Simplified surgical procedures and bioactive agents in the regenerative treatment of periodontal intraosseous defects

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UNIVERSITY OF FERRARA
2016

CONTENT

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals:

I. FARINA R, SIMONELLI A, RIZZI A, PRAMSTRALLER M, CUCCHI A, TROMBELLI L (2013) Early post-operative healing following buccal Single Flap Approach to access intraosseous periodontal defects.
Clinical Oral Investigation **17**, 1573–1583.

II. FARINA R, SIMONELLI A, MINENNA L, RASPERINI G, TROMBELLI L (2014) Single-flap approach in combination with enamel matrix derivative in the treatment of periodontal intraosseous defects.
International Journal of Periodontics and Restorative Dentistry **34(4)**, 497-506.

III. SCHINCAGLIA GP, HEBERT E, FARINA R, SIMONELLI A, TROMBELLI L (2015) Single versus Double Flap Approach in Periodontal Regenerative Treatment.
Journal of Clinical Periodontology **42(6)**, 557-66

IV. FARINA R, SIMONELLI A, MINENNA L, RASPERINI G, SCHINCAGLIA GP, TOMASI C, TROMBELLI L (2015) Change in the gingival margin profile following Single Flap Approach in periodontal intraosseous defects.
Journal of Periodontology **30**, 1-16.

V. SIMONELLI A, FARINA R, RIZZI A, TROMBELLI L (2013) Treatment of an intraosseous periodontal defect associated with a cemental tear with the Single Flap Approach.
Dental Cadmos **81**, 365-373.

VI. RIZZI A, FARINA R, SIMONELLI A, SCHINCAGLIA GP, TROMBELLI L (2013) Trattamento ricostruttivo di un difetto parodontale infraosseo associato a deiscenza vestibolare: Single Flap Approach in associazione ad un preparato a base di β-fosfato tricalcico, al fattore di crescita di derivazione piastrinica e ad una matrice tridimensionale in collagene.
Dental Cadmos **81(5)**, 299-307.

CHAPTER 1

General introduction

PERIODONTAL DISEASE

Definition and prevalence

Any inherited or acquired disorder of the tissues surrounding and supporting the teeth (periodontium) can be defined as a "periodontal disease". These diseases may be of developmental, inflammatory, traumatic, neoplastic, genetic or metabolic origin (Armitage 2004, Jordan 2004). However, the term "periodontal disease" usually refers to the common inflammatory disorders of "gingivitis" and "periodontitis" that are caused by microbial biofilm or dental plaque that forms adjacent to the teeth on a daily basis.

Periodontitis is a destructive inflammatory disease of the tooth supporting tissues (i.e. alveolar bone, cementum, periodontal ligament) which leads to non-reversible connective tissue attachment and alveolar bone loss. If left untreated, periodontitis will result early tooth loss (Pihlstrom et al. 2005). Periodontitis can be defined as a complex and multi-factorial condition, meaning that the process of onset and progression of periodontitis involves multiple etiological factors that act simultaneously and lead to a shift from a healthy periodontal status to a diseased condition. These factors can be clustered into five major groups: i) microbiological factors, ii) factors related to the genetic of the host, iii) lifestyle factors such as tobacco smoking, stress, diet, iv) systemic conditions such as diabetes, obesity and metabolic disorders, v) other still unknown factors.

Severe periodontitis is the sixth most prevalent disease of humankind (Kassebaum et al. 2014). Although prevalence estimates differ on the basis of how the disease is defined, the prevalence, severity, and rate of disease progression clearly varies worldwide (Löe et al. 1978, Löe et al. 1986). A recent systematic review indicate that the global prevalence of severe chronic periodontitis in adults is 11.2% (Kassebaum et al. 2014). In patient sample of residents in northern Italy, the prevalence of moderate to severe periodontitis ranged from 41.21% to 87,44% in patients of 20-29 years and 60-75 tears, respectively (Aimetti et al. 2015). The impact of periodontal diseases on an affected individual is increasingly apparent and becomes more significant with progression of the diseases, beginning with gingival recession and associated dentin hypersensitivity at an early stage. The disease then progresses toward tooth mobility, pathological migration, and, eventually, tooth loss, thereby affecting chewing and speech functions, aesthetics, psychological aspects, and quality of life (Ng et al. 2006, Patel et al 2008), as well as increasing financial burden (Jin, 2009).

INTRAOSSEOUS PERIODONTAL DEFECTS

Definition and prevalence

Loss of alveolar bone support represent the anatomical sequelae to the spread of periodontitis; in this context, the apical downgrowth of subgingival plaque can result in the formation of osseous periodontal lesions (i.e bony defects). Bony defects are generally classified as supraosseous (or horizontal), intraosseous (or vertical), and inter-radicular (or furcation), depending on the pattern of bone resorption. These type of defects are assessed by a combination of radiographic and clinical means and have different prognosis, evolution and treatment.

Intraosseous defects (also known as intrabony defects) are defined by the apical location of the base of the pocket with respect to the residual alveolar crest (Papapanou & Tonetti 2000). Intraosseous defects are a common finding in periodontal patients. The prevalence of intraosseous defects in adults was investigated on dried skulls (Larato 1970) as well as through clinical (Söder et al. 1995, Vrotsos et al. 1999) and radiographic assessments (Nielsen et al. 1980, Papapanou et al. 1988, Wouters et al. 1989, Soikkonen et al. 1998, Dundar et al. 2008). At the patient-level, the presence of at least one intraosseous defect was detected with an incidence ranging between 25.5% and 51% in subject samples representative of the general population or specific age cohorts (Larato 1970, Wouters et al. 1989, Soikkonen et al. 1998), between 18% and 23% in patients seeking dental care (Nielsen et al. 1980, Dundar et al. 2008), and of 45.1% in a periodontally compromised cohort (Söder et al. 1995). In general, most studies reported a substantially lower prevalence of intraosseous defects at anterior sextants compared to posterior sextants, with heterogeneous data regarding the dental arch and tooth types at greater risk of developing these lesions within the anterior region (Larato 1970, Papapanou et al. 1988, Soikkonen et al. 1998, Vrotsos et al. 1999, Müller et al. 2005, Dundar et al. 2008).

Intraosseous defects are generally classified according to their morphology: in this respect, one-wall, two-wall, three-wall defects may be identified on the basis of the number of residual bony walls. Frequently, intraosseous defects present a complex anatomy consisting of a combination of the previous options, with a three-wall component in the most apical portion of the defect, and two- and/or one-wall components in the more superficial portions. Of particular interest is a special morphology: the crater, a cup-shaped defect located in the interdental alveolar bone with bone loss nearly equal on the roots of two contiguous teeth and more coronal position of the buccal and ligual alveolar crest.

Other important morphological characteristics of intraosseous periodontal defects are the width of the intrabony component, measured as the angle that the bony wall of the defect forms with the long axis of the tooth (Steffensen & Weber 1989), as well as the depth of the intrabony component. In these respect, wide/narrow- and deep/shallow- defects may be identified.

Periodontal regeneration of intraosseous defects

Periodontal intraosseous defects are considered a clinical challenge. Teeth with deep pockets associated with deep intraosseous defects have a high risk of further progression of bone loss and eventually tooth loss (Papapanou & Wennstrom 1991). In this context, intraosseous defects are generally characterized by severe bone loss, deep bleeding pockets, tooth mobility and tooth migration. These alterations have a severe impact on tooth prognosis that is often considered "questionable" or "hopeless" by clinicians. The aim of periodontal therapy is to change the prognosis from "questionable" to "fair" or "favorable" in order to maintain teeth over time and improve patient comfort and function.

The treatment of periodontitis, which also encompasses intraosseous defects, is based on the removal of the supra- and sub-gingival biofilm, achieved by patient-performed oral hygiene associated with professional non-surgical periodontal debridement. During non-surgical periodontal debridement dental plaque and calculus are mechanically removed from tooth-crown and root surfaces by the use of various manual or powered instruments with the aim to reduce the total bacterial load and change the environmental conditions of the microbial niches (Heitz-Mayfield et al. 2002). Table 1 summarizes the studies on non-surgical therapy for the treatment of intraosseous defects

Recently, minimally-invasive non-surgical periodontal therapy (MINST) has been introduced as a concept aiming to obtain extensive subgingival debridement with minimal tissue trauma (Ribeiro et al. 2011). MINST is based on the following principles: (i) thorough debridement of the root surface up to the bottom of the periodontal pocket, avoiding root planing and gingival curettage; (ii) use of a magnification system; (iii) prevalent use of a ultrasonic device with specific thin tips, complemented by Gracey minicurettes; (iv) caution is taken to preserve the integrity of soft tissues. Compared to traditional non-surgical periodontal debridement, MINST showed good clinical results (Nibali et al. 2011, Ribeiro et al. 2011). At 6 months, a CAL gain of 2.56 mm and a PD reduction of 3.13 were reported by Ribeiro et al. (Ribeiro et al. 2011). Similarly, an average reduction in the radiographic vertical defect depth of 2.93 mm, accompanied by a

CAL gain of 2.8 mm and a PD reduction of 3.12 mm, was observed at 12 months following treatment by Nibali et al. (Nibali et al. 2015).

Additional therapeutic options beyond non-surgical debridement, including periodontal surgery, should be considered only when initial treatment has been only partly efficacious in reducing PD and gingival bleeding, thus not ensuring the local conditions for an acceptable long-term prognosis of the treated teeth. Since intraosseous defects may be associated with persistent deep pockets and bleeding following conventional non-surgical treatment, these lesions are frequently considered as sites requiring surgical therapy.

Surgical options are based on the open flap debridement of the defect-associated root surface and the degranulation of the intraosseous component of the lesion. When performed alone, this procedure is classified as *open flap debridement* (OFD) (Rosling et al. 1976) otherwise, when combined to the surgical elimination of the defect with ostectomy/osteoplasty, is classified as *resective surgery*. OFD has been traditionally included as the control procedure in clinical trials evaluating regenerative techniques, such as guided tissue regeneration (GTR) (Cortellini et al. 1996, Needleman et al. 2005) and use of biologic factors, including enamel matrix derivative (EMD) (Heijl et al. 1997). Even when used as the control procedure, OFD resulted in significant clinical benefits (Cortellini et al. 1996, Needleman et al. 2005).

Regenerative treatment represents a proven method to improve clinical parameters, periodontal prognosis and tooth retention of elements associated with intraosseous periodontal defects (Nyman et al. 1982). In detail, regenerative periodontal therapy aims to: i) create a new periodontal attachment apparatus (i.e. new bone, cementum, periodontal ligament); ii) maximize treatment outcomes in term of CAL and PD, obtaining maintainable shallow probing sites; iii) obtain a minimal increase in gingival recession, especially in aesthetic areas (Farina et al. 2015). Histological assessment is the only method to demonstrate periodontal regeneration by determining the nature of the attachment apparatus resulting from the treatment. Due to ethical concerns, human studies on periodontal regeneration use surrogate-clinical methods to evaluate the outcomes of regenerative therapy such as assessment of periodontal probing (PD and CAL) and bone levels (re-entry procedures, bone sounding and radiographs) (Garret 1996).

Factors influencing the outcomes of periodontal regenerative procedures

The efficacy of regenerative procedures has been extensively evaluated in several clinical trials (Murphy et al 2003, Needeleman et al. 2005, Trombelli et al. 2002, Reynolds et al. 2003, Esposito et al. 2009). The results suggest that regenerative treatments hesitate in significant improvements of the pre-operatory conditions but also a great variability in term of clinical outcomes must be expected. The outcomes of regenerative periodontal therapy may be influenced by several factors that are broadly divided into patient, tooth, defect and surgical technique factors.

Patient-related factors: Clinical studies demonstrated that poor plaque control and residual periodontal infection are associated with compromised outcomes after regenerative surgery (Cortellini et al. 1994, 1996, Heden et al. 1999, Tonetti et al 1996, 2002). A full-mouth plaque score (FMPS) and full-mouth bleeding score (FMBS) $\leq$ 20% have been reported as measures of acceptable preoperative oral hygiene (Cortellini et al. 2007, 2009). Among patient/related factors, other aspects that need to be considered are "smoking status" and "systemic disease status" (in particular the presence of diabetes mellitus). In this context, cigarette smoking is an environmental factor clearly associated with compromised regenerative outcomes (Patel et al. 2012, Trombelli et al. 1997). Recent studies, comparing regenerative outcomes and complication rates in smokers versus non-smokers, demonstrate that smokers have less reduction in PD (Stavropoulos et al. 2004, Yilmaz et al 2010), smaller gains in CAL (Stavropoulos et al. 2004, Yilmaz et al 2010 Cortellini et al. 1996, Eickholz et al. 2004), less bone fill/ bone gain (Patel et al. 2012, Klein et al. 2001) compared with non-smokers. Control of these variables should be achieved before initiating regenerative procedures: clinicians have to discuss with the patient the opportunity to further improve hygiene and discontinue the smoking habit.

