the available grafting procedures may result in healing that can be termed ''true periodontal regeneration'', with new bone, cementum and periodontal ligament (PDL) regrowth. However, by means of both GTR and grafting procedures, a complete and predictable reconstruction of periodontal tissues is still difficult to obtain. Furthermore, there is a substantial variation in the clinical response to the same reconstructive procedure, which may be partly due to the limited biological potential inherent in the graft biomaterials and membranes in affecting the healing dynamics of the periodontal wound.

The main goal of periodontal regenerative procedures is the optimization and

Conflict of interest and source of funding statement

The Authors declare they have no conflict of interests.

This review was supported by the Research Centre for the Study of Periodontal Diseases, University of Ferrara, Italy, and by a grant from the University of Ferrara, Italy (STAMINA).

The 6th European Workshop on Periodontology was supported by an unrestricted educational grant from Straumann AG.

enhancement of the biological mechanisms of periodontal wound healing in order to maximize the extent of the restored periodontal apparatus (i.e. alveolar bone, PDL and cellular cementum). In essence, the nature and extent of the regeneration depends on the cells re-populating the wound that are responsible for the growth and differentiation of new tissue (Melcher 1976). In this respect, the chemotaxis, differentiation and synthetic activity of periodontal cells and their undifferentiated progenitors are modulated by biologically active molecules that reside in the extracellular matrix (Taba et al. 2005). Recently, advances in the areas of cellular and molecular biology have allowed the elucidation of functions of growth factors (GFs) and their participation in the different phases of periodontal wound healing. Recent in vitro and in vivo studies have confirmed that GFs can improve the capacity of tissues to regenerate, improving cellular chemoattraction, differentiation and proliferation. GFs, as used in tissue repair models, have been shown to regulate important cellular events involved in wound healing by binding to specific cell surface receptors, mimicking their function during embryonic life. Recently, specific GFs have also been used to treat different periodontal defects in humans.

The aim of this review is to determine the effect of the use of bioactive agents (BAs) for the treatment of intra-osseous and furcation defects. In particular, the effectiveness of the available BAs will be evaluated when used in addition to OFD either alone or in association with grafts and/or GTR.

Material and Methods

Definition of BAs

Under the term BAs, we have considered and analysed two classes of potent biologically active molecules. Firstly, we have considered a wide range of GFs including bone morphogenetic proteins (BMPs). Secondly, we have considered a number of other available BAs including platelet-rich plasma (PRP) preparations as well as two commercially available products: enamel matrix derivative (cEMD, Emdogain®; Straumann AG, Basel, Switzerland) and a 15-amino-acid peptide (P-15, PepGen p-15®; DENSPLY CeraMed Dental, Lakewood, CO, USA).

Definition of ''graft'' *versus* ''carrier''

Commonly, reported carriers (or delivery devices) for BAs are collagen in the form of a sponge, membrane or gel and gelatin with varying degrees of cross-linking, as well as biodegradable synthetic polymers, such as poly(lactide-co-glycolide) and propylene-glycol alginate (Wang et al. 2005). BAs can be incorporated into these materials and potentially released in a controlled, sustained manner to enhance tissue regeneration.

Although the physico-chemical characteristics of the carrier may substantially modulate the effect that the BA may exert on the regenerative response, generally, the delivery device is not directly active per se in the regeneration process. In contrast, for some BA the active molecule is introduced into the defect site by a bone substitute or graft biomaterial with putative biological properties on periodontal wound healing. When this was the case, we regarded that as an association of the BA with a graft and, therefore, the available evidence was included in the ''association with grafts and/or GTR'' section.

Study population

When considering the clinical effects of Bas, we have only included aggressive and chronic periodontitis patients where the loss of periodontal attachment had been due to infective/inflammatory periodontal diseases. The evaluation of the clinical effects was related to different periodontal lesions: intra-osseous and furcation (inter-radicular) defects.

Eligibility criteria for study inclusion

A literature search, for articles published up to and including December 2007, was performed using the MEDLINE database and the Cochrane Oral Health Group Specialist Register. We used a combination of MeSH terms and keywords designed to identify all pertinent articles dealing with BAs in periodontal therapy. Only studies (published or in press) in English were included. We also scanned the reference lists of review articles, relevant texts, previous workshops and all primary studies identified.

We first screened all the BAs that have been shown to have an in vitro, pre-clinical and/or a clinical effect on periodontal regeneration. However, for the purpose of this review, we only considered the BAs that have been clinically tested.

To assess the clinical effectiveness of each BA, different study designs and methodology that provide different levels of evidence were considered: proof-of-principle (descriptive) studies, randomized clinical trials (RCTs) and/or systematic reviews (SRs).

For any BA, the available evidence related to their clinical use was analysed in three different sections: proof of principle, clinical effectiveness and association with grafts and/or GTR.

In the ''proof of principle'' section, we reported the descriptive studies showing the outcomes, both clinical and histological, derived from the clinical use of the BA. For this reason, we have pooled case reports, case series, case–control studies or arms of RCTs where the BA was used even though the trial was not specifically designed to assess the effectiveness of the BA per se.

In the ''clinical effectiveness'' section, we aimed to determine the clinical effectiveness of the BA when used with an OFD procedure compared with OFD either alone or with the BA carrier. In other words, we selected proper studies where the study design aimed to elucidate both the effect of the treatment as well as the mere contribution of the BA. Hence, in this section we only included RCTs or SRs where the comparison OFD+BA *versus* OFD (w/wo BA carrier) was assessed.

In the ''association with grafts and/or GTR'' section, we reviewed the evidence (descriptive studies, RCTs and SRs) to determine: (i) the clinical effect of the association of the BA with graft and/or GTR; (ii) the clinical adjunctive effect of the BA when combined with graft and/or GTR with respect to the graft and/or GTR alone; and (iii) the clinical adjunctive effect of the graft and/or GTR when combined with the BA with respect to the BA alone.

For each BA when used alone or in association with graft/GTR, we also evaluated *patient-centred outcomes* as: adverse effects related to the additional use of BA; post-operative complications; change in aesthetic appearance; estimation of patient well-being derived from additional use of BA; cost-effectiveness (including evaluation of additional treatment time and costs for placement of the BA); and risk benefit derived from the use of the BA. *Long-term results* of the reconstructive procedure (i.e. follow-up period $\geqslant$2 years) were also assessed.

Results

Table 1 shows all the BAs that have been analysed as well as the level of

scientific evidence about their clinical use. Data concerning the clinical outcome of the great majority of BAs are supported by descriptive studies. Except for cEMD, RCTs aimed at evaluating the clinical effectiveness of the available BAs either per se or in association with graft/GTR are scarce, with a limited number of patients and, therefore, are not suitable for meta-analysis. Therefore, we decided to carry out the present review with a narrative layout rather than a SR approach.

Platelet-derived growth factor (PDGF)

Proof of principle

No case report/series evaluating the effectiveness of PDGF alone, as applied by a delivery device, in the treatment of intra-osseous and furcation defects, are at present available.

Clinical effectiveness

No RCTs evaluating the clinical effectiveness of PDGF alone, as applied by a delivery device, in the treatment of intra-osseous or furcation defects, are at present available. However, an RCT assessed the combined application of recombinant human PDGF-BB (rhPDGF-BB) in association with recombinant human insulin-like growth factor-1 (rhIGF-1), delivered by a methylcellulose gel, in intra-osseous defects (Howell et al. 1997a). The combinations were applied in a low dose (50 μg/ml of each GF) and in a high dose (150 μg/ml of each GF), while the control treatment was represented by conventional periodontal flap surgery or surgery plus vehicle. Only the high dose of rhPDGF-BB/rhIGF-1 resulted in a statistically significant new bone formation and defect fill with respect to controls after 6–9 months of healing (Howell et al. 1997a).

Association with grafts and/or GTR

The association of rhPDGF-BB with grafts (bone substitutes) has been evaluated in vitro (Mott et al. 2002, Papadopoulos et al. 2003, Vavouraki et al. 2003, Bateman et al. 2005) as well as in human clinical studies for the treatment of furcation as well as intra-osseous defects (Camelo et al. 2003, Nevins et al. 2003, McGuire et al. 2006, Nevins et al. 2007).

In two case reports/series of a limited number of patients, a demineralized freeze-dried bone allograft (DFDBA) saturated with different concentrations of rhPDGF-BB (0.5, 1.0 and 5.0 mg/ml) was applied to class II furcation defects (Camelo et al. 2003, Nevins et al. 2003). When considering the 9-month defect-associated clinical parameters, the rhPDGF-BB–DFDBA treatment resulted in a robust improvement with respect to the pre-surgery values. In particular, the horizontal probing depth (PD) reduction ranged from 3.4 mm (Nevins et al. 2003) to 3.5 mm (Camelo et al. 2003), the vertical PD reduction ranged from 4.00 mm (Nevins et al. 2003) to 4.25 mm (Camelo et al. 2003) and the gain in clinical attachment level (CAL) varied from 3.2 mm (Nevins et al. 2003) to 3.75 mm (Camelo et al. 2003). An exploratory analysis of the rhPDGF-BB dose–response impact in the rhPDGF-BB–DFDBA association in class II furcation defects revealed no significant differences between different doses (Nevins et al. 2003). No systemic or local adverse reactions were registered (Nevins et al. 2003). Moreover, the histological analysis on biopsy specimens suggested the potential of rhPDGF-BB–DFDBA association to restore a complete functional periodontal apparatus, including new bone, cementum and PDL. At present, the data available do not allow us to determine the extent of the adjunctive clinical effect of the BA over the sole effect of the graft in the treatment of furcation defects.

A case report also suggested that rhPDGF-BB association with graft biomaterials, such as DFDBA, may lead to substantial PD reduction, CAL gain and radiographic defect fill (Nevins et al. 2003). Recently, the clinical and radiographic effect of the combination of rhPDGF-BB with a freeze-dried bone allograft (FDBA) in periodontal intra-osseous defects has been investigated in a human case series (Nevins et al. 2007). Surgical re-entries were performed up to 11 months post-surgery, revealing complete bone fill and an improvement in clinical and radiographic parameters (Nevins et al. 2007).

A multicentre randomized triple-blind controlled trial conducted on 180 patients evaluated the clinical and radiographic reconstructive outcomes of two different doses of rhPDGF-BB (0.3 and 1.0 mg/ml) in combination with β-tricalcium phosphate (β-TCP) compared with β-TCP alone in the treatment of deep intra-osseous defects (Nevins et al. 2005). After 6 months of healing, both

Table 1. List of the bioactive agents included in the present review and level of evidence about their clinical effect in intra-osseous (I) and furcation (F) defects when used alone or in combination with grafts and/or guided tissue regeneration (GTR) (in parenthesis: number of available studies)

Bioactive agent	Bioactive agent alone		Bioactive agent in association with grafts		Bioactive agent in association with GTR		Bioactive agent in association with grafts+GTR	
	proof of principle	clinical effectiveness	proof of principle	clinical effectiveness	proof of principle	clinical effectiveness	proof of principle	clinical effectiveness
PDGF	–	I(1)*	I(2),F(2)	I(1)	–	–	–	–
IGF	–	I(1)†	–	–	–	–	–	–
FGF	–	I(1)‡	–	–	–	–	–	–
BMPs	–	–	–	–	–	–	I(1)	–
cEMD	I(60), F(5)	I(16)	I(8)	I(12)	I(1), F(1)	I(5)	I(2)	I(1)
PRP	I(2)	–	I(2)	I(5)	I(2)	–	–	I(7), F(1)
Peptide P-15	–	–	I(4), I+F(1)	I(4)	–	–	I(1)	–

*Only in association with IGF.

†Only in association with PDGF.

‡Non-retrievable data (Murakami 2007).

PDGF, platelet-derived growth factor; IGF, insulin-like growth factor; FGF, fibroblast growth factor; BMP, bone morphogenetic protein; cEMD, commercially available enamel matrix derivative; PRP, platelet-rich plasma.

test and control treatments provided an improvement in clinical and radiographic parameters. However, when test groups were compared with control groups, the addition of either a low or a high dose of rhPDGF-BB to β-TCP failed to enhance the extent of CAL gain significantly. Despite this observation, the rate of gain in CAL was shown to be more rapid in the 0.3 mg/ml rhPDGF-BB+β-TCP group over the control group (significantly different at 3 months). On the other hand, both test groups were significantly more effective than the control group (β-TCP+buffer) in the improvement of radiographically determined linear bone growth and percentage of bone defect fill. Moreover, a statistically significant difference was detected between test groups for both linear bone growth (1.0 mg/ml rhPDGF-BB: 1.5 mm; 0.3 mg/ml rhPDGF-BB: 2.6 mm; $p<0.002$) and percentage of bone defect fill (1.0 mg/ml rhPDGF-BB: 34%; 0.3 mg/ml rhPDGF-BB: 57%; $p=0.002$), favouring the 0.3 mg/ml rhPDGF-BB dose (Nevins et al. 2005).

The evaluation of PDGF in association with GTR is limited to pre-clinical animal models (Wang et al. 1994, Cho et al. 1995, Park et al. 1995), showing a strong potential of the combined approach in the regeneration of the lost periodontal apparatus compared with GTR alone (Park et al. 1995). Unfortunately, the available evidence does not allow us to determine the adjunctive clinical effect of PDGF over GTR.

Patient-centred outcomes

For the combined use of rhPDGF-BB and rhIGF-1, the most frequent adverse events were associated with the periodontal surgery and included pain and discomfort at the operative site, sensitivity and oedema. Treatment-emergent abnormal laboratory events were reported for five out of 38 patients and included elevated liver enzymes (SGOT and SGPT), lymphocytosis and haematuria, which were present at both baseline and 28 days following surgery (Howell et al. 1997a).

No adverse events that could be related to the use of rhPDGF-BB in combination with β-TCP were reported (Nevins et al. 2005).

Long-term results

When some of the rhPDGF-BB+β-TCP-treated defects ($N=4$) included in a multicentre clinical trial (Nevins et al. 2005) were re-evaluated at 24 months, a clinical stability of the reconstructive outcome was observed (McGuire et al. 2006). A tendency towards an increased bone growth and defect fill was observed between 6 months and 2 years (McGuire et al. 2006).

Fibroblast growth factor (FGF)

Proof of principle

No clinical studies evaluating the effect of FGF in the treatment of intra-osseous or furcation defects are at present available.

Clinical effectiveness

Recent data of Phase II clinical trial demonstrated that FGF-2 may be effective in regenerating periodontal tissue (Murakami 2007, non-retrievable data).

Association with grafts and/or GTR

No clinical studies evaluating the effect of the association of FGF with grafts and/or GTR in the treatment of intra-osseous or furcation defects are at present available.

Patient-centred outcomes

No data on patient-centred outcomes are at present available.

Long-term results

No data on long-term results are at present available.

IGF

Proof of principle

No clinical studies evaluating the use of IGF alone in the treatment of intra-osseous or furcation defects are at present available.

Clinical effectiveness

A single RCT evaluated the clinical effectiveness of two different doses of rhIGF-1 in association with rhPDGF-BB in the treatment of periodontal intra-osseous defects (Howell et al. 1997a). For details, see the paragraph ''PDGF – clinical effectiveness''.

Association with grafts and/or GTR

No studies investigating the association of IGF-1 with grafts and/or GTR are at present available.

Patient-centred outcomes

For details, see the paragraph ''PDGF – patient-centred outcomes''.

Long-term results

No data on long-term results are at present available.

BMPs

BMPs are members of the TGF-β superfamily, with the exception of BMP-1, which is a pro-collagen C-protease (Kessler et al. 1996). To date, several BMPs have been identified and characterized (Ripamonti & Renton 2006). In general, BMPs exert multiple effects on bone by: (1) acting as mitogens on undifferentiated mesenchymal cells and osteoblast precursors; (2) inducing the expression of the osteoblast phenotype (e.g. increasing alkaline phosphatase activity in bone cells); and (3) acting as chemoattractants for mesenchymal cells and monocytes as well as binding to extracellular matrix type IV collagen.