Tooth-related factors: There are conflicting views regarding the endodontic treatment of teeth undergoing regenerative therapy but it must be assumed that frank infective processes should be controlled if a regenerative procedure is to be considered an appropriate treatment option. To date, also the effect of tooth mobility on regenerative therapy is still controversial. Increased tooth mobility seems to negatively affect the clinical outcomes of periodontal regeneration (Cortellini et al. 2001). However, any tooth with horizontal mobility < 1mm can be successfully treated with regenerative procedures without pre-surgical splinting (Trejo et al. 2004, Tonetti et al. 2002).

Site-specific factors: Periodontal regeneration has been shown to be effective in the treatment of one-, two- and three-wall intraosseous defects or combinations thereof, from very deep to very shallow, from very wide to very narrow (Needleman et al. 2005, Murphy et al. 2003). Defect morphology plays a crucial role in determining the final clinical outcomes following periodontal regenerative therapy of intraosseous defects. In particular, three morphological characteristics may influence the amount of CAL gain and bone fill after regenerative therapy: depth of the intrabony component, width of the intrabony component and number of residual bony walls. Regarding the former characteristic, the deeper the defect, the greater the expected amount of the clinical improvement (Ehmke et al. 2003, Garrett et al. 1988, Silvestri et al. 2003, Tonetti et al. 1993, Tonetti et al. 1996). Comparing the healing following regenerative treatment of deep and shallow intraosseous defects, both defects may express a regenerative potential up to the complete resolution of the intrabony component. However, following the treatment of deep defects we would expect to achieve linear amounts of attachment gain larger than those obtained following the treatment of shallow defects. When considering the width of the intrabony component, several studies demonstrated that wider defects are associated with reduced amounts of CAL gain and bone gain at 1-year (Garrett et al. 1988, Tonetti et al. 1993, Tonetti et al. 1996). This trend was confirm from a recent study that demonstrate a negative association between the radiographic angle of the defect and the CAL gains observed 1-year, following regenerative treatment with enamel matrix derivative (Tsitoura et al. 2004). Regarding the influence of residual bony walls on the clinical outcomes following regenerative surgery, a great variability between studies can be found. While some studies reported a correlation between the number of defect walls and the clinical outcomes of the procedure (Silvestri et al. 2003, Tonetti et al. 2002, Heijl et al. 1997), other studies failed to demonstrate such an effect (Trombelli et al. 1997, Heden 2000, Bratthall et al. 2001, Minabe et al. 2002).

Surgical technique: Development of periodontal-regenerative medicine in the last 25 years has followed two distinctive, yet totally intertwined paths. The interest of researchers has so far focused on novel surgical approaches on the one hand and on regenerative materials or products on the other. These two aspects will be discussed in the following chapters.

SURGICAL APPROCHES FOR RECONSTRUCTIVE PROCEDURES

When considering the technical aspects of periodontal regenerative procedures, the surgical management of the supracrestal soft tissues seems of paramount importance in

affecting the final clinical outcomes. The surgical manipulation of the soft tissues overlying an intraosseous defect must be aimed at the complete preservation of the marginal tissues in order to achieve and maintain primary closure of the flap and optimal wound stability. In this context, the increased predictability of reconstructive procedures is strictly dependent upon two key factors: i) the surgical design and flap management for better survival of flap, and ii) the suturing technique to optimize primary closure, thus ensuring the primary condition for blood clot stabilization and maturation in a biological environment protected from biomechanical and microbiological challenge (Takei 1991). Moreover, proper flap design and incision placement is of utmost importance to achieve complete flap closure and flap-to-root seal at the time of suturing and during post-surgical healing as well as minimal or absent exposure and subsequent contamination and/or exfoliation of the grafted materials.

Different surgical options in terms of flap designs and related suture technique have been developed over the years with the aim of optimizing the primary flap closure, thus promoting wound stability and blood clot maturation: the Papilla Preservation Technique (PPT) introduced by Takei (Takei et al. 1985), the Interproximal Tissue Maintenance (ITM) (Murphy 1996), the Modified Papilla Preservation Technique (MPPT) (Cortellini et al. 1995) and the Simplified Papilla Preservation Technique (SPPT) (Cortellini et al. 1999). All these surgical techniques, representing the "classical" surgical approaches to intraosseous defects, are based on the elevation of a double mucoperiosteal flap involving both buccal and oral aspects and on the complete preservation of the interdental tissues. The application of this concept as MPPT for GTR-treatment of intraosseous defects results in 70% of complete flap closure in interdental areas and greater CAL gain compared to surgical approaches not characterized by the preservation of the interdental papilla.

In the last decade, a growing interest for more operator-friendly and patient-oriented surgery has urged clinical investigators to focus their interest in the development of less-invasive and simply approaches. The term "simplify" means the act of making something less complex. Its etymology originates from the Medieval Latin verb *simplificare*, which, in turn, derives from the terms *simplex* (simple), and *facere* (make). A clinical procedure is considered "simplified" when is characterized by more favorable conditions for either the patient or the clinical operator. For the operator, a simplified procedure should: (i) require limited surgical equipment; (ii) be characterized by a steep learning curve; (iii) limit the need for the use of additional treatments/devices (through the maximization of the inherent healing potential of the treated lesion). For the patient, a simplified procedure should have

a reduced impact on: (i) post treatment daily activities; (ii) post-treatment pain and discomfort (also reducing the required compliance for post-treatment regimens); (iii) pre-existing esthetics. For both patient and operator, a simplified procedure should reduce: (i) chair-side time needed for treatment administration and follow-up visits; and (ii) treatment costs.

A simplified surgical procedure in the treatment of intraosseous periodontal defects: the Single Flap Approach

The Single Flap Approach (SFA) is a simplified surgical procedure specifically indicated for periodontal reconstructive procedures in intraosseous defects characterized by an extension prevalent on the buccal or oral side (Trombelli et al. 2007, 2009, 2010). The basic principle behind the SFA consists of the elevation of a limited mucoperiosteal flap to allow surgical access from either the buccal or oral aspect only, depending on the main buccal or oral extension of the lesion (as diagnosed by pre-operative bone sounding and periapical radiographs), and leaving the interproximal supracrestal gingival tissues intact. Therefore, a pre-requisite to apply the SFA principles is that the morphology of the defect is compatible with a thorough root/defect debridement when accessed by either buccal or oral side only. Whenever the bucco-oral extension of the defect prevents the successful removal of the oral biofilm from the root surface as well as the complete degranulation of the intraosseous component of the defect, conventional double-flap approaches are to be performed. However, data derived from the distribution of intrabony defects according to the bone morphology (Vrotsos et al. 1999, Tal 1984) combined with observation from a prospective trial (Cortellini & Tonetti 2011) seem to suggest that a single-flap (usually buccal) access to intrabony defects may be feasible in a relevant proportion of surgically treated defects. The SFA may pose several clinical advantages. First, it may facilitate flap repositioning and suturing; the flap can easily be stabilized to the undetached papilla, thus optimizing wound closure for primary intention healing. Second, by limiting the surgical trauma on the vascular supply of the interproximal supracrestal soft tissues due to a limited flap elevation, a faster wound-healing process, particularly at the level of the incision line, is promoted. Wound stabilization and preservation of an intact interdental papilla may also minimize the post-surgery shrinkage of gingival tissues and, therefore, limit the esthetic impairment of the patient. In contrast, a limited surgical access may potentially result in an inadequate root/defect debridement and difficulty in graft/membrane placemen (Trombelli et al. 2012). The SFA represents a valuable reconstructive procedure *per se* (Trombelli et al. 2010, 2012). A pilot study (Trombelli et al. 2012) indicated that the SFA is at least as

clinically effective as the elevation of a flap at both buccal and oral aspects according to the papilla preservation techniques (Cortellini et al. 1995, 1999). In addition, the SFA was effective also when used in association with various reconstructive technologies, including graft materials, membranes and bioactive agents (Trombelli et al. 2009, 2010, Farina et al. 2013, 2014). Cortellini & Tonetti (Cortellini & Tonetti 2009) published a similar surgical approach, namely the modified MIST, which is based on the elevation of a single buccal flap and that was successfully used alone or in combination to different regenerative technologies to treat deep intraosseous defects (Cortellini & Tonetti 2011). Moreover, the SFA was shown to be associated with low mean postoperative REC increases, with five of six prospective clinical trials reporting mean variations within 1 mm at 6 months after surgery (Trombelli et al. 2009, 2010, 2012, Farina et al. 2013, 2014, Schincaglia et al. 2015). Also, encouraging results were reported by two randomized controlled trials in which limited post-surgery REC increase was observed for sites treated with SFA compared to sites accessed with papilla preservation techniques (Trombelli et al. 2012, Schincaglia et al. 2015). Similarly, low REC increase was reported when intraosseous defects were accessed with a flap design similar to the SFA and treated with different modalities (Cortellini & Tonetti 2011).

SFA mainly consists of an envelope flap. Sulcular incisions are performed on the buccal or oral side (for defects with a prevalent extension on the buccal or oral side, respectively) following the gingival margin of the teeth included in the surgical area. The mesio-distal extension of the flap is kept as limited as possible while ensuring proper access for defect debridement (as well as positioning/application of a reconstructive technology, if indicated). The priority in terms of flap extension, therefore, is given to provide adequate surgical access, sometimes extending the incision to involve the papillae of adjacent teeth in order to limit the use of vertical releasing incisions. In the interproximal area (i.e., at the level of the interdental papilla) overlying the intraosseous defect, an oblique or horizontal, butt-joint incision is made following the profile of the underlying bone crest. The distance between the tip of the papilla and the apico-coronal level of the interdental incision is based on the apico-coronal dimension of the supracrestal soft tissues. Pre-operatively, probing measurements are carefully performed to properly assess the horizontal component of the bone loss and, therefore, the apico-coronal dimension of the soft tissues overlying the bone crest. The greater the distance from the tip of the papilla to the underlying bone crest, the more apical (i.e., close to the base of the papilla) the incision in the interdental area. This is done to provide an adequate amount of untouched supracrestal soft tissue

connected to the undetached papilla to ensure flap adaptation and suturing as well as to warrant proper access to the intraosseous defect for debridement and, when needed, graft/membrane positioning.

The defect is approached by elevating a flap only on the buccal or oral side and leaving the opposite portion of the inter-dental supracrestal soft tissues undetached. The full-thickness elevation of the marginal portion of the flap should be performed with a microsurgical periosteal elevator. Partial-thickness dissection, if needed, must be limited to the apical portion of the flap to ensure flap replacement and suturing without tension. Once root and defect debridement has been completed, a horizontal internal mattress suture is placed coronal to the mucogingival junction between the flap and the base of the undetached papilla in order to provide the flap re-positioning. Then, a vertical or horizontal internal mattress suture (or an interrupted suture) is placed between the most coronal portion of the flap and the most coronal portion of the intact papilla to ensure primary closure. Suture removal is performed 14 days after surgery.

REGENERATIVE PROCEDURES WITH BIOLOGICAL AGENTS

There are several techniques - used alone or in combination- considered to achieve periodontal regeneration. Among these, root surface conditioning, bone grafts or bone substitutes and biological agents. Localized delivery of biological agents to the periodontium is an emerging approach that has become a powerful tool in periodontal regenerative medicine and in particular in the management of periodontal defects (Chen et al. 2010). Current research in this area is focused on identifying relevant biological agents, optimal dosages and the best approach of delivering.