Proof of principle

No clinical studies evaluating the use of BMPs in the treatment of intra-osseous or furcation defects are at present available.

Clinical effectiveness

No RCTs are at present available evaluating the adjunctive clinical effect of BMPs in the treatment of intra-osseous or furcation defects.

Association with grafts and/or GTR

The evaluation of the effect of a combination of BMPs with a graft/bone substitute relates to one single histomorphometric study in humans where the association of BMP-3 (osteogenin) and two different biomaterials (purified bovine collagen and DFDBA) has been evaluated (Bowers et al. 1991). Test treatments consisted of the association of BMP-3 with DFDBA or bovine collagen; control groups consisted of the grafts used alone. The histologic analysis, conducted at 6-month re-entry, revealed that osteogenin combined with DFDBA significantly enhanced regeneration of a new attachment apparatus and component tissues. DFDBA plus osteogenin and DFDBA alone formed significantly more new attachment apparatus and component tissues than either

the tendon-derived matrix plus osteogenin or the tendon-derived matrix alone (Bowers et al. 1991).

No clinical studies evaluating the association between BMPs and GTR are at present available.

Patient-centred outcomes

No data on patient-centred outcomes are at present available. In particular, in the only study dealing with the use of BMP (BMP-3) for periodontal defects, safety assessment for either local or systemic adverse events was not performed (Bowers et al. 1991).

Long-term results

No data on long-term results are at present available.

PRP

Under the term ''PRP,'' we have grouped different preparations characterized by a high concentration of platelets, obtainable by a single- or a double-step centrifugation of autologous blood (Tamimi et al. 2007). These preparations have also been referred as ''autologous platelet concentrate'', ''platelet pellet'' or ''platelet gel'' (Marx 2001).

Proof of principle

Case reports/series have suggested a beneficial effect of PRP in periodontal regenerative procedures (Papli & Chen 2007). In a study that compared the effect of GTR *versus* PRP, the PRP-treated arm included five intra-osseous defects. PRP treatment resulted in a PD reduction of 3.0 mm, a CAL gain of 2.2 mm and a recession (REC) increase of 0.8 mm after 52 weeks of healing. Radiographic measures revealed a bone height gain of 3.2 mm with a defect angle increase at 1 year (Papli & Chen 2007). The combined application of PRP and human mesenchymal stem cells isolated from the iliac crest in intra-bony defects was recently explored in one patient with multiple intra-osseous defects. Re-examination revealed a 4 mm CAL gain and a 4 mm reduction in PD, with radiographic evidence of defect fill (Yamada et al. 2006).

Clinical effectiveness

No RCTs are at present available evaluating the adjunctive clinical effect of PRP in the treatment of intra-osseous or furcation defects.

One controlled clinical trial evaluated the effect of PRP in post-extraction defects located distally to the second mandibular molars that were related to the surgical extraction of the impacted third molars. They found significantly greater PD reduction and CAL gain for the PRP-treated group compared at 18 weeks post-surgery (Sammartino et al. 2005). It should be considered that the study population seems to mostly include young periodontally healthy individuals and that experimental sites presented incidental, and not periodontitis-induced, loss of periodontal attachment.

Association with grafts and/or GTR

The use of PRP combined with several types of grafts, such as bovine-derived xenografts with (Döri et al. 2008) or without cEMD (Lekovic et al. 2002, Hanna et al. 2004, Okuda et al. 2005, Ouyang & Qiao 2006, Yilmaz et al. 2007), allografts (Demir et al. 2007, Yassibag-Berkman et al. 2007) and autografts (Czuryszkiewicz-Cyrana & Banach 2006), has been evaluated only in the treatment of periodontal intra-osseous defects. Overall, the association of PRP and graft biomaterials led to a statistically significant improvement of the defect-associated clinical parameters with respect to the pre-surgical condition. A significant CAL gain, ranging from 2.1 mm (Yassibag-Berkman et al. 2007) to about 5.0 mm (Döri et al. 2008), was observed. However, when the additional effect of PRP over the graft was assessed, some studies suggested a significantly positive enhancement of the regenerative outcomes of the graft by the use of PRP (Hanna et al. 2004, Okuda et al. 2005, Ouyang & Qiao 2006), while other studies reported no additional benefit of PRP over the graft alone (Demir et al. 2007, Yassibag-Berkman et al. 2007, Döri et al. 2008) (Table 2).

The combination of PRP with GTR resulted in a significant CAL gain (Mauro et al. 2003, Keles et al. 2006) and radiographically determined bone height (Keles et al. 2006) in the treatment of periodontal intra-osseous defects. The design of the available studies did not allow for the determination of the adjunctive effect of PRP+GTR over either GTR alone or PRP alone.

The combination of PRP with both grafts and GTR has been evaluated by several authors both in intra-osseous (Camargo et al. 2002, Lekovic et al. 2002, Camargo et al. 2005, Christgau et al. 2006, Döri et al. 2007a, b, Yassibag-Berkman et al. 2007) and in furcation defects (Lekovic et al. 2003). In intra-osseous defects, this combination showed a significant CAL gain and osseous defect fill, ranging from 2.5 mm (Yassibag-Berkman et al. 2007) to 5.0 mm (Christgau et al. 2006) and from 4.8 mm (Camargo et al. 2002) to 5.0 mm (Lekovic et al. 2002), respectively. The combination of PRP+GTR with bovine porous bone matrix led to a significantly greater CAL gain and defect bone fill over an OFD procedure (Camargo et al. 2005). When the additional effect of PRP was evaluated, all the available studies reported no additional benefit of PRP over the GTR+graft alone (Christgau et al. 2006, Döri et al. 2007a, b) (Table 3).

In a split-mouth study, the effect of PRP combined with bovine-derived porous bone mineral (BPBM) and GTR has been compared with OFD in class II furcation defects (Lekovic et al. 2003). The results suggested a significantly greater CAL gain, vertical and horizontal osseous defect fill of PRP/graft/GTR-treated defects with respect to OFD-treated ones (Lekovic et al. 2003). At present, the available evidence does not allow for the determination of the adjunctive effect of PRP over graft+GTR alone in the furcation defects (Table 3).

Patient-centred outcomes

No adverse events were reported following application of PRP in periodontal defects. In contrast, clinical observations suggest a more rapid healing with less post-operative pain in PRP-treated sites compared with controls (Papli & Chen 2007). Moreover, an uneventful post-operative healing was described when PRP was used in conjunction with grafts (e.g. Lekovic et al. 2002, Hanna et al. 2004, Okuda et al. 2005, Yassibag-Berkman et al. 2007). When the combined use of PRP with both grafts and GTR was evaluated, non-PRP-grafted sites exhibited a higher incidence of membrane exposure with respect to PRP-grafted sites (Christgau et al. 2006, Döri et al. 2007a, b)

Long-term results

No data on long-term results are at present available.

Table 2. Randomized clinical trials (RCTs) evaluating the effectiveness of platelet-rich plasma (PRP) in association with grafts with respect to either graft alone or PRP alone in the treatment of intra-osseous defects

PRP association with	Adjunctive effect of PRP over graft (PRP+graft *versus* graft)		Adjunctive effect of graft over PRP (PRP+graft *versus* PRP)	
	reference	result	reference	result
Autogenous bone grafts	No available RCTs		No available RCTs	
Allogeneic bone grafts	No available RCTs		No available RCTs	
Xenografts	Hanna et al. (2004)	↑ (PD reduction, CAL gain)	No available RCTs	
	Okuda et al. (2005)	↑ (PD reduction, CAL gain, relative CAL gain) = (IBD change)		
	Ouyang & Qiao (2006)	↑ (PD reduction, CAL gain, bone probing reduction, defect bone fill, radiographic gain in alveolar bone mass)		
	Döri et al. (2008)	= (PD reduction, CAL gain, post-operative REC increase) (graft was used in association with cEMD)		
Alloplastic materials	Yassibag-Berkman et al. (2007)	= (PD reduction, CAL gain, clinical and radiographic bone fill) ↓ (clinical bone fill at 12 months)	No available RCTs	
	Demir et al. (2007)	= (PD reduction, CAL gain, defect fill)		

Result: ↑, positive adjunctive effect of combined treatment with respect to the single therapy; =, null adjunctive effect of combined treatment with respect to the single therapy; ↓, detrimental effect of combined treatment with respect to the single therapy. In parentheses: the study parameters where the positive or null adjunctive effect of the combined treatment was reported.
PD, pocket probing depth; CAL, clinical attachment level; REC, recession depth; IBD, radiographic intra-bony defect depth.

Table 3. Randomized clinical trials (RCTs) evaluating the effectiveness of platelet-rich plasma (PRP) in association with grafts+guided tissue regeneration (GTR) with respect to either graft+GTR alone or PRP alone in the treatment of intra-osseous defects

PRP association with	Adjunctive effect of PRP over graft+GTR (PRP+graft+GTR *versus* graft+GTR)		Adjunctive effect of graft+GTR over PRP (PRP+graft+GTR *versus* PRP)	
	reference	result	reference	result
Resorbable membranes	Christgau et al. (2006)	= (PD reduction, CAL gain, post-operative REC increase, relative CAL gain) ↑ (radiographic bone density gain at 6 months)	No available RCTs	
	Döri et al. (2007a)	= (PD reduction, CAL gain, post-operative REC increase)		
Non-resorbable membranes	Döri et al. (2007b)	= (PD reduction, CAL gain, post-operative REC increase)	No available RCTs	

Result: ↑, positive adjunctive effect of combined treatment with respect to the single therapy; =, null adjunctive effect of combined treatment with respect to the single therapy. In parentheses: the study parameters where the positive or null adjunctive effect of the combined treatment was reported.
PD, pocket probing depth; CAL, clinical attachment level; REC, recession depth.

cEMD

cEMD is a compound consisting mainly (about 90%) of amelogenin (Hoang et al. 2002) and traces of related proteins derived from porcine tooth buds, including albumin, amelin and enamelin (Maycock et al. 2002), in a propylene-glycol alginate carrier. Although cEMD was stated not to contain any of the known GFs (Gestrelius et al. 1997), TGF-β1 (or a TGF-β-like substance) might act as the principal bio-active factor in cEMD to elicit cell type-specific growth modulation (Bosshardt 2008).

Proof of principle

Several reports have demonstrated a substantial CAL gain after regenerative procedures supported by the application of cEMD in intra-osseous defects (Heden et al. 1999, Mellonig 1999, Rasperini et al. 1999, 2005, Sculean et al. 1999a, b, c, 2000a, 2001a, b, 2003a, b, 2004a, 2005a, 2006a, b, 2007a, b, Heard et al. 2000, Heden 2000, Lekovic et al. 2000, Manor 2000, Martu et al. 2000, Parashis & Tsiklakis 2000, Parodi et al. 2000, Yukna & Mellonig 2000, Bratthall et al. 2001, Pietruska 2001, Pietruska et al. 2001, Cardaropoli & Leonhardt 2002, Windisch et al. 2002, Trombelli et al. 2002a, Bonta et al. 2003, Forabosco et al. 2003, Schwarz et al. 2003, Silvestri et al. 2003, Froum et al. 2004, Gurinsky et al. 2004, Parodi et al. 2004, Sanz et al. 2004, Trejo & Weltman 2004, Tsitoura et al. 2004, Parashis et al. 2004, 2006, Vandana et al. 2004, Cortellini & Tonetti 2005, 2007a, b, Harrel et al. 2005, Majzoub et al. 2005, Sipos et al. 2005, Bokan et al. 2006, Bosshardt et al. 2006, Heden & Wennstrom 2006, Kuru et al. 2006, Zucchelli et al. 2006, Farina et al. 2007, Guida et al. 2007, Miliauskaite et al. 2007,

Ozcelik et al. 2007a). Often, the improvement in clinical measurements was accompanied by a radiographically assessed defect fill. The clinical reconstructive outcomes of cEMD may be associated with the preservation of supracrestal soft tissue (Trombelli et al. 2002a) or, more recently, a minimally invasive surgical approach (Harrel et al. 2005, Cortellini and Tonetti 2007a, b).

In a preliminary case series of 10 patients, eight buccal and eight lingual degree II furcation involvements were treated with cEMD. At 6 months, the mean horizontal CAL of the buccal defects was reduced from 4.0 ± 1.3 to 2.6 ± 1.4 mm, and the mean vertical CAL was reduced from 5.2 ± 2.0 to 4.0 ± 1.6 mm. At the lingual defects, the mean horizontal CAL was reduced from 3.6 ± 1.3 to 3.1 ± 1.1 mm, and the mean vertical CAL was reduced from 5.6 ± 2.0 to 4.3 ± 1.8 mm. At 12 and 36 months, the clinical parameters remained similar, without any further clinical improvement (Donos et al. 2003a). The clinical effectiveness of cEMD in the treatment of furcation defects was investigated in one arm of a multicentre RCT comparing cEMD and GTR on class II furcation defects of mandibular molars (Jepsen et al. 2004, Meyle et al. 2004, Hoffmann et al. 2006). In the cEMD group (45 defects), the median reduction of the horizontal furcation depth, as assessed at re-entry at 14 months, amounted to 2.8 mm. Among the 45 cEMD-treated sites, eight exhibited a complete furcation closure at 8–14 months, 27 partial closure, nine no change and one deterioration. The frequency of patients experiencing either no pain or swelling at 1 week post-surgery was 62% and 44%, respectively (Jepsen et al. 2004). In the mid-furcation site, PD changed from 3.5 mm at baseline to 3.0 mm at 14 months, while CAL changed from 7.5 mm at baseline to 7.0 mm at 14 months (Meyle et al. 2004).

Clinical effectiveness

The clinical effectiveness of cEMD for the treatment of intra-osseous defects has been reviewed recently (Venezia et al. 2004, Trombelli 2005). Three SRs are currently available to determine the additional effect of cEMD with respect to OFD (Trombelli et al. 2002b, Giannobile & Somerman 2003, Esposito et al. 2005). Overall, the results derived from all SRs indicate that there were significant differences between cEMD and OFD in the post-surgical changes of CAL, PD and radiographic marginal bone levels. There was a significant gain in CAL for cEMD compared with OFD defects, with a weighted mean difference (WMD) ranging from 1.20 mm (95% CI: 0.71–1.69, $p < 0.0001$) (Esposito et al. 2005) to 1.33 mm (95% CI: 1.01–1.42, $p < 0.001$) (Trombelli et al. 2002b). A significant reduction in PD was also observed, with a WMD ranging from 0.77 mm (95% CI: 0.54–1.00, $p = 0.0009$) (Esposito et al. 2005) to 1.60 mm (95% CI: 0.59–2.62, $p < 0.001$) (Trombelli et al. 2002b). There was no significant difference in the changes of marginal bone (WMD 1.08 mm; 95% CI: -0.72 to 2.89) and REC between cEMD and OFD (WMD 0.04 mm; 95% CI: -0.32 to 0.40) (Esposito et al. 2005). However, in both SRs, the analysis showed statistically significant heterogeneity in the results among studies for both CAL and PD changes, implying that the differences between the outcomes of included studies are greater than that would occur by chance. In other words, the studies appear too dissimilar (some studies favour cEMD, and some studies show no difference between treatments) in certain respects to be sensibly combined, and overall summary values should be interpreted with caution.

No RCTs are available in order to determine the adjunctive effect of cEMD over OFD in the treatment of furcation defects.

Association with grafts

The clinical use of cEMD in combination with grafting of bone substitutes is limited to studies on periodontal regenerative procedures of intra-osseous defects. Several types of graft, such as autogenous bone grafts (Leung & Jin 2003, Trombelli et al. 2006, Guida et al. 2007), allogeneic bone grafts (Rosen & Reynolds 2002, Gurinsky et al. 2004), xenografts (Lekovic et al. 2000, 2001b, Camargo et al. 2001, Scheyer et al. 2002, Velasquez-Plata et al. 2002, Sculean et al. 2002b, 2003c, Zucchelli et al. 2003, Döri et al. 2005, 2008) and alloplastic materials (Sculean et al. 2002a, 2005a, b, 2007a, Döri et al. 2005, Bokan et al. 2006, Kuru et al. 2006, Jepsen et al. 2008), have been investigated in conjunction with cEMD. RCTs evaluating the effectiveness of the combination of cEMD with grafting procedures are summarized in Table 4.