Definition

Biological agents (BAs) are potent biological active molecules. Several classifications of BAs have been proposed through the years (Trombelli & Farina 2008, Bosshardt 2008). In particular, in the systematic review by Trombelli e Farina (Trombelli & Farina 2008) the BAs are divided in two categories:

i. Growth Factors (GFs): GFs are proteins that bind to specific cell surface receptors and mediate cross talk between the cells in the extracellular matrix via their autocrine and paracrine effects. The biological basis of using GFs is

because of their inherent property of inducing chemotaxis and/or mitogenesis of mesenchymal and somatic cell populations, initiating a cascade of events that ultimately lead to proliferation and differentiation of these cells (Cochran e Wozney 1999). 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. The GFs employed for periodontal and/or bone regeneration include: Insulin-like growth factors (IGFs), fibroblast growth factors (FGFs), epidermal growth factors (EGF), platelet-derived growth factors (PDGFs), vascular endothelial growth factors (VEGF), parathyroid hormone (PTH), transforming growth factor-β (TGF-β) and bone morphogenetic proteins (BMPs) are among the growth factors that have been tested in animal experiments. The efficacy of the GFs previously described in periodontal regeneration has been reviewed exhaustively both for clinical and for pre-clinical applications (Caffesse & Quinones 1993, Ripamonti & Reddi 1997, Shimono et al. 2003, Ripamonti & Renton 2006).

ii. Other biological mediators: including platelet-rich plasma (PRP) preparations as well as other commercially available products such as Enamel Matrix Proteins (EMPs, Emdogain®, Straumann, Basel, Switzerland).

This dissertation will be focused on two of the most commonly used BAs: PDGF and EMPs. In particular, the clinical effectiveness of PDGF and EMPs in the regenerative treatment of intraosseous periodontal defects will be evaluated.

Enamel Matrix Proteins (EMPs)

To better understand the mechanism of action of EMPs, it is critical to understand the composition of its protein complex. EMPs are secreted by ameloblasts, columnar epithelial cells involved in the formation of the enamel layer of the teeth, and include:

- Amelogenins proteins: a family of hydrophobic proteins derived from different splice variants and controlled post-secretion from the expression of a single gene.
- Non-amelogenin: including ameloblastin (also called amelin, sheathlin), amelotin, tuftelin and enamelin (Bartlett et al. 2006, Margolis et al. 2006).

Traditionally, EMPs are associated with amelogenesis: amelogenins self-aggregate into supramolecular aggregates, so-called nanospheres, and play a crucial role in regulating the initiation, maturation and orientation of hydroxyapatite crystals during the formation of

enamel (Fincham 1983, Hu 2001). It is important to know that EMPs have functions that go beyond enamel bio-mineralization: EMPs also play a pivotal role in the differentiation of progenitor cells into cementoblasts that specifically produce acellular extrinsic fibre cementum (Bosshardt 2008).

EMPs are available as a therapeutic agent under the brand name of Emdogain® (Straumann, Basel, Switzerland). Emdogain® consists of an enamel matrix derivative (EMD) (a mixture of EMPs, mainly amelogenins), water and a carrier (propylene glycol alginate, PGA) gel at an acidic pH to permit application via syringe to the affected site. It was first tested successfully for periodontal regeneration in animal models in 1986 and later approved for the market in Europe (1995), in the United States (1996) and in Japan (1998). The application of EMPs in the form of Emdogain® has set a modern standard for periodontal regeneration therapy (Esposito et al. 2004). A meta-analysis of the literature reveals that EMD seems to be safe in clinical settings, was able to regenerate lost periodontal tissues in previously diseased sites based on clinical parameters, and was better than OFD and GTR in terms of histological studies in both animals and humans.

Mechanism of action

If, as several observation suggest (Hammarström 1997, Sphar & Hammarström 1999), amelogenin deposition precedes cementum formation, than EMD treatment probably mimics odontogenesis and works by restarting dormant developmental programs in cells for regeneration of the tooth-supporting apparatus. It has been assumed that the most important mechanism of action of EMD is to initiate periodontal regeneration through the recruitment of cementoblasts from surrounding healthy tissues to the root surface and stimulate these cells to form root-cementum (Chen et al. 2010). The newly formed root cementum will then lead to regeneration of periodontal fibres and alveolar bone. Interestingly, many recent studies are reporting that amelogenins also can interact directly with cell types such osteoblasts, fibroblasts and oral epithelial cells, suggesting that these molecules have a more direct role in the regrowth of periodontal tissues (Lyngstadaas et al. 2001, Kawase et al. 2001, Veis et al. 2000)

Cellular effects of EMD

Periodontal ligament (PDL) fibroblasts have been heavily studied in the presence of EMD for effects on specific cell functions associated with the regenerative process including recruitment, proliferation and differentiation. The majority of studies on PDL cells indicate

that EMD enhances proliferation (Gestrelius et al. 1997, Lyngstadaas et al. 2001, Okubo et al. 2003). This property combined with increased migration contributes to the beneficial wound-healing effects observed for PDL cells stimulated by EMD in vitro (Hoang et al. 2000, Rincon et al. 2003). Effects of EMD on PDL cell attachment are most inconsistent ranging from no effect (Gestrelius et al. 1997, Palioto et al. 2004) to having a positive effect (Lyngstadaas et al. 2001), particularly when using an EMD-coated colture well (Suzuki et al. 2001). Numerous GFs, cytokines, extracellular components and transcription factors have been shown to be up-regulated by PDL cells exposed to EMD (Brett et al. 2002, Haase et al. 2001). Multiple studies have supported the findings that mRNA expression and protein synthesis of the TGF-β by PDL cells are stimulated by EMD (Lyngstadaas et al. 2001, Okubo et al. 2003). EMD has further been shown to stimulate alkaline phosphatase (ALP) activity and to promote mineral nodule formation of PDL cells (Gestrelius et al. 1997). Changes in phenotype of PDL cells toward cementoblasts have also been reported on EMD-coated surfaces (Cattaneo et al. 2003, Inoue et al. 2004).

The treatment of gingival and dermal fibroblast with EMD has also been under investigation. Again, the majority of studies indicate enhanced proliferation following treatment with EMD (Kawase et al. 2001, Keila et al. 2004). Kawase et al. (Kawase et al. 2001) showed that EMD substantially stimulated the proliferation of human gingival fibroblasts in a dose-dependent manner over 3 days. EMD dose-dependently stimulated synthesis in human gingival fibroblastic cells. The proliferation of gingival al dermal fibroblasts has been shown to be independent of the cAMP pathway (Kawase et al. 2001) and dependent on the presence of serum growth factors and the activation of the extracellular sugnal-regulated kinase cascade (Zeldich et al 2007). EMD has been reported to stimulate tissue inhibitor of matrix metalloproteinase-3 (TIMP-3) production, which could improve the MMP-TIMP balance in gingival tissue and curb extracellular matrix destruction caused by periodontal disease (Zeldich et al 2007). Gingival fibroblast mRNA expression of matrix proteoglycans and hyaluronan synthesis, among other proteins, also appear to be stimulated by EMD (Keila et al. 2004, Haase et al 2001). Compared with human PDL fibroblasts, human gingival fibroblasts attached and spread much less and slower (Van der Pauw et al. 2000). Furthermore, when compared with PDL cells, ALP activity and in vitro mineralization seems to be negligible in fibroblasts (Keila et al. 2004). Thus, EMD affects dermal and gingival fibroblasts differently than it does in PDL cells.

Several studies have demonstrate that the effects of EMD on epithelial cells are primarily inhibitory regarding proliferation (Kawase et al. 2000, 2001). Kawase et al. (Kawase et al.

2000) examined the effects of EMD on the proliferation of oral epithelial cells (SCC25, a carcinoma-derived cell line). Their results showed that EMD, in a dose-dependent manner, inhibited cell division and concomitantly arrested cell cycle at the G1 phase. However, no apoptosis was observed. The Authors concluded that EMD acts as a cytostatic rather than a cytotoxic agent on epithelial cells. The evidence suggest that the suppression of epithelial cell growth may be mediated by TGF-β. However, this principle may not be applicable to another special type of epithelial cells, the epithelial cells rests of Malassez (ERM). Rincon et al. (Rincon et al. 2005) showed that DNA synthesis by the ERM was significantly increased after ERM stimulation. The ERM represent a special group of cells, known to respond to inflammatory mediators by at least cell proliferation, and may be involved in periodontal regeneration.

The recruitment of vascular cells is also required for the regeneration of periodontal tissues. Interestingly, EMD appeared to stimulate the angiogenetic activity of human umbilical vein endothelial cells (HUVECs) and human microvascular endothelial cells (HMVECs). Proliferation and migration of endothelial cells were shown to be stimulated at low concentrations of EMD (0.1, 10, and 25μg/mL) and inhibited at higher concentrations (50-100μg/mL). Bertl reported that EMD up-regulates mRNA expression of the angiogenic factor *ANG-2* and adhesion molecules *ICAM-1* and *E-selectin* in HUVECs (Bertl 2009). This study also found that TGF-β was present in EMD-conditioned media, and that its presence maybe responsible the decrease in proliferation / viability of HUVECs by EMD. A study by Schleuter et al. found that EMD stimulated angiogenesis by stimulating endothelial cells directly and also indirectly by stimulating PDL cells expression of the angiogenic factors VEGF A, B, and C (Schlueter et al. 2007). Furthermore, this study demonstrated that EMD enhanced the bidirectional communication between HMVECs and PDL cells during angiogenesis associated with healing (in other words, HMVECs appear to enhance migration of PDL cells to sites of healing).

The effect of EMD on bone cells and bone growth has been studied by a number of groups with the following results. A study by Schwartz et al. indicated that EMD affects osteoblasts differently at different stages in their maturation: proliferation is stimulated by EMD in the early stages of osteoblastic maturation (pre-osteoblasts), while differentiation is enhanced as cells mature in the lineage (Schwartz et al. 2000). For the case of more mature osteoblasts, enhanced proliferation and differentiation has been also observed in a number of other studies. The effect of EMD on gene expression of osteoblastic and osteoblast-like cell lines has been studied by microarray analysis, polymerase chain

reaction (He et al. 2004) and enzyme-linked immunosorbent assay. Several hundred genes appear to be up- or downregulated by EMD, with upregulation of TGF-b (Reseland et al. 2006) IL-6 (Reseland et al. 2006, Jiang et al. 2001) osteoprotegerin (OPG) (He et al. 2004) collagen (He et al. 2004, Weishaupt 2008) and bone sialoprotein (BSP) (He et al. 2004, Weishaupt 2008) being reported in a number of studies. Normal bone remodeling depends on a delicate balance between bone formation and resorption. Bone resorption is regulated by a system constituting RANK and its ligand RANKL, which are members of the tumor necrosis factor ligand and receptor families, and OPG. RANKL is expressed by bone marrow stromal cells, osteoblasts and certain fibroblasts, whereas RANK is expressed by osteoclast precursors and mature osteoclasts. The binding of RANK to RANKL induces osteoclast differentiation and activity, and regulates their survival. OPG, which is produced by bone marrow stromal cells, osteoblasts, and certain fibroblasts, however, is a soluble decoy receptor for RANKL that competes for this binding. Thus, OPG is a natural inhibitor of osteoclast differentiation and activation. Any interference with this system can shift the balance between bone apposition and resorption. The expression of M-CSF plays an essential role in this regulatory system. Interestingly, EMPs have an influence on this system by modulating the expression of OPG and RANKL. While a few studies suggest an up-regulation of RANKL, most studies show a down-regulation of RANKL and an up-regulation of OPG. This suggests that EMPs modulate the RANKRANKL- OPG system most likely towards bone apposition. Of interest in this context is the observation that amelogenin knock-out mice show increased hard tissue resorption (Hatakeyama et al. 2003). Furthermore, it has also to be taken into consideration that some of the growth factors and cytokines that are up-regulated by EMPs directly up-regulate OPG and down-regulate RANKL production. Thus, EMPs appear to be indirectly involved in the regulation of bone remodelling.

The effect of EMD on bacterial cells has also been studied. The first paper that appear on this topic was by Sculean (Sculean et al. 2001). They evaluate the effects of EMD on ex vivo dental plaque vitality. Plaque samples from 24 patients with chronic periodontitis were covered with various solutions for 2 min, followed by vitality measurements. When EMD was used, 54% of the bacteria remained vital. However, when EMD+PGA (Emdogain®) was used, only 21.4% of the bacteria remained vital. When PGA, the carrier, was used alone, the vitality of the bacteria declined to only 19.6%. NaCl, used as a negative control, and chlorhexidine as a positive control showed 76.8% and 32.3% vitality, respectively. These results suggest that Emdogain® (EMD+PGA) has an antibacterial

effect and that PGA contributed most to this activity. The aim of a study of Spahr (Spahr et al. 2002) was to evaluate the effect of EMD on the in vitro growth of gram-negative periodontal pathogens like *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia*. Their results revealed a marked inhibitory effect of EMD+PGA on the growth of these gram-negative bacteria. Interestingly, alone had the same inhibitory effect. Arweiler et al. (Arweiler et al. 2002) examined the antibacterial efficacy of EMD on established supra-gingival plaque in periodontally healthy dental students. Biofilm vitality was 86.7%, 70.4%, 67.5%, and 56.2% after application of NaCl, EMD+PGA, PGA alone, and chlorhexidine, respectively. This study shows that EMD+PGA, PGA alone, and chlorhexidine possess significantly high antimicrobial properties when compared with a standard NaCl solution. As in the study of Sculean (Sculean et al. 2001), the PGA alone appeared to contribute primarily to the antibacterial properties. Newman (Newman et al. 2003) studied the in vitro effects of EMD on *P. gingivalis* and showed that EMD+PGA, and PGA alone had antimicrobial effects. An amelogenin fraction of EMD did not show an antibacterial effect on *P. gingivalis*, but stimulated the growth of this bacterium. Thus, the authors clearly and correctly concluded that the antimicrobial effects could be attributed to the vehicle PGA. Walter et al. (Walter et al. 2006) came to the same conclusion.