The effectiveness of cEMD plus an autogenous bone graft was demonstrated clinically and radiographically in a case report regarding the reconstruction of an intra-osseous defect with furcation involvement (Leung & Jin 2003). Recently, a case series explored the clinical effectiveness of the combination between cEMD and autogenous cortical bone particulate in deep, non-self-containing intra-osseous defects in humans. The results suggested a clinical and statistical benefit of the cEMD–graft combination in the periodontal reconstruction, with a CAL gain of 4.3 mm and limited post-surgical REC increase (Trombelli et al. 2006). At present, no clinical studies evaluating the adjunctive effect of the combination cEMD+autogenous bone graft over either OFD or autogenous bone graft alone are available. In a recent RCT, the combined cEMD+autograft approach resulted in a significantly smaller post-operative REC at 12 months and increased proportion of defects with a substantial ($\geqslant 6$ mm) CAL gain when compared with cEMD alone (Guida et al. 2007).

An explorative case series was conducted to assess the clinical effect of the combination of cEMD with either DFDBA or FDBA in the reconstruction of periodontal intra-osseous defects (Rosen & Reynolds 2002). Although both combinations were effective in the improvement of the defect-associated clinical parameters at 6 months post-surgery, the results showed a trend towards a better effect of FDBA–cEMD over DFDBA–cEMD (relative 6-month CAL gain: 57.3% *versus* 47.1%) (Rosen & Reynolds 2002). At present, no clinical studies evaluating the adjunctive effect of the association cEMD+allogeneic bone graft over either OFD or allogeneic bone graft alone are available. The cEMD–DFDBA combination showed no clinical benefit with respect to cEMD. However, when part of the defects were surgically re-entered, the combination of cEMD+DFDBA therapy yielded statistically significant improvements in bone fill and prevalence of sites showing substantial bone fill when compared with cEMD alone (Gurinsky et al. 2004).

At present, all the studies evaluating the combination of cEMD with a xenograft have used a BPBM (BioOss®; Geistlich Pharma AG, Wolhusen, Switzerland). The histologic examination in humans revealed that the cEMD–

Table 4. Randomized clinical trials (RCTs) evaluating the effectiveness of commercially available enamel matrix derivative (cEMD) in association with grafts with respect to either graft alone or cEMD alone in the treatment of intra-osseous defects

Combined graft	Adjunctive effect of cEMD over graft (cEMD+graft *versus* graft)		Adjunctive effect of graft over cEMD (cEMD+graft *versus* cEMD)	
	reference	result	reference	result
Autogenous bone grafts	No available RCTs		Guida et al. (2007)	↑ (post-operative REC increase, prevalence CAL gain ⩾6 mm) = (PD reduction, CAL gain, radiographic defect depth)
Allogeneic bone grafts	No available RCTs		Gurinsky et al. (2004)	↑ (bone fill, prevalence substantial bone fill) = (PD reduction, CAL gain, post-operative REC increase, % defect resolution)
Xenografts	Scheyer et al. (2002)	= (PD reduction, CAL gain, post-operative REC increase, bone fill, % defect resolution)	Lekovic et al. (2000)	↑ (PD reduction, CAL gain, defect fill) = (post-operative REC increase)
	Sculean et al. (2002b)	= (PD reduction, CAL gain, post-operative REC increase)	Velasquez-Plata et al. (2002)	↑ (post-operative REC increase, bone fill) = (PD reduction, CAL gain, % bone fill, % defect resolution)
			Zucchelli et al. (2003)	↑ (CAL gain, post-operative REC increase, defect fill) = (PD reduction)
Alloplastic materials	Sculean et al. (2002a)	= (PD reduction, CAL gain, post-operative REC increase)	Sculean et al. (2005a)	= (PD reduction, CAL gain)
			Bokan et al. (2006)	= (PD reduction, CAL gain, post-operative REC increase)
			Kuru et al. (2006)	↑ (PD reduction, relative attachment gain, post-operative REC increase, radiographic bone gain)
			Jepsen et al. (2008)	= (CAL gain, PD reduction, bone gain)

Result: ↑, positive adjunctive effect of combined treatment with respect to the single therapy; =, null adjunctive effect of combined treatment with respect to the single therapy. In parentheses: the study parameters where the positive or null adjunctive effect of the combined treatment was reported. PD, pocket probing depth; CAL, clinical attachment level; REC, recession depth.

BPBM-treated defects healed with a new connective tissue attachment (i.e. new cellular cementum with inserting collagen fibres) and new bone (Sculean et al. 2003c). All the clinical studies showed significantly positive results in terms of PD reduction and CAL gain with respect to pre-surgery (Lekovic et al. 2000, 2001b, Camargo et al. 2001, Scheyer et al. 2002, Velasquez-Plata et al. 2002, Sculean et al. 2002b, 2003c, Zucchelli et al. 2003, Döri et al. 2005, 2008). CAL gain, as assessed between 6 and 12 months post-surgery, ranged from 1.99 mm (Camargo et al. 2001) to 5.80 mm (Zucchelli et al. 2003). Defect fill at surgical re-entry ranged from 2.67 mm (Camargo et al. 2001) to 4.00 mm (Velasquez-Plata et al. 2002). In one RCT, the cEMD–BPBM combination was shown to exert a significant adjunctive effect over OFD alone in terms of 6-month PD reduction, CAL gain and defect fill (Camargo et al. 2001). When compared with BPBM alone, the cEMD–BPBM combination did not show any additional benefit on both clinical parameters (Scheyer et al. 2002, Sculean et al. 2002b) and re-entry measurements (Scheyer et al. 2002). When compared with cEMD alone, the cEMD–BPBM combination was found to significantly improve the extent of PD reduction and CAL gain (Lekovic et al. 2000, Zucchelli et al. 2003), post-operative REC (Velasquez-Plata et al. 2002, Zucchelli et al. 2003) and defect fill (Lekovic et al. 2000, Velasquez-Plata et al. 2002, Zucchelli et al. 2003).

The alloplastic materials that were clinically investigated in association with cEMD in periodontal regenerative procedures are bioactive glass (Sculean et al. 2002a, 2005a, b, 2007a, Kuru et al. 2006), β-TCP (Döri et al. 2005, Bokan et al. 2006) and biphasic calcium phosphate (Jepsen et al. 2008). Overall, the cEMD–bioactive glass showed (at 6–12 months) post-surgery improvements in the defect-associated clinical parameters with respect to pre-surgery. CAL gain ranged from about 3.00 mm (Sculean et al. 2002a, 2005a, 2007a) to 5.17 mm (Kuru et al. 2006). In one study where radiographic measurements were performed, the radiographic bone gain amounted to a mean of 2.76 mm (Kuru et al. 2006). The histologic evaluation in humans revealed that in defects treated with cEMD+bioactive glass, the healing occurred predominantly with new PDL and cementum formation (Sculean et al. 2005b). At present, no studies evaluating the adjunctive effect of the association between cEMD and bioactive glass over OFD are available. The cEMD–bioactive glass combination did not show any clinical additional benefit with respect to bioactive glass alone (Sculean et al. 2002a). Contrasting results were obtained by RCTs evaluating the adjunctive clinical effect of the cEMD–bioactive glass association over cEMD alone. While Sculean et al. (2005a) failed to show any difference between treatments, Kuru et al. (2006) reported significantly greater PD reduction as well as relative attachment level and radiographic bone gain for the combined treatment.