EMD and angiogenesis

Angiogenesis is one of the most critical events in wound healing as an increase in vascularization results in rapid and complete healing (Yuan et al. 2003). As such, numerous studies have investigated the effects of EMD on angiogenesis. Schlueter et al. (Schlueter et al. 2007) demonstrated that treatment of human micro-vascular endothelial cells with EMD resulted in an increased proliferation and a 100% increase in chemotaxis. These Authors also tested various weight fractions of EMD isolated by size-exclusion chromatography and found that certain fractions were associated with proliferation and angiogenic activities of microvascular cells. Aspriello et al. (Aspriello et al. 2011) tested the effects of EMD on VEGF expression and micro-vessel density in gingival tissue biopsies of periodontal pockets of 28 patients at 48 h post-surgery. It was found that sites treated with EMD showed induced proliferation, viability and angiogenesis as well as increased endothelial VEGF expression (Aspriello et al. 2011). Similarly, in another study by Thoma et al. (Thoma et al. 2011), the angiogenic effect of EMD was analyzed using an in vivo angiogenesis assay of silicon tubes implanted sub-cutaneously in mice with: i) EMD parent; ii) nine pools of EMD proteins; iii) VEGF; iv) amelogenin. The higher

angiogenic potential of the EMD parent was at a weight of 125 ng, resulting in a 4.3-fold increase compared to the negative controls. All these studies investigating angiogenesis following use of EMD show an increase in either migration of endothelial cells, VEGF production and/or vessel formation in various cell cultures and *in vivo* models.

Clinical effectiveness of EMD

Several reports have demonstrate a substantial CAL gain after regenerative procedures supported by the application of EMD in intraosseous defects (Trombelli & Farina 2008). The clinical effectiveness of EMD for the treatment of intraosseous defects has been reviewed recently (Venezia et al. 2004, Kao et al 2015).

EMD versus OFD. The first RCT to compare the effectiveness of EMD versus OFD was published by Heijl (Heijl et al. 1997) Clinical reductions in PD, increases in CAL, and increases in linear bone growth with EMD were statistically superior to improvements observed with OFD. Subsequently, several additional studies evaluated the efficacy of EMD versus OFD, with the majority confirming that OFD followed by EMD application resulted in substantial improvements in clinical measurements and bone fill with EMD in the management of intraosseous defects (Tonetti et al. 2002, Sculean et al. 2004, Silvestri et al. 2000). Neither postoperative antibiotics nor EDTA root conditioning improved the clinical outcome of EMD therapy (Sculean et al. 2006, Parashis et al. 2006).

EMD versus GTR. Of the 11 studies comparing the clinical management of intrabony defects with EMD versus GTR, all but one failed to show any significant difference (Sanz et al. 2004, Crea et al. 2008, Sculean et al. 2006, Silvestri et al. 2000). The noted exception was an RCT that compared the two therapeutic modalities in deep, non-contained intraosseous defects (Siciliano et al. 2011). In these defects, GTR with titanium reinforcement was superior. The latter results suggest that, in situations in which defect configuration is broad or lacking in wall containment, a supported barrier membrane may be critical in the success of EMD-associated regeneration. Additionally, no added clinical advantage was observed when EMD was combined with GTR (Sculean et al. 2001, 2004).

EMD alone versus EMD used in combined therapy. There are several studies in which EMD has been used in combined therapy (Sculean et al. 2007, Guida et al. 2007, Zucchelli et al. 2003, Döri et al. 2013). Histologic evidence of periodontal regeneration has been demonstrated when EMD is used in combination with autogenous bone, a bovine-derived natural bone mineral (NBM), bioactive glass, NBM + PRP, nanocrystalline hydroxyapatite

(NHA), or biphasic calcium phosphate (Sculean et al. 2003, 2005). The majority of the studies indicate no added benefits in either clinical and radiographic gains when EMD is used with the addition of graft materials (Sculean et al. 2007, Guida et al. 2007, Döri et al. 2013).

These updated studies confirmed the conclusions of meta-analyses of RCTs that there are few additional benefits of EMD when used in conjunction with other regenerative materials/approaches (Tu et al. 2010). The exceptions are limited reports that indicate that improved PD, CAL, and/or bone fill is achievable when EMD augments the effect of bone (Yilmaz et al. 2010) or bone graft enhances the effects of EMD (Zucchelli et al. 2003).

Post-operative wound healing after application of EMD: Several clinical studies (Cueva et al. 2008, Castellanos et al. 2006, Tonetti et al. 2004) have demonstrated that the application of Emdogain® results in the enhancement of the post-operative wound healing, both after regenerative periodontal therapy and mucogingival surgery. Moreover, a number of *in vitro* findings further favor the idea that EMD is capable of promoting wound healing and resolution of inflammation: (i) the reduction in the expression/production of a number of interleukins and possibly TNF, suggests that EMD is able to attenuate the inflammatory phase (Miron et al. 2014); (ii) the increase in OPG/RANKL ratio may reduce local bone resorption and favor the resolution of bone loss and formation of new bone; (iii) EMD was shown to stimulate gingival fibroblast proliferation and extracellular matrix production and prevent their apoptosis (Miron et al. 2014). These effects could enhance the proliferative/maturation phase of wound healing. In addition, a number of key molecules implicated in tissue repair are up-regulated by EMD in various cell types; (iv) The increase in expression of VEGF, a known angiogenic growth factor responsible for new blood vessel formation, may contribute to tissue repair by providing faster renewal of blood supply within the wound (Yuan et al. 2003, Schlueter et al. 2007); (v) Lastly, TGF-β is considered an important growth factor in orchestrating wound healing (Barrientos et al. 2008). A number of in vitro studies have demonstrated that EMD increases the expression and/or release of TGF-β in gingival fibroblasts and PDL cells, an effect that increases their activity and generation of their associated tissues (Bosshardt 2008, Nokhbehsaim et al. 2011, Okubo et al. 2003). Thus, the combination of these events that may take place following application of EMD *in vivo* could be the mechanism by which this preparation contributes to the healing/ regeneration of tissues and resolution of inflammation.

Platelet-derived growth factors (PDGF)

One of the most extensively studied GFs is platelet-derived growth factor (PDGF). This growth factor has been well characterized and has broad wound healing activities in both hard (bone) and soft (gingival) tissues (Kaigler et al. 2011). In the late 1980's, Lynch and co-workers first discovered in an animal study that PDGF promotes regeneration of periodontal tissues including bone, cementum and periodontal ligament (Lynch et al. 1989). Since then, numerous studies have been published providing a deeper understanding of the mechanism of action and therapeutic potential of this molecular mediator. PDGF is a naturally occurring protein that is found abundantly in the bone matrix in at least three different forms: PDGF-AA, PDGF-AB and PDGF-BB (Alvarezet al. 2006). This GF is locally released by blood platelets during clotting following soft or hard tissue injury (Pierce et al 1991). Once it is released from the platelets, PDGF binds to specific cell surface receptors promoting rapid cell migration (chemotaxis), and proliferation (mitogenesis), in the area of injury (Ronnstrand et al. 2001). *In vitro* and *in vivo* studies have demonstrated that PDGF is a potent chemotactic and myogenic factor for gingival and periodontal ligament fibroblasts, cementoblasts and osteoblasts (Lynch et al. 1989, Centrella et al. 1992). In this context, an *in vitro* study reported that at a concentration of 10-20 ng/ml, PDGF-BB stimulated the proliferation of fibroblasts and osteoblasts, whereas a higher concentration (50 ng/ml) was required for the adhesion of periodontal ligament fibroblasts to diseased roots (Canalis et al. 1989). Additionally, recombinant human platelet-derived growth factor (rhPDGF) has been shown to promote regeneration of periodontal tissues in animal studies (Lync et al. 1989), human histologic reports (Nevins et al. 2003, Camelo et al. 2003) and human clinical trials (Nevins et al. 2005, 2013).

Preclinical studies using PDGF-BB for periodontal regeneration

In order to evaluate the potential and safety of this therapy, extensive in vivo preclinical studies have been performed using PDGF alone or in combination with other GFs such as insulin-like growth factor (IGF) to treat periodontal defects. In a previously mentioned study, Lynch and co-workers first published evidence of the regenerative potential of PDGF-BB when used to treat naturally occurring periodontal defects in dogs (Lynch et al. 1989). Most notably, this study showed increased cellular activity after treatment with PDGF-BB, leading to increased bone, cementum and periodontal ligament regeneration.

Clinical studies using PDGF-BB for periodontal regeneration

An early human clinical trial to evaluate the effect of rhPDGF/IGF treatment applied to osseous periodontal defects was reported by Howell and co-workers (Howell et al. 1997). The experimental sites received direct application of the GFs contained in a methylcellulose matrix to improve retention. A statistically significant increase in alveolar bone formation was seen in the growth factor treated sites at nine months post-operatively, as compared to untreated control sites. Average bone height for the PDGF/IGF group was 2.08 mm and 43.2% osseous defect fill was achieved, as compared to 0.75 mm new bone height and 18.5% fill in the control sites.

Based on the principles of tissue engineering, the use of a growth factor enhanced matrix for periodontal regeneration consisting of rhPDGF-BB in combination with an osteoconductive scaffold (i.e., autograft, allograft, xenograft, or a synthetic matrix, such as beta-TCP) was proposed (Stephan et al. 2000). The rationale underlying this approach is that PDGF stimulates angiogenesis, promotes cell migration into the bone defect from the surrounding tissue margins, and up-regulates cell proliferation (Hollinger et al. 2008). The matrix, in addition to its role as a growth factor delivery vehicle, provides mechanical support for migrating cells and contributes to the formation of new bone, cementum and/or periodontal ligament. The most commonly used osteoconductive scaffold is the β-TCP, a resorbable ceramic biomaterial commonly used in oral reconstructive surgery. The results of a large, multicenter clinical trial evaluating the effectiveness of rhPDGF-BB combined with a porous β-TCP matrix have been recently reported (Nevins et al. 2005). This study included 180 participants with one interproximal periodontal defect 4 mm or deeper after debridement. Other noteworthy inclusion criteria included a baseline probing depth of 7 mm or greater, sufficient keratinized gingiva to allow complete coverage of the defect, radiographic defect base at least 3 mm coronal to the apex of the tooth, and no evidence of localized aggressive periodontitis. Grade I and Grade II furcation defects were acceptable for inclusion in the study. Smokers who consumed up to one pack per day were also included in the study. Three treatment groups were evaluated:

1) β-TCP plus 0.3 mg/ml rhPDGF-BB (Group I),

2) β-TCP plus 1.0 mg/ml rhPDGF-BB (Group II),

3) β-TCP plus buffer alone (Group III).

Defects were classified as 1-wall, 2-wall or 3-wall / circumferential, indicating the extent of involvement and severity. At the time of surgery, β- TCP granules were saturated with rhPDGF-BB before the graft was placed in the defect site. Patients were followed for a period of six months and outcome measures included evaluation of soft tissue changes and assessment of bone growth. Safety was monitored throughout the trial by assessing the frequency and severity of clinical and/or radiographic adverse events.