When cEMD was used in conjunction with β-TCP in the treatment of intra-osseous defects, a substantial CAL gain (about 4.00 mm) was reported at 12

months post-surgery (Döri et al. 2005, Bokan et al. 2006). When compared with OFD alone, cEMD+β-TCP showed a significant adjunctive effect in terms of CAL gain (2.1 *versus* 3.7 mm, respectively) (Bokan et al. 2006). No studies are currently available evaluating the additional effect of cEMD when used with β-TCP with respect to β-TCP alone. In one RCT, cEMD–β-TCP treatment showed clinical results similar to cEMD alone (Bokan et al. 2006).

Recently, a comparison between cEMD plus biphasic calcium phosphate and cEMD alone was reported for the treatment of intra-osseous defects (Jepsen et al. 2008). The results showed no significant differences in hard and soft tissue measurements as well as patient-centred outcomes between treatments.

Association with GTR

Clinically, the combination of cEMD with GTR has been explored in the treatment of intra-osseous (Sculean et al. 2001b, 2004a, Minabe et al. 2002, Sipos et al. 2005) and furcation defects (Donos et al. 2004). RCTs evaluating the effectiveness of the combination of cEMD with GTR are summarized in Table 5.

The clinical trials concerning cEMD–GTR application in human intra-osseous defects include both resorbable (Sculean et al. 2001b, 2004a, Minabe et al. 2002) and non-resorbable membranes (Sipos et al. 2005). cEMD combined with resorbable membranes resulted in a CAL gain ranging from about 3.00 mm (Minabe et al. 2002) to 3.40 mm (Sculean et al. 2001b) at 12 months after surgery. Sculean et al. (2001b) suggested a positive adjunctive effect of the combined technique over the OFD procedure.

The association of cEMD and GTR (resorbable membrane) did not seem to improve the reconstructive outcomes obtained by GTR alone at 1 year post-surgery (Sculean et al. 2001b, Minabe et al. 2002). When compared with cEMD alone, the cEMD+GTR (resorbable membranes) combination did not show any additional benefit on clinical and radiographic parameters both in the short term (Sculean et al. 2001b, 2004a, Minabe et al. 2002) and during maintenance (Sculean et al. 2004a). Consistently, the combination of cEMD with a non-resorbable (e-PTFE) membrane failed to demonstrate a significant adjunctive effect over cEMD alone in terms of CAL gain and probing bone level (Sipos et al. 2005).

When cEMD was either combined with a non-resorbable (e-PTFE) membrane or used alone to treat class III furcation defects in a case report study, the clinical outcomes appeared similar for both treatments (Donos et al. 2004).

Association with grafts and GTR

The use of cEMD in combination with a BPBM and a resorbable collagen membrane was histologically investigated in a human case report (Sculean et al. 2004b). At 7 months post-surgery, the histologic analysis revealed the presence of a newly formed periodontal apparatus, including bone, cementum and PDL.

In a 50-patient case series, the use of cEMD in association with DFDBA and a resorbable membrane was explored (Harris et al. 2007). At 4–8 months after surgery, the clinical measurements revealed a CAL gain of 5.0 mm (Harris et al. 2007). In a split-mouth study, intra-osseous defects were treated by means of either cEMD+BPBM+GTR (collagen/polylactic acid membrane) or OFD (Lekovic et al. 2001a). At 6 months post-surgery, the combined technique resulted in a significant CAL gain (3.8 mm) and defect fill (4.8 mm), with significantly better results over OFD (Lekovic et al. 2001a).

At present, no data are available in order to determine the adjunctive effect of cEMD+graft+GTR compared with either the graft+GTR alone or cEMD alone.

Patient-centred outcomes

The clinical safety of cEMD was first supported by clinical studies where the changes in specific antibody levels against cEMD components were assessed in patients undergoing either single or multiple periodontal surgical exposures to cEMD. The results showed no increase in antibody-mediated reaction to cEMD, indicating a low immunogenic potential of cEMD (Zetterstrom et al. 1997). In addition, only a slight, non-significant activation of the immune system occurred during the first year following cEMD application. Neither cellular immunity nor humoral immune response was significantly modified as far as could be tested (Nikolopoulos et al. 2002).

Table 5. Randomized clinical trials (RCTs) evaluating the effectiveness of enamel matrix derivative (cEMD) in association with guided tissue regeneration (GTR) with respect to either GTR alone or cEMD alone in the treatment of intra-osseous defects

EMD association with	Adjunctive effect of cEMD over GTR (cEMD+GTR *versus* GTR)		Adjunctive effect of GTR over cEMD (cEMD+GTR *versus* cEMD)	
	reference	result	reference	result
Resorbable membranes	Sculean et al. (2001b)	= (PD reduction, CAL gain, post-operative REC increase)	Sculean et al. (2001b)	= (PD reduction, CAL gain, post-operative REC increase)
	Minabe et al. (2002)	= (PD reduction, CAL gain, post-operative REC increase, bone gain)	Minabe et al. (2002)	= (PD reduction, CAL gain, post-operative REC increase, bone gain)
			Sculean et al. (2004a)	= (PD reduction, CAL gain, post-operative REC increase)
Non-resorbable membranes	No available RCTs		Sipos et al. (2005)	= (PD reduction, CAL gain, post-operative REC increase, PBL change)

Result: ↑, positive adjunctive effect of combined treatment with respect to the single therapy; =, null adjunctive effect of combined treatment with respect to the single therapy. In parentheses: the study parameters where the positive or null adjunctive effect of the combined treatment was reported. PD, pocket probing depth; CAL, clinical attachment level; REC, recession depth.

Patients who underwent flap surgery with cEMD demonstrated the same types and frequencies of post-surgical experiences compared with non-cEMD-treated patients (Zetterstrom et al. 1997), and multiple applications of cEMD did not produce any negative effect on periodontal wound healing, as determined by clinical signs and symptoms (Heard et al. 2000).

In one trial reporting on the aesthetic aspect following surgical treatment, no statistically significant difference between cEMD and OFD was found. Moreover, the frequencies of subjects reporting pain, intensity and duration of pain, use of analgesic tablets, oedema, haematoma, wound dehiscence and root sensitivity were similar for both treatments (Tonetti et al. 2004). Recently, the adjunctive effect of cEMD, in terms of patient perception of oral health-related quality of life, has been evaluated. At 1 week post-surgery, OFD plus cEMD treatment was compared with OFD alone and non-surgical therapy. The results indicated that patient perceptions on the immediate post-operative period were significantly better in the non-surgical and OFD+cEMD groups when compared with the OFD group (Ozcelik et al. 2007b).

Recently, a case report described two examples of external inflammatory root resorption following surgical root surface debridement and the use of cEMD (St George et al. 2006). The treatment in both cases involved raising a full-thickness flap, removal of granulation tissue from the defect and root surface debridement and conditioning with EDTA gel. External inflammatory root resorption was observed on the treated teeth 6–24 months after therapy.

Long-term results

When analysed long term, the outcomes of the cEMD-based regenerative surgery seem to be stable over time when used either alone (Sculean et al. 2003a, 2006b, 2007a, b, Parodi et al. 2004, Rasperini et al. 2005, Heden & Wennstrom 2006, Farina et al. 2007) or in association with bioactive glass (Sculean et al. 2007a). The stability of the reconstructive outcome could be similarly maintained in GTR+cEMD- and GTR-treated defects at 5 years (Sculean et al. 2004a). Moreover, no significant differences in terms of disease recurrence were observed when the reconstructive outcomes obtained by either cEMD+bioactive glass or cEMD alone were compared in the long term (Sculean et al. 2007a).

Peptide P-15 (P-15)

P-15 is a 15-amino-acid peptide (sequence: GTPGPQGIAGQRGVV) that mimics part of the sequence of the α1 chain of type I collagen (Bhatnagar et al. 1997). The biological rationale for the use of P-15 in periodontal reconstructive procedures resides in its steric similarities to the cell-binding site of type I collagen, and its capacity to enhance the rate and the extent of the attachment and migration of periodontal cells to root (Lallier et al. 2003) or biomaterial surfaces (Bhatnagar et al. 1999, Lallier et al. 2001).

Proof of principle

No clinical studies evaluating the use of P-15 in the treatment of intra-osseous or furcation defects are at present available.