Excellent healing was observed for all defects treated with rhPDGF-BB. The study results demonstrated that there was a significantly greater clinical attachment level gain at three months for the 0.3 mg/ml rhPDGF-BB (Group I), as compared to the β-TCP controls (Group III), indicating an early benefit of rhPDGF-BB treatment. At six months, the clinical attachment level gain for the lower rhPDGF-BB concentration group continued to be greater than the control group, although statistical significance was not achieved. Additionally, rhPDGF-BB treatment resulted in significantly less gingival recession at three months, as compared to the untreated control group. This difference was no longer apparent at six months, however, as the control group exhibited a slight gain in gingival height over time. Increasing the rhPDGF-BB concentration appeared to reduce the effectiveness of the growth factor-enhanced matrix. No statistically significant differences were observed in clinical attachment level or gingival recession for the higher rhPDGF-BB concentration (Group II), as compared to the β-TCP controls. Radiographic assessment revealed that bone fill was significantly increased at six months for the lower rhPDGF-BB concentration, as compared to both the higher rhPDGF-BB concentration and the control group. A subgroup analysis further indicated that rhPDGF-BB treatment improved bone fill in smokers and for all defect types (1, 2, 3 wall and circumferential). Similarly, linear bone growth was also significantly greater for Group I, as compared to Groups II and III. No significant differences were observed in the number or severity of adverse events among the three groups, indicating that both rhPDGF-BB and the β-TCP matrix were safe and adequately tolerated in the defect site. The results of this study demonstrate that the use of rhPDGF-BB in combination with a synthetic β-TCP matrix accelerates the rate of bone regeneration and improves bone fill and clinical attachment level in surgically treated periodontal defects.

Based on the results that the use of rhPDGF incorporated in β-TCP is effective and safe for the long-term use, a commercial product containing PDGF and β-TCP called GEM 21S (Osteohealth, Shirley, NY, USA) has been approved for the treatment of intraosseous and furcation periodontal defects in the USA and Canada, but isn’t currently available in

Europe. These positive outcomes were maintained over time as reported in a patient case series (McGuire et al. 2006). Four patients, selected from centers participating in the original pivotal clinical trial, exhibited significantly enhanced results for sites treated with 0.3 mg/ml rhPDGF and β-TCP in a long-term (24 month) evaluation. These results remained significantly improved over results observed for the β-TCP control group.

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Table 1. Clinical studies evaluating the effectiveness of non-surgical therapy in the treatment of intraosseous defects: experimental design, treatment approach and main results.

First Author	Year of publication	Experimental design	n° defects	radiographic depth of intraosseous component	baseline PD (mean, mm)	Treatment approach	Localization of intraosseous defects	Follow-up (months)	Clinical outcomes			Radiographic outcomes	Patient-centered outcomes
									CAL	PD	REC		
Isidor	1985	split-mouth, prospective clinical trial	13	≥ 15% of root length	7.6	root planing with manual instruments	lateral incisors canines premolars	12	mean gain: 1.6 mm	reduced to 4.2 mm	mean increase: 1.8 mm	mean gain in bone height: 0.9% (no alteration in 50% of defects; change comprised between -10% and +10% in the other 50% of defects)	NA
Renvert	1985	split-mouth, prospective clinical trial	25	NA	6.7	root planing with manual instruments	all tooth types	6	mean gain: 0.8 mm	reduced to 5.2 mm	mean increase: 0.8 mm	NA	NA
Hwang	2008	case series	39 (sites)	≥ 3 mm	6.57	scaling and root planing using mechanical and manual instruments	all tooth types	6	NA	NA	NA	increase in radiographic density in 83.3% of the analyzed regions	NA
Nibali	2011	retrospective study	126	mean: 3.8 mm	6.5	scaling and root planing using mechanical and manual instruments with/without systemic or local antibiotics	all tooth types	range: 12-18	mean gain: 1.42 - 1.50 mm	mean reduction: 2.24 - 2.29 mm	mean increase: 0.5 - 0.7 mm	vertical defect depth reduced to 3.08 mm (persistent radiographic defect depth ≥2.0 mm in 71% of defects)	NA

First Author	Year of publication	Experimental design	n° defects	radiographic depth of intraosseous component	baseline PD (mean, mm)	Treatment approach	Localization of intraosseous defects	Follow-up (months)	Clinical outcomes			Radiographic outcomes	Patient-centered outcomes
									CAL	PD	REC		
Ribeiro	2011	parallel-arm RCT	13 patients with ≥ 1 defect	≥ 4 mm	6.35	scaling and root planing with minicurets and an ultrasonic device with specific thin tips under an operating microscope (minimally-invasive non-surgical periodontal therapy, MINST)	single-rooted teeth	6	mean gain: 2.56 mm	mean reduction: 3.13 mm	mean increase: 0.45 mm	NA	low extent of discomfort, root hypersensitivity, and edema during the first post-therapy week no hematoma, high fever, or interference with daily activities mean n° of analgesic medications: 0.31 92.30% of patients very satisfied at 6 months

First Author	Year of publication	Experimental design	n° defects	radiographic depth of intraosseous component	baseline PD (mean, mm)	Treatment approach	Localization of intraosseous defects	Follow-up (months)	Clinical outcomes			Radiographic outcomes	Patient-centered outcomes
									CAL	PD	REC		
			14 patients with ≥ 1 defect	≥ 4 mm	7.07	Minimally Invasive Surgical Technique (MIST; Cortellini & Tonetti 2007)	single-rooted teeth	6	mean gain: 2.85 mm	mean reduction: 3.51 mm	mean increase: 0.48 mm	NA	low extent of discomfort, root hypersensitivity, and edema during the first post-therapy week no hematoma, high fever, or interference with daily activities mean n° of analgesic medications: 0.40 92.85% of patients very satisfied at 6 months
Nibali	2015	retrospective study	35	≥ 3 mm mean: 6.7 mm	7.8 (at deepest site)	MINST (Ribeiro et al. 2011)	all tooth types	12	mean gain: 2.8 mm	mean reduction: 3.1 mm	increased from 0.6 mm to 0.8 mm	reduction of intraosseous component: 2.93 mm supraosseous component increased from 2.1 mm to 2.6 mm	NA

CAL: clinical attachment level; NA: not available; PD: probing depth; REC: gingival recession.

AIMS OF THE THESIS

The general purpose of this Ph.D. thesis was to evaluate the effectiveness of bioactive agents (BAs) alone or in combination with graft materials in the regenerative treatment of periodontal intraosseous defects accessed with a simplified surgical procedure (the *Single Flap Approach*, SFA; Trombelli et al. 2007, 2009).

The following studies were performed in order to answer specific research/clinical questions:

1. What is the clinical effectiveness of BAs when used in combination with the SFA versus traditional double flap approaches in the treatment of periodontal intraosseous defects?
2. When treating an intraosseous defect with BA, does the SFA improve postoperative morbidity of the intervention compared to traditional double flap approaches?
3. What is the quality of early post-operative healing at the incision margin following regenerative treatment of periodontal intraosseous defects performed with the SFA with/without reconstructive devices (including bioactive agents)?
4. Which factors (either related to the patient or the treated site) may influence the quality of early wound healing following regenerative treatment of periodontal intraosseous defects with BAs in combination with the SFA?
5. What is the rationale for combining BAs and graft materials when approaching an intraosseous defect with the SFA?

CHAPTER 2

Early postoperative healing following buccal *Single Flap Approach* to access intraosseous periodontal defects

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Clinical Oral Investigations (2013) **17**, 1573-1583

Abstract

Aim: This study aims to to evaluate the early postoperative healing of papillary incision wounds and its association with (i) patient/site-related factors and technical (surgical) aspects as well as with (ii) 6-month clinical outcomes following buccal Single Flap Approach (SFA) in the treatment of intraosseous periodontal defects.

Methods: Forty-three intraosseous defects in 35 patients were accessed with a buccal SFA alone or in combination with a reconstructive technology (graft, enamel matrix derivative (EMD), graft+EMD, or graft+membrane). Postoperative healing was evaluated at 2 weeks using the Early Wound-Healing Index (EHI).

Results: EHI ranged from score 1 (i.e., complete flap closure and optimal healing) to score 4 (i.e., loss of primary closure and partial tissue necrosis). SFA resulted in a complete wound closure at 2 weeks in the great majority of sites. A significantly more frequent presence of interdental contact point and interdental soft tissue crater, and narrower base of the interdental papilla were observed at sites with either EHI> 1 or EHI= 4 compared to sites with EHI= 1. No association between EHI and the 6-month clinical outcomes was observed.

Conclusions: At 2 weeks, buccal SFA may result in highly predictable complete flap closure. Site-specific characteristics may influence the early postoperative healing of the papillary incision following SFA procedure. Two-week soft tissue healing, however, was not associated with the 6-month clinical outcomes.

INTRODUCTION

Ideally, during the early phases of healing following the elevation of a gingival flap, flap manipulation should ensure the stabilization of the root surface–adhering blood clot in a biologic environment protected from mechanical and microbiologic challenges. Unfortunately, a dehiscence of the wound margins may occur as a result of a compromised vascular supply due to surgical manipulation and/or tensile forces acting on wound margins. Wound dehiscence may compromise wound stability, which in turn would jeopardize the cascade of biologic events leading to periodontal regeneration (Linghorne & O'Connell 1950, Yumet & Polson 1985, Wikesjö & Nilvéus 1990, Haney et al. 1993). Furthermore, when flap surgery is used in association with regenerative technologies, the postoperative loss of primary closure may lead to the partial or complete exfoliation of the implanted graft, contamination of the membrane surface, or premature clearence of the biological agent. In this respect, the significance of primary (unexposed) intention healing as a determinant of periodontal wound healing following regenerative procedures has been universally recognized (Wikesjö et al. 1992, Polimeni et al.2006). In particular, the first postoperative weeks seem to be critical for the maintenance of wound stability (Wikesjö et al. 1992, Hiatt et al. 1968, Werfully et al 2002).

The surgical management of the supracrestal soft tissues, including flap design and suturing technique, seems of paramount importance in controlling the chances of wound failure during the early phases of healing, thus preserving clot stability (Wikesjö et al. 1991). Over the years, new surgical techniques specifically designed to optimize functional and esthetic outcomes of reconstructive procedures in the interdental area have been developed and, at least in part, validated. In essence, all the proposed "papilla preservation procedures" approached the main goal of optimizing the primary closure in the interdental area, thus ensuring the central conditions for blood clot stabilization and maturation (Trombelli 2010, Trombelli & Farina 2012). Such approaches, either used alone (Wachtel et al. 2003, Trombelli et al. 2010, 2012, Cortellini & Tonetti 2011) or in association with reconstructive devices (Wachtel et al. 2003, Trombelli et al. 2010, Cortellini & Tonetti 2001, 2005, 2007, 2009, 2011, Cortellini et al. 1995, 1996, 1999, 2008) were associated with a variable incidence of preserved primary flap closure within the first postoperative weeks.

Recently, we proposed a minimally invasive surgical procedure, the Single Flap Approach (SFA), designed for reconstructive procedures of intraosseous periodontal defects

(Trombelli et al. 2007). The basic principle behind SFA is the unilateral elevation (on the buccal or oral side) of a limited mucoperiosteal flap to allow surgical access depending on the main, buccal or lingual, extension of the intraosseous defect leaving adjoining gingival tissues intact. Buccal SFA has been shown to be similarly effective to the double flap (i.e., buccal and palatal) approach in supporting clinical improvements as a stand-alone protocol (Trombelli et al. 2012), and has been successfully combined with various regenerative technologies, including bone biomaterials with or without provisions for GTR (Trombelli et al. 2007, 2009, 2010). Preliminary observations revealed that the quality of wound healing in the first postoperative weeks after SFA may substantially differ among patients and sites, ranging from complete flap closure to wound dehiscence due to the complete necrosis of the interproximal tissues (Trombelli et al. 2010). Which factors are implicated in the incidence of early postoperative complications, such as wound dehiscence, following SFA needs to be further explored.

The present study was performed to evaluate the early postoperative healing of papillary incision wounds following SFA in the treatment of intraosseous periodontal defects, as assessed by the Early Wound-Healing Index (EHI) (Wachtel et al. 2003). The influence of patient-related and site-specific characteristics as well as technical (surgical) factors on EHI was explored. Also, the association between the EHI and the 6-month clinical outcomes of the procedure was evaluated.

MATERIALS & METHODS

The study was designed as a single-center clinical trial and conducted at the Research Centre for the Study of Periodontal and Peri-Implant Diseases, University of Ferrara, Italy. All the clinical procedures were performed in accordance with the Declaration of Helsinki and the Good Clinical Practice Guidelines (GCPs). Each patient provided a written informed consent before participation.

Patient and defect eligibility

Patient were consecutively included in the study if positive for each of the following inclusion criteria: (a) diagnosis of chronic or aggressive periodontitis; (b) no pregnancy or lactation; (c) no systemic diseases that contraindicated periodontal surgery; (d) no use of medications affecting periodontal status; (e) no assumption of anticoagulants, non-steroidal

anti-inflammatory drugs (NSAIDs), corticosteroids, or biological agents for the treatment of rheumatoid arthritis (e.g., TNF-α blockers, IL-1 blockers, IL-6 blockers); (f) presence of ≥ 1 deep (probing pocket depth ≥ 5 mm, radiographic depth ≥ 3 mm) interproximal intraosseous periodontal defect; (g) limited to no extension of the defect on the lingual or palatal side as assessed by preoperative bone sounding; (h) full-mouth plaque score (O'Leary et al. 1972) and full-mouth bleeding score < 20% at the time of the surgical procedure.

Third molars, teeth with degree III mobility, furcation involvement or inadequate endodontic treatment and/or restoration were excluded from the study.

Experimental protocol

Pre-surgery procedures

Each patient underwent a full-mouth session of scaling and root planing using mechanical and hand instrumentation and received personalized oral hygiene instructions. Temporary splinting and/or occlusal adjustment were performed for teeth with degree I or II mobility at re-evaluation following non-surgical instrumentation. The surgical phase was delayed until the achievement of minimal residual inflammation at the defect site.

Surgical procedures

All surgeries were performed by one experienced periodontal surgeon (LT) using 2.5 magnifying loops. The site of surgery was anesthetized using mepivacaine-epinephrine 1:100,000. Transcrevicular probing (bone sounding) was always performed pre-surgery to determine the characteristics of the bony defect, including the defect morphology and extension, the probing bone level and the horizontal component of bone loss.

The surgical access was performed by the elevation of a buccal mucoperiosteal flap according to previously detailed principles of the SFA (Figure 1) (Trombelli et al. 207, 2009, 2010, 2012). Briefly, a buccal envelope flap without vertical releasing incisions was performed. Sulcular incisions were made following the gingival margin of the teeth included in the surgical area. The mesio-distal extension of the flap was kept limited while ensuring access for defect debridement. An oblique or horizontal, butt-joint incision was made at the level of the interdental papilla overlying the intraosseous defect; the greater the distance from the tip of the papilla to the underlying bone crest, the more apical (i.e., close to the base of the papilla) the buccal incision in the interdental area. However, the

interdental incision was performed at least 1 mm coronal to the underlying bone crest. This provided an adequate amount of pristine supracrestal soft tissue connected to the undetached oral papilla to ensure subsequent flap adaptation and suturing, and permitted proper surgical access to the intraosseous defect. For each defect, a microsurgical periosteal elevator (P-TROM periosteal elevator, Hu-Friedy, Milan, Italy) was used to raise a flap only on the buccal side, leaving the oral portion of the interdental supracrestal soft tissues undetached. Root and defect debridement were performed using hand and ultrasonic instruments. At the completion of root and defect instrumentation, defects were left to fill with a blood clot or treated with a reconstructive technology (hydroxyapatite-based graft or enamel matrix derivative, EMD) or a combination of different technologies (graft+EMD or graft+resorbable collagen membrane). The choice of the reconstructive strategy was based on patient-related and defect-specific characteristics (Trombelli 2005) and left to the operator's judgment. At wound closure, a horizontal internal mattress suture (Vicryl 6.0, Ethicon, Sommerville, NY) was placed between the buccal flap and the base of the attached oral papilla to ensure repositioning of the buccal flap. Wound closure was achieved by means of a second internal mattress suture (vertical or horizontal) which was placed between the most coronal portion of the flap and the most coronal portion of the oral papilla. When needed (i.e. in case of a large, thick interdental papilla), an interrupted suture was performed to ensure primary intention healing at the incision line. Primary flap closure was always obtained at suturing.

Post-surgery procedures

Sutures were removed at 2 weeks post-surgery. The patients were asked to abstain from mechanical oral hygiene procedures in the surgical area for 4 weeks. A 0.12% chlorhexidine mouthrinse (10 mL BID/6 wks) was used to support local plaque control. The patients were then supplemented with an antimicrobial AmF/SnF_2 mouthrinse (meridol mouthrinse, GABA International, Therwil, Switzerland) and toothpaste (meridol toothpaste, GABA International, Therwil, Switzerland) regimen. Each patient was inserted in a monthly recall program for 3 months and was reviewed according to personal needs thereafter. Each session included reinforcement of oral hygiene procedures and supragingival plaque removal. Subgingival scaling was performed following completion of the study at 6 month post-surgery.

Recordings

One calibrated masked examiner (AS) performed all the following clinical recordings.

Pre-surgery assessment

The interdental site presenting the defect was characterized according to the following parameters:

- interdental contact point, recorded as present or absent;
- interdental soft tissue crater, recorded as present when invagination of soft tissues was observed at the interdental col overlying the intraosseous defect.

The following measurements were performed immediately before surgery using a manual pressure sensitive probe (UNC 15, Hu-Friedy, Chicago, IL, USA) with 1-mm increments at the site showing the greatest loss of clinical attachment:

- pocket probing depth (PPD), measured from the gingival margin to the bottom of the pocket;
- local bleeding score (BS): recorded as positive when bleeding on probing was present at the surgical site;
- clinical attachment level (CAL), measured from the cemento-enamel junction (CEJ) to the bottom of the pocket;
- gingival recession (REC), measured from the CEJ to the gingival margin.

The gingival recession was also recorded at the buccal aspect of the tooth presenting the intraosseous defect (bREC). The amount of interdental keratinized tissue (iKT) was measured as the distance from the tip of the interdental papilla to the mucogingival junction.

On digital photographs showing the buccal aspect of the tooth presenting the intraosseous defect and taken as much perpendicular as possible to the long axis of the tooth, the tip of the papilla (A) was first identified. Lines were then traced passing through A and tangent to the profile of the crowns of the teeth adjacent to the intraosseous defect to identify reference points (B_1 and B_2) (Figure 2). The following measurements were obtained in mm:

- width of the interdental papilla (pW): distance between B1 and B2;

- height of the interdental papilla (pH): distance between A and the midpoint of the line connecting B1 and B2.

To account for photographic magnification, pW and pH were referred to the increments of the UNC 15 probe as depicted in the same photograph (Figure 2).

Intra-surgery assessments

The distance between the tip of the interdental papilla and the papillary incision margin at the interdental site (T-I) was measured using a UNC 15 periodontal probe.

At completion of the intra-surgical debridement, the following parameters were assessed:

- configuration of the intraosseous defect (i.e., number of walls);
- depth of the intrabony component (IBD), measured with a UNC 15 periodontal probe as the distance between the deepest point of the defect and the most coronal point of the alveolar crest.

Post-surgery assessments

At suture removal, performed at 2 weeks post-surgery, the following parameters were assessed:

- membrane exposure, biomaterial exfoliation, and other complications;
- early wound healing of the incision at the level of the interdental papilla, evaluated according to the EHI (Wachtel et al. 2003) as reported in the following scale: 1) complete flap closure, no fibrin line in the interproximal area; 2) complete flap closure, fine fibrin line in the interproximal area; 3) complete flap closure, fibrin clot in the interproximal area; 4) incomplete flap closure, partial necrosis of the interproximal tissue; and 5) incomplete flap closure, complete necrosis of the interproximal tissue. Intra-examiner agreement for EHI recordings, as assessed on a sample of 34 defect sites and expressed as Kendall τ coefficient, was 0.97.

At 6 months after surgery, PPD, BS, CAL, REC, and bREC were assessed.

Statistical analysis

The statistical analysis was performed at the Department of Clinical and Experimental Medicine, University of Ferrara, by an operator (A.C.) expert in the elaboration of data from studies in the periodontal field. A statistical software (STATISTICA; StatSoft, Italia s.r.l., Vigonza, Italy) was used for data analysis.

Descriptive statistics on early postoperative healing was based on the entire defect population (n= 43). However, to assess the association between EHI and patient-related and site specific characteristics, the patient was considered as the statistical unit. Therefore, in patients contributing two ore more defect sites, only one defect site was selected at random and considered for analysis. EHI was regarded as the primary outcome variable. The patient-related (age, gender, smoking status, diabetic status) and site-specific characteristics (tooth type, presence of interdental contact point, pW, pH, iKT, supracrestal component of the pocket, presence of interdental soft tissue crater, IBD, defect configuration with respect to the number of bony walls) as well as the technical aspects (T-I, additional use of a reconstructive technology) were regarded as influencing (independent) variables. Patients were categorized according to EHI (= 1, >1, >2 or >3). In order to assess which factors were associated with optimal wound healing, patients with EHI= 1 were compared to patients with EHI> 1. Similarly, patients with EHI= 1 and patients with EHI> 3 were compared to determine which variables were associated with wound failure.

6-month changes in CAL, PPD, REC and bREC were also calculated and referred to EHI.

Data were expressed as mean ± standard deviation (SD). Comparisons were performed using the χ^2-test or the two-tailed Fisher's exact test, and the Student's t-test for independent observations. Odds ratios were calculated for factors which were significantly associated with optimal wound healing (EHI= 1 vs EHI> 1). Such factors were also entered into a multiple regression model to explain the variability in early soft tissue healing.

Level of significance was set at 5%.

RESULTS

Study population

Thirty-five patients [24 males and 11 females; mean age: 51.4 ± 8.5 years, age range: 34 - 65 years; 2 poorly controlled type I diabetics (i.e., HbA1c≥ 7.0% at the last exam prior to surgery); 7 current smokers and 4 former smokers] were included in the study. Two patients assumed anti-aggregants (Cardioaspirin® 100 mg; Bayer S.p.A., Milan, Italy). Patients contributed 43 intraosseous defects. In all cases, buccal SFA ensured an adequate surgical access for root and defect instrumentation. Twenty-eight patients contributed 1 defect, 6 patients contributed 2 defects, and 1 patient contributed 3 defects. Defect characteristics are summarized in Table 1.

Twenty-five defects (18 patients) were left to spontaneous healing, whereas in 18 defects (17 patients) different reconstructive technologies were associated to SFA (Table 2). All patients complied with the recall program until the 6-month visit.

Early postoperative healing

EHI was 1.9 ± 1.1 and ranged from 1.0 to 4.0. Thirty-six sites exhibited a complete wound closure at 2 weeks, showing a EHI= 1 (23 defects), EHI= 2 (9 defects) or EHI= 3 (4 defects). Seven defects showed an incomplete flap closure (EHI= 4). None of the defects showed a EHI= 5. For sites where SFA was associated with a reconstructive technology, no evidence of membrane exposure or exfoliation of the biomaterial were either observed by the clinical examiner or referred by the patient at the 2-week visit.

Factors associated with early postoperative healing

Factors associated with early postoperative healing are reported in Table 3.

No association between EHI and age, gender or smoking status was observed. The 2 sites in the 2 diabetic patients showed a EHI of 1 and 3, respectively. The 4 sites in the 2 patients assuming antiaggregants showed a EHI of 1.

When compared to patients exhibiting a EHI= 1, patients with either EHI> 1 or EHI> 3 showed a significantly more frequent presence of interdental contact point and interdental soft tissue crater, and lower pW (Table 3, Figure 3). Moreover, patients with EHI> 3 showed a significantly different distribution according to tooth type (i.e., a greater proportion of posterior teeth) than patients with EHI= 1 (Table 3). The presence of the

interdental contact point, the presence of an interdental soft tissue crater, and pW< 5 mm were significantly associated with an increased risk of having EHI> 1 (Table 4). When site-specific characteristics associated with early wound healing (EHI= 1 vs EHI> 1) (i.e., presence of interdental contact point, presence of interdental soft tissue crater, pW) were entered into a multiple regression analysis, the model was statistically significant (**p**< 0.001, R^2= 0.47).

Early postoperative healing and 6-month outcomes

At 6 months, treatment resulted in a significant CAL gain as well as a significant PPD reduction (p< 0.001 for both comparisons). Also, significant increases in REC and bREC were observed at 6 months compared to pre-surgery (p= 0.004 and p= 0.016, respectively) (Table 5). The number of patients showing CAL loss or no change in CAL, CAL gain 1÷2 mm, CAL gain 3÷4 mm, or CAL gain ≥ 5 mm was 3, 6, 15, and 11, respectively. At 6 months, 74.3% of patients (26 over 35 patients) showed a PPD≤ 4. The frequency of BS-positive patients shifted from 26, as assessed immediately before surgery, to 13, as assessed at 6 months post-surgery (p= 0.002).

No statistically significant difference in the changes of CAL, PPD, REC and bREC were observed between patients with EHI= 1 and either patients with EHI> 1 or EHI> 3 (Table 6).