Clinical effectiveness

No RCTs evaluating the adjunctive clinical effect of P-15 in the treatment of intra-osseous or furcation defects are currently available.

Association with grafts and/or GTR

The clinical use of P-15 with an organic bovine-derived hydroxyapatite matrix (ABM) was reported in case reports/series (Yukna et al. 2002a, b, Barros et al. 2006). In intra-osseous defects, Yukna et al. (2002b) reported significant clinical changes (CAL gain and PD reduction) from pre-surgery to 6 months, confirmed by the surgical re-entry of the treated defects. The 6-month histologic evaluation of intra-osseous defects grafted with an enhanced ABM/P-15 graft showed evidence of regeneration (new cementum, bone and PDL), with no evidence of root resorption or ankylosis (Yukna et al. 2002a). In pure intra-osseous and combined intra-osseous/furcation defects, similar positive clinical results were obtained 12 months after surgery (Barros et al. 2006). No significant differences in soft and hard tissue changes were found when two different forms of ABM/P-15 graft (hydrogel *versus* particulate form) were compared in the treatment of intra-osseous defects (Matos et al. 2007).

Intra-osseous defects treated with ABM/P-15 exhibited statistically significant clinical and radiographic 6-month improvements, and performed better than either OFD (CAL gain and defect fill) (Yukna et al. 1998, Radhakrishnan & Anusuya 2004, Bhongade & Tiwari 2007) or ABM alone (defect fill) (Yukna et al. 2000) (Table 6). No RCTs are currently available evaluating the additional effect of P-15–graft combination with respect to P-15 alone.

When the association of ABM/P-15 with either a porous or a non-porous e-PTFE membrane was compared in intra-osseous defects, both groups exhibited similarly positive results, with no significant differences between membranes (Walters et al. 2003).

No RCTs are currently available evaluating the additional effect of ABM/P-15 in combination with GTR with respect to either OFD alone, P-15 alone or a combination with ABM and GTR.

Patient-centred outcomes

In one study, no untoward effect or patient complaints were recorded after the use of ABM/P-15 in intra-osseous defects. The association appeared to be clinically well tolerated by the periodontal tissues, and exfoliation of the graft particles was not observed (Yukna et al. 1998).

Long-term results

When longitudinally followed in a single study, the reconstructive outcomes of P-15/ABM remained stable at 3 years (Yukna et al. 2002b).

Discussion

Study methodology

To evaluate the clinical application of a BA in periodontal reconstructive procedures, we based our analysis on data emerging from both descriptive studies as well as RCTs or SRs. This approach was justified by the limited clinical evidence that is currently available on most BAs. Hence, we analysed *proof-of-principle* studies in order to provide evidence on the potential use of the BA when applied alone or in combination with other reconstructive techologies, while we used RCTs/SRs to support the clinical effectiveness of the BA alone or in combination compared with non-BA procedures. Except for cEMD,

Table 6. Randomized clinical trials (RCTs) evaluating the effectiveness of peptide P-15 (P-15) in association with grafts with respect to either open flap debridement (OFD) or graft alone in the treatment of intra-osseous defects

P-15 association with	Adjunctive effect of P-15+graft over OFD (P-15+graft *versus* OFD)		Adjunctive effect of P-15 over graft (P-15+graft *versus* graft)	
	reference	result	reference	result
ABM	Yukna et al. (1998)	↑ (CAL gain, defect fill, relative defect fill) = (PD reduction, post-operative REC increase)	Yukna et al. (2000)	↑ (defect fill, relative defect fill, number of sites with relative defect fill ⩾90%) = (PD reduction, CAL gain, post-operative REC increase)
	Radhakrishnan & Anusuya (2004)	↑ (PD reduction, CAL gain, defect depth reduction, defect fill, defect resolution)		
	Bhongade & Tiwari (2007)	↑ (PD reduction, CAL gain, defect fill) = (post-operative REC increase)		

Result: ↑, positive adjunctive effect of combined treatment with respect to the single therapy; =, null adjunctive effect of combined treatment with respect to the single therapy. In parentheses: the study parameters where the positive or null adjunctive effect of the combined treatment was reported. PD, pocket probing depth; CAL, clinical attachment level; REC, recession depth.

where several RCTs on the clinical benefit of the agent were recently analysed in SRs (Trombelli et al. 2002b, Giannobile & Somerman 2003, Esposito et al. 2005), limited information, mostly relying on few and heterogeneous RCTs, are available for the majority of the considered BAs to be pooled and suitable for meta-analysis.

Among all the considered BAs, data on the clinical use were only found for PDGF, BMPs, PRP, cEMD and P-15.

PDGF

The use of rhPDGF-BB used in association with an allogenic bone graft (either DFDBA or FDBA) has shown substantial CAL gain and PD reduction in case reports on the treatment of class II furcation (Camelo et al. 2003, Nevins et al. 2003) and intra-osseous (Nevins et al. 2003, 2007) defects. When the association of two different doses of PDGF-BB (0.3 and 1.0 mg/ml) with β-TCP was compared with β-TCP alone in deep intra-osseous defects, the rate of gain in clinical attachment was shown to be more rapid in the low-dose PDGF+β-TCP group with respect to the control group at 3 months post-surgery. However, no significant differences were found in the extent of CAL gain after 6 months of healing. Both PDGF formulations were significantly more effective than the control group (β-TCP+buffer) in the improvement of radiographically determined linear bone growth and percentage of bone defect fill at 6 months (Nevins et al. 2005). Further studies are needed to determine whether and to what extent rhPDGF-BB+graft may be effective for periodontal reconstructive procedures in different periodontal lesions.

BMPs

BMPs, in particular rhBMP-2, have shown a robust biological potential for bone regeneration in pertinent animal models (Wikesjö et al. 2005). However, limited cementum formation with functionally anchored PDL fibres has also been observed (Wikesjö et al. 1999, 2003a, Sorensen et al. 2004). Previous studies in humans demonstrated the absence of relevant systemic adverse events and clinical manifestations due to an immune response following the use of rhBMP-2 when used for maxillary sinus elevation (Boyne et al. 1997, Boyne et al. 2005), extraction socket preservation and alveolar ridge augmentation (Howell et al. 1997b, Cochran et al. 2000). However, the use of BMPs may lead to local adverse events, including root resorption (Sigurdsson et al. 1995, Wikesjö et al. 1999, Selvig et al. 2002, Wikesjö et al. 2003a, Sorensen et al. 2004) and/or ankylosis (Sigurdsson et al. 1995, 1996, King et al. 1998, King & Hughes 1999, Wikesjö et al. 1999, 2003a, b, Selvig et al. 2002, Saito et al. 2003, Sorensen et al. 2004). A histomorphometric study in humans revealed that BMP-3 (osteogenin) plus DFDBA significantly enhanced the regeneration of a new attachment apparatus and component tissue (Bowers et al. 1991). Although positive results have been reported when BMPs (in particular, rh/BMP-2) was used for alveolar bone reconstruction in oral and maxillofacial applications (Herford et al. 2007), the clinical effect of BMPs when used either alone or in combination with grafts and/or GTR for the treatment of intra-osseous or furcation defects remains undetermined.

PRP

As a platelet concentrate, PRP contains a number of different GFs including PDGF, TGF-β and IGF (Okuda et al. 2003) that may potentially exert a positive effect on cell lines involved in periodontal wound healing. One case report on five patients has suggested a beneficial effect of PRP when used for treating intra-osseous defects (Papli & Chen 2007). However, RCTs evaluating the adjunctive clinical effect of PRP in the treatment of intra-osseous and furcation defects are still lacking.

There is currently a great deal of interest in oral and maxillofacial bone-grafting procedures, which involve the use of PRP to enhance bone formation and, in particular, increase the rate of bone graft healing (Marx et al. 1998, Kassolis et al. 2000, Aghaloo et al. 2006). The use of PRP combined with several types of grafts for the treatment of intra-osseous defects resulted in a substantial CAL gain (Lekovic et al. 2002, Hanna et al. 2004, Okuda et al. 2005, Czuryszkiewicz-Cyrana & Banach 2006, Ouyang & Qiao 2006, Demir et al. 2007, Yassibag-Berkman et al. 2007, Yilmaz et al. 2007, Döri et al. 2008). However, when the additional effect of PRP over the graft was evaluated, contrasting results were reported, ranging from a significant enhancement for PRP (Hanna et al. 2004, Okuda et al. 2005, Ouyang & Qiao 2006) to a null effect (Demir et al.