DISCUSSION

The present study was performed to evaluate the early postoperative healing following buccal SFA for the treatment of deep intraosseous periodontal defects. Also, the association between EHI and (a) patient-related and site-specific characteristics as well as technical (surgical) factors, and (b) 6-month clinical outcomes was evaluated. Forty-three intraosseous defects in 35 patients were accessed with SFA as a stand-alone protocol or in combination with a reconstructive technology. Primary flap closure was obtained at suturing in 100% of sites. A semi-quantitative assessment of postoperative wound healing was performed at 2 weeks using the EHI (Wachtel et al. 2003). Within their limits, the results of the study indicate that (a) SFA resulted in a complete wound closure at 2 weeks in the great majority of sites; (b) a significantly more frequent presence of interdental contact point and interdental soft tissue crater, and narrower base of the interdental papilla

were observed in groups with either EHI> 1 or EHI> 3 compared to the group with EHI= 1, and a significantly greater proportion of posterior teeth was observed in group with EHI> 3 compared to the group with EHI= 1; (c) 2-week soft tissue healing was not associated with 6-month clinical outcomes.

In the present study, the assessment of early postoperative healing at the incision margin was performed according to EHI (Wachtel et al. 2003). EHI evaluates the condition of the wound margin using a 5-point scale: the scores 1 to 3 are compatible with complete flap closure, whereas the scores 4 and 5 indicate partial or complete tissue necrosis leading to incomplete flap closure. Although EHI has never been validated either clinically or histologically, this semi-quantitative scale currently represents the only available method to objectively determine the early healing phase of a periodontal wound. Noteworthy, the relevance of EHI as either clinical endpoint or predictor on the outcomes of a regenerative procedure had never been investigated.

SFA showed substantial reconstructive outcomes when used alone or in association with different reconstructive technologies (Trombelli et al. 2007, 2009, 2010, 2012), and appeared to be at least similarly effective compared to conservative 2-flap papilla preservation techniques (Trombelli et al. 2012). The rationale for the application of the SFA resides in the preservation of an intact interdental papilla, which may facilitate flap repositioning and suturing, thus optimizing wound closure for primary intention healing, as well as accelerate the re-establishment of the local vascular supply. Previous studies where a similar flap design was used to provide access for endodontic surgery in periodontally healthy patients, showed a high incidence of sites healed by primary intention (Velvart 2002). In our material, 36 over 43 (83.7%) sites overlying deep intraosseous defects showed complete flap closure at 2 weeks, with 53.5% of sites presenting optimal wound closure (EHI= 1) at clinical assessment. Consistently, when evaluating the 2-week soft tissue healing at sites accessed with a modified papilla preservation technique, Wachtel et al. reported a high proportion (89-96%) of sites showing a EHI≤ 2. Overall, these results confirm that a minimally-invasive surgical access, such as SFA (Trombelli et al. 2007), may be associated with a high prevalence of sites maintaining wound closure at 2 weeks following surgery even in the presence of a substantial alteration of the periodontal anatomy.

Although groups with EHI=1 and EHI>3 did not show a significantly different distribution in additional reconstructive technologies, a relevant proportion of patients treated with

reconstructive devices showed a sub-optimal (i.e., EHI> 1) postoperative healing. On the other hand, 88.9% of the patients treated with SFA as a stand-alone protocol showed successful soft tissue healing (EHI 1/3). This finding seem to suggest an effect of placing different biomaterials on periodontal wound healing following a standardized approach for the treatment of intraosseous defects such as SFA. It is possible to hypothesize that the adjunctive use of reconstructive devices may reduce the probability to obtain optimal early healing at sites accessed in accordance with the SFA principles. The results from previous studies evaluating the early postoperative healing of sites approached with SFA in combination with the use of a graft biomaterial + membrane support this consideration (Trombelli et al. 2010). The presence of a membrane may result in a transient impairment of the revascularization process of the gingival flap during the early phase of healing (Vergara et al. 1997). In this respect, a relationship between reduced blood perfusion in a mucoperiosteal flap covering a membrane and the incidence of wound dehiscences has been reported (Zanetta-Barbosa et al. 1993). Obviously, the influence of reconstructive devices on early wound healing following SFA need to be explored in specifically designed controlled clinical trials.

No association between EHI and age, gender or smoking status was observed. In contrast with our finding, a delayed and impaired healing process following gingival biopsies was observed in older compared with younger patients (Holm-Pedersen & Löe 1971). The effect of age on the early healing of gingival incision wounds and its clinical relevance on the reconstructive outcomes need to be further investigated since there is wide consensus that wound healing is negatively affected by the ageing process (Ashcroft et al. 2002, Gosain & Di Pietro 2004).

Higher risk for sub-optimal wound closure was associated with a narrower base of the interdental papilla and the presence of either interdental contact point or interdental soft tissue crater. At the buccal aspect, blood vessels in the gingival tissues are oriented mainly in an apico-coronal direction (Mörmann et al. 1979). A horizontal incision performed in the gingiva results in a transient reduction of blood perfusion to the gingival tissues coronal to the incision margin (Mörmann et al. 1979, Retzepi et al. 2007). When considering the characteristics of the vascular system of the interdental tissues, which consists of a mixed pattern of anastomosing capillaries and loops (Kohl & Zander 1961), it is therefore reasonable to admit that the dimensions as well as the morphological characteristics of the soft tissues occupying the interdental space may affect the re-establishment of the normal blood perfusion after gingival incision.

Our interest in the evaluation of factors associated with early postoperative healing of gingival wounds is based on the importance of early wound stability for periodontal healing following flap elevation (Linghorne & O'Connell 1950, Wikesjö & Nilvéus 1990, Haney et al. 1993, Wikesjö et al. 1991, Wirthlin et al. 1980, Polson & Proye 1983). Our exploratory analysis, however, showed no significant differences in terms of 6-month CAL gain and PPD reduction at sites with EHI = 1 versus sites with EHI either >1 or >3. Although the additional use of specific reconstructive technologies may influence the early postoperative healing, it is reasonable to hypothesize that such technologies may exert their beneficial effect on the regenerative process (Trombelli et al. 2002, Esposito et al. 2009, Needleman et al. 2006, Trombelli & Farina 2008, Tu et al. 2012) which become clinically manifest at longer term. In this respect, the potential negative effect on early wound healing can be compensated by a clinical and histological beneficial effect at longer observation intervals as stressed in our previous study (Trombelli et al. 2010). On the other hand, the fact that different outcomes in terms of early wound healing have been observed following different reconstructive strategies also indicate that this flap approach may be more indicated when some, and not other, reconstructive technologies are used. It should also be considered that sites with wound dehiscence were resolved within 1 month post-surgery in absence of manifest membrane exposure or graft exfoliation. Therefore, the observed dehiscences may have exerted a limited detrimental effect on 6-month outcomes. On the other hand, it may well be that EHI could not be enough sensible to detect substantial differences in early postoperative healing that would significantly affect the 6-month results. Previous studies where a significant effect of early wound healing on the regenerative outcome had been observed, the definition of "wound failure" by far exceeded the severity of the clinical conditions included in the EHI score (Haney et al. 1993, Selvig et al. 1992, Sigurdsson et al. 1994, Trombelli et al. 197). Further studies on large cohorts are therefore needed to validate the EHI as a methodology tool to assess the impact of early wound healing on clinical endpoints of periodontal regenerative surgery. Also, a potential confounding effect due to patient-related and site-specific factors, other than EHI, that have been shown to affect the reconstructive endpoints (Tonetti et al. 1996, Tal 1984) can not be excluded.

In the present study, only intraosseous defects accessed with a buccal SFA were considered for analysis. SFA with a buccal approach is indicated in intraosseous defects involving the interproximal aspect and exhibiting limited to no extension on the lingual/palatal side and when buccal access allows an appropriate root and defect debridement and the application

of the proper reconstructive technology. A previous study demonstrated that, in interproximal intraosseous defects, the oral bone crest is often higher than the buccal bone crest (Tal 1984). Therefore, the data reported in the present study can be considered representative of a common clinical condition suitable for the application of the SFA. Obviously, an SFA based on a buccal flap provides better surgical access for soft tissue management, root/defect debridement and grafting, membrane positioning, and suturing procedures compared to an SFA with an oral approach. The present study design did not allow the exclusion of different outcomes that could be obtained with SFA if an oral approach had been used.

Within the limitations of the study, the present results indicate that buccal SFA may result in highly predictable (> 80%) complete flap closure and a substantial (about 50%) prevalence of sites with optimal healing at 2 weeks following surgery. Our findings also indicate that local, site-specific characteristics may influence the early postoperative healing of the papillary incision.

Acknowledgements: This study was supported by the Research Centre for the Study of Periodontal and Peri-Implant Diseases, University of Ferrara, Italy, and by a grant from Regione Emilia-Romagna (Programma Ricerca Regione - Università 2007-09).

Conflict of interest: The Authors declare that they have no conflict of interest.

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TABLES

Table 1. Characteristics of the defect sites as assessed immediately before and during surgery.

	Incisors (n)	**Canines** (n)	**Premolars** (n)	**Molars** (n)
Tooth type	14	18	7	4
	Positive / present (n)		**Negative / absent (n)**	
Interdental contact point	26		17	
Interdental soft tissue crater	12		31	
	Mean	**SD**	**Min**	**Max**
Width of the interdental papilla (pW)	5.0	1.6	2.0	11.0
Height of the interdental papilla (pH)	3.5	1.4	0.5	7.5
Interdental keratinized tissue (iKT)	9.3	2.3	4.0	14.0
	combined 1/2 wall (n)	**2-wall (n)**	**combined 2/3 wall (n)**	**3-wall (n)**
Defect configuration	4	3	26	10

Table 2. Treatment approaches.

Treatment approach associated with buccal SFA	n° of defects (n= 43)	n° of patients (n= 35 [a])
spontaneous healing	25	18
hydroxyapatite-based graft [b] + resorbable collagen membrane [c]	7	7
hydroxyapatite-based graft [b]	6	6
Enamel Matrix Derivative (EMD) [d]	3	3
hydroxyapatite-based graft [b] + EMD [d]	2	2

[a] one patient received a hydroxyapatite-based graft in one site, and a combination of a hydroxyapatite-based graft [†] and EMD in another site
[b] Bio-Oss® spongiosa granules 0.25-1.0 mm; Geistlich Pharma, AG, Wolhusen, Switzerland, or Biostite®, GABA Vebas, S. Giuliano Milanese, Milan, Italy
[c] Paroguide®, GABA Vebas, S. Giuliano Milanese, Milan, Italy
[d] Emdogain® gel, Straumann, Basel, Switzerland

Table 3. Characterization of patients with EHI =1, >1, >2, and > 3.

	EHI					
	= 1	**> 1**	*p value* (=1 vs >1)	**> 2**	**>3**	*p value* (=1 vs >3)
Distribution according to EHI (1 / 2 / 3 / 4 / 5)	17/0/0/0/0	0/8/3/7/0	-	0/0/3/7/0	0/0/0/7/0	-
n	17	18	-	10	7	-
Patient-related factors						
age (years)	53.1 (± 7.9)	49.8 (± 9.0)	0.260	47.1 (± 9.1)	47.3 (± 7.6)	0.117
gender (males/females)	11 / 6	13 / 5	0.725	9 / 1	7 / 0	0.130
smoking status (current smokers / former smokers / never smoked)	2 / 2 / 13	5 / 2 / 11	0.522	3 / 2 / 5	2 / 1 / 4	0.642
Site-specific characteristics						
tooth type (incisors + canines / premolars / molars)	15 / 2 / 0	10 / 4 / 4	0.055	4 / 3 / 3	2 / 3 / 2	0.005
interdental contact point (present / absent)	5 / 12	15 / 3	0.002	8 / 2	6 / 1	0.023
width of the interdental papilla (pW) (mm)	5.8 (± 1.7)	4.4 (± 1.5)	0.011	4.3 (± 1.5)	3.8 (± 1.1)	0.004
height of the interdental papilla (pH) (mm)	3.9 (± 1.2)	3.1 (± 1.7)	0.140	3.5 (± 2.0)	3.1 (± 1.8)	0.304
interdental keratinized tissue (iKT) (mm)	9.7 (± 2.6)	8.6 (± 1.9)	0.171	8.6 (± 2.3)	7.9 (± 2.3)	0.116
supracrestal component of the pocket (PPD - IBD) (mm)	2.2 (± 2.3)	3.1 (± 2.4)	0.312	2.9 (± 2.9)	2.9 (± 3.4)	0.672
interdental soft tissue crater (present / absent)	2 / 15	9 / 9	0.027	5 / 5	4 / 3	0.038
depth of the intrabony component (IBD) (mm)	6.5 (± 2.8)	5.9 (± 2.5)	0.516	5.6 (± 1.6)	5.3 (± 1.8)	0.210
defect configuration (mainly 2-3 wall / mainly 1-2 wall)	15 / 2	13 / 5	0.402	8 / 2	6 / 1	1
Technical aspects						
distance between the tip of the papilla and the incision margin (T-I) (mm)	3.2 (± 1.2)	2.9 (± 1.9)	0.560	3.3 (± 2.1)	3.3 (± 2.4)	0.913
additional reconstructive technology (none / graft + membrane / other)	11 / 2 / 4	7 / 5 / 6	0.330	3 / 4 / 3	2 / 3 / 2	0.174

Table 4. Odds ratios as calculated for site-specific characteristics impairing optimal wound healing (EHI= 1 vs EHI> 1).