2007, Yassibag-Berkman et al. 2007, Döri et al. 2008) (Table 2). The discrepancy may be partly due to differences in the methods used to obtain the PRP preparations, which may in turn have affected the content of platelets and inflammatory cytokines as well as the contamination of the platelet preparation with leucocytes and erythrocytes (Weibrich et al. 2003). However, recent data have shown that similar reconstructive outcomes could be obtained when two different PRP preparations were used in addition to bone graft to treat experimental calvarial defects in rabbits (Hatakeyama et al. 2008). An alternative explanation may involve the potential synergistic effect between PRP and specific types of bone substitutes.

No additional benefit of PRP has been shown when used with graft+GTR over the graft+GTR alone for intra-osseous defects (Christgau et al. 2006, Döri et al. 2007a, b) (Table 3).

cEMD

Several studies support the clinical effectiveness of cEMD for periodontal regeneration (Table 1). Recent SRs demonstrated a significant effect of cEMD in terms of CAL gain and PD reduction when compared with OFD (Trombelli et al. 2002b, Giannobile & Somerman 2003, Esposito et al. 2005). However, general conclusions about the clinical relevance (i.e. magnitude of the additional effect) of cEMD are limited by the high level of heterogeneity found across the studies, i.e. while some studies showed a significant additional effect of cEMD, other studies failed to show any difference (Trombelli 2005).

Because of its gel-like consistency, cEMD possesses limited space-making potential, which, in turn, may potentially affect its regenerative capacity (Mellonig 1999). Hence, a combined approach based on cEMD plus a graft biomaterial has been suggested, particularly when the regenerative treatment is directed towards deep, non-contained intra-osseous defects (Froum et al. 2001). The rationale for this approach was that, while cEMD would exert a biological effect on the cascade of events leading to periodontal regeneration, the use of the graft may, to a certain extent, hinder the collapse of the flap into the bone defect during the early healing phase. Several studies investigated the combined effect of cEMD plus a graft biomaterial in the treatment of intra-osseous defects. Overall, available data indicate that the graft (autogenous bone particles, FDBA/DFDBA, BPBM, bioactive glass) may improve the clinical performance (in terms of either CAL gain, PD reduction or bone fill) when used in combination with cEMD with respect to cEMD alone (Table 4). In contrast, when cEMD is used to enhance the reconstructive potential of the graft (i.e. cEMD+graft *versus* graft alone), limited evidence seems to indicate no adjunctive effect of cEMD over the reconstructive potential of the graft (Table 4). Unfortunately, available RCTs do not provide evidence on patient and defect characteristics where the combined cEMD+graft approach rather than either cEMD or graft alone would optimize the treatment outcome.

The histological effectiveness of cEMD combined with GTR principles in the regeneration of intra-osseous and furcation defects has been demonstrated previously in animal models (Araujo & Lindhe 1998, Sculean et al. 1998, 2000b, Donos et al. 2003b, Sallum et al. 2004, Onodera et al. 2005). However, while some studies indicated a more predictable healing following the combined treatment with respect to the single therapies (Araujo & Lindhe 1998, Donos et al. 2003b), other reports failed to show any differences between single or combined approaches (Sculean et al. 1998, 2000b, Sallum et al. 2004, Onodera et al. 2005). When clinically tested, the cEMD+GTR combination did not show any additional clinical advantage over either GTR or cEMD alone (Table 5).

P-15

ABM/P-15 is a combination of a natural ABM with a synthetic cell-binding peptide (P-15). The peptide component is a synthetic analogue of the 15 amino-acid sequence of type I collagen that is uniquely involved in the binding of cells, particularly fibroblasts and osteoblasts (Lallier et al. 2003). Clinical results indicate that when ABM/P-15 was used to treat intra-osseous defects, a significant benefit after the use of P-15-enhanced ABM was reported in clinical and radiographic parameters when compared with either OFD alone (Yukna et al. 1998, Radhakrishnan & Anusuya 2004, Bhongade & Tiwari 2007) or ABM alone (Yukna et al. 2000) (Table 6). Although promising, these results need to be confirmed by further large-cohort, controlled trials specifically designed to assess the relative role of ABM and/or P-15 in the observed clinical improvements.

Patient-centred outcomes

Limited information, mostly related to cEMD and PRP, is currently available on the safety related to the clinical use of BAs in periodontal reconstructive procedures. In particular, only a few studies have investigated either local or systemic adverse effects derived from the topical application of the agents. Cost-effectiveness as well as risk benefit have never been addressed for any considered BA.

Long-term results

Results stemming from long-term observations after cEMD use in intra-osseous defects indicate that the outcomes of the cEMD-based regenerative surgery are stable over time when used either alone (Sculean et al. 2003a, 2006b, 2007a, b, Parodi et al. 2004, Rasperini et al. 2005, Heden & Wennstrom 2006, Farina et al. 2007) or in association with bioactive glass (Sculean et al. 2007a). A 3-year follow-up study showed the stability of CAL as obtained by the use of the ABM/P-15 combination in intra-osseous defects (Yukna et al. 2002b). However, due to limited information on the long-term outcome, it is still unclear whether and to what extent the stability of periodontal support and tooth survival rate are affected by the application of BAs.

Conclusions

BAs, including GFs, are a general term used to denote a class of molecules or compounds that may stimulate a variety of cellular events such as proliferation, chemotaxis, differentiation and the production of extracellular matrix proteins. Such events are essential requirements for periodontal regeneration. It is therefore conceivable and likely that BAs individually or in combination with other technologies may be relevant to regenerate PDL, new bone and cementum.

The conclusions of the present review are as follows:

1. Evidence shows that cEMD either alone or in combination with grafts

can be effectively used to treat intra-osseous defects, and the clinical results appeared stable in the long term.

2. The additional use of a graft (autogenous bone, DFDBA, BPBM, bioactive glass) seems to enhance the clinical outcome of cEMD over cEMD alone. However, available evidence suggests no adjunctive effect of cEMD plus a graft (either BPBM or bioactive glass) over the graft alone.
3. Neither does GTR seem to enhance the cEMD reconstructive outcome, nor does cEMD appear to enhance the outcome of GTR.
4. Additional beneficial effects have also been reported with the combined use of rhPDGF-BB and P-15 with a graft biomaterial over the use of a graft biomaterial alone for the treatment of intra-osseous defects.
5. When the additional effect of PRP over a graft was evaluated, contrasting results were reported, ranging from a significant enhancement for PRP to a null effect.
6. Scientific evidence is currently limited to support the use of BAs either alone or in association with a graft/GTR for the treatment of furcation defects.
7. Patient-centred outcomes, including adverse effects, cost-effectiveness and risk benefit, were investigated in a limited number of studies.
8. Other BAs have been experimentally tested to treat periodontal defects, including rhBMP-2, OP-1, transforming growth factor β, bFGF, IGF-1, cementum-derived growth factor, neurotrophins, vascular endothelial growth factor and parathyroid hormone-related protein. Unfortunately, although the great majority of these molecules have shown a biologic activity on cells that are involved in the periodontal regenerative process, limited, if any, clinical information is at present available, indicating that these factors may be safely and effectively used for treating periodontal defects.

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Clinical Relevance

Scientific rationale for the study: BAs are molecules or compounds that play a key role in the modulation of cellular events, including periodontal regeneration. The addition of BAs was shown to enhance the periodontal wound-healing dynamics.
Principal findings: PDGF-BB, IGF-1, PRP preparations, BMPs, cEMD and peptide P-15 have been clinically used for treating periodontal defects.
Practical implications: At present, only cEMD possesses solid evidence for its clinical use in intra-osseous defects either alone or in combination with grafts or GTR.