Factor	**OR**	**95% CI**	*p value* (2-tailed Fisher's exact test)
Presence of the interdental contact point	12.0	1.9 - 88.7	0.002
Presence of an interdental crater	7.5	1.1 - 65.1	0.027
Papillary width (pW) < 5 mm	11.4	1.9 - 80.2	0.002

Table 5. Clinical parameters as assessed immediately before and 6 months after surgery.

	PRE-SURGERY	**6 MONTHS**	*p value*	**CHANGE** [a]
	Mean (± SD)	Mean (± SD)		Mean (± SD)
Clinical attachment level (CAL)	10.4 (± 2.6)	7.0 (± 2.4)	< 0.001	3.4 (± 2.0)
Pocket probing depth (PPD)	8.9 (± 2.0)	4.2 (± 1.3)	< 0.001	4.7 (± 2.0)
Gingival recession (REC)	1.5 (± 1.4)	2.8 (± 2.1)	0.004	1.3 (± 1.4)
Gingival recession at the buccal aspect (bREC)	1.2 (± 1.5)	2.2 (± 1.8)	0.016	1.0 (± 1.3)

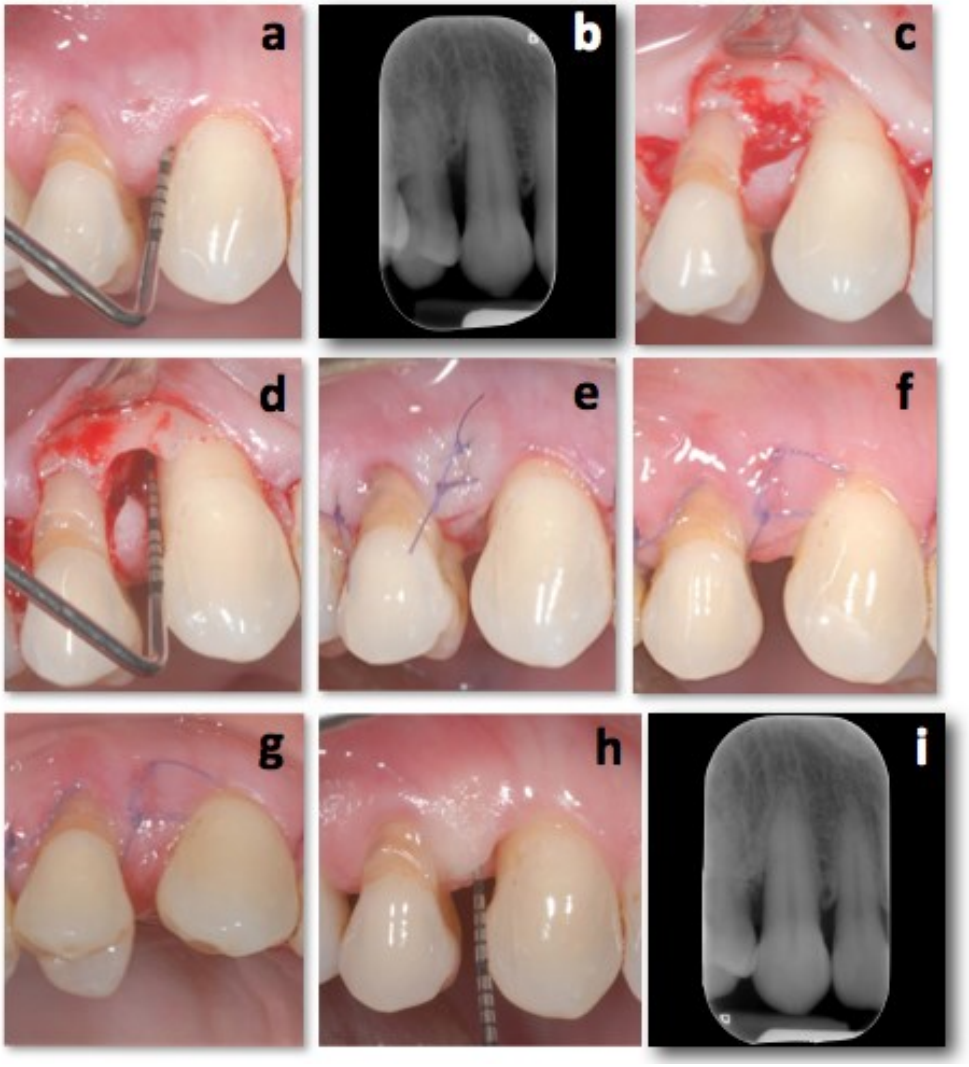
[a] positive change values indicate CAL gain, PPD reduction, REC and bREC increases.

Table 6. 6-month change in clinical parameters in patients with different early wound healing (as assessed by the EHI).

	EHI					
	= 1	**> 1**	*p value* (=1 vs >1)	**> 2**	**>3**	*p value* (=1 vs >3)
Distribution according to EHI (1 / 2 / 3 / 4 / 5)	17/0/0/0/0	0/8/3/7/0	-	0/0/3/7/0	0/0/0/7/0	-
n	17	18	-	10	7	-
Clinical parameters						
Clinical attachment level (CAL) change (mm)	3.3 (± 2.4)	3.6 (± 1.7)	0.742	3.2 (± 1.9)	3.0 (± 1.5)	0.696
Probing pocket depth (PPD) change (mm)	4.6 (± 2.4)	4.8 (± 1.7)	0.790	4.4 (± 1.9)	4.1 (± 2.0)	0.642
Gingival recession (REC) change (mm)	1.3 (± 1.7)	1.2 (± 1.2)	0.885	1.2 (± 1.1)	1.1 (± 1.2)	0.809
Gingival recession at the buccal aspect (bREC) change (mm)	0.6 (± 1.5)	1.3 (± 0.9)	0.149	1.6 (± 0.9)	1.6 (± 0.9)	0.086

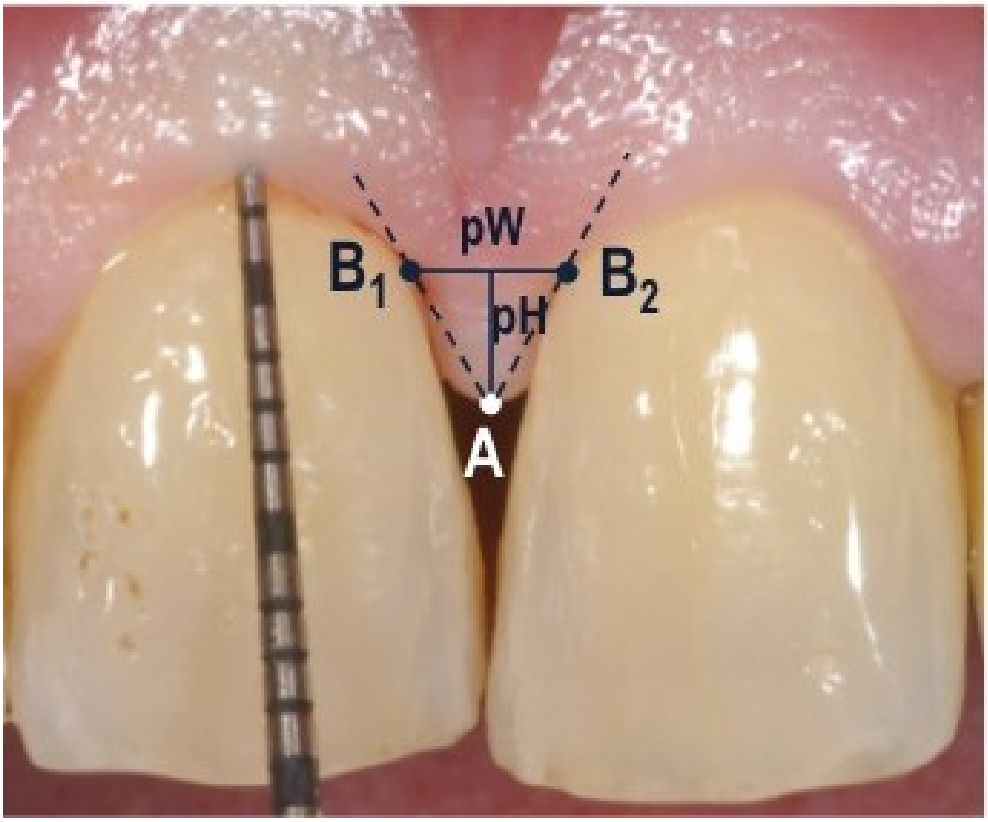
FIGURES

Figure 1. Surgical access according to the Single Flap Approach (Trombelli et al. 2007, 2008, 2009, 2010, 2012).



a)Preoperative clinical attachment loss at the disto-buccal aspect of a lateral right maxillary canine. **b**) Radiographic aspect at pre-surgery. **c**) Sulcular incisions are made following the gingival margin of the teeth included in the surgical area. An oblique or horizontal, butt-joint incision is made at the level of the interdental papilla. **d**) The elevation of a buccal mucoperiosteal flap allows for proper root/defect debridement. Note the untouched interdental papilla. **e**) Wound closure is obtained with a horizontal internal mattress suture at the base of the papilla, first, and another internal mattress suture (or interrupted suture) at the most coronal portion of the papilla, secondly. **f**) Soft tissue healing at suture removal performed at 2 weeks following surgery**. g**) Complete wound closure with absence of fibrin line at the incision margins as observed at 2 weeks after surgery. **h**) Clinical aspect at 6 months following surgery. i) Radiographic aspect at 6 months following surgery.

Figure 2. Assessment of papillary width (pW) and height (pH).



On digital photographs showing the buccal aspect of the tooth presenting the intraosseous defect and taken as much perpendicular as possible to the long axis of the tooth, lines were then traced passing through the tip of the papilla (A) and tangent to the profile of the crowns of the teeth adjacent to the intraosseous defect to identify reference points (B_1 and B_2). pW was measured in mm as the distance between B_1 and B_2. pH was measured as the distance between A and the midpoint of the line connecting B_1 and B_2. To account for photographic magnification, pW and pH were referred to the increments of the UNC 15 probe as depicted in the same photograph.

Figure 3. Pre-surgery view of cases showing EHI 1, 2, 3 and 4 at 2 weeks.

CHAPTER 3

Single-Flap Approach in combination with enamel matrix derivative in the treatment of periodontal intraosseous defects

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The International Journal of Periodontics & Restorative Dentistry
(2014) **34**, 497-506

Abstract

Twenty-four periodontal intraosseous defects were accessed with a buccal Single Flap Approach (SFA) and treated with enamel matrix derivative (EMD) or EMD+ deproteinized bovine bone mineral (DBBM) according to the operator' discretion. Both EMD with or without DBBM were clinically effective in the treatment of periodontal intraosseous defects accessed with a buccal SFA. The adjunctive use of DBBM in predominantly 1-wall defects seemed to compensate, at least in part, the unfavorable osseous characteristics on the outcomes of the procedure.

INTRODUCTION

Enamel matrix derivative (EMD) is a biologically active agent capable to promote periodontal regeneration when applied onto the periodontally-compromised root surface after surgical debridement. Systematic reviews demonstrated a significant adjunctive effect of EMD when compared with access flap in the treatment of intraosseous defects (Trombelli et al. 2002, Giannobile et al. 2003, Esposito et al. 2009, Koop et al. 2012). Because of its gel-like consistency, however, EMD has a limited space-making effect, which, in turn, may potentially affect its regenerative capacity (Mellonig 1997). Hence, a combined approach based on EMD 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). Data from recent reviews indicate that the additional use of a graft may improve the clinical performance of EMD in the treatment of intraosseous defects (Trombelli & Farina 2008)

The *Single Flap Approach* (SFA) (Trombelli et al. 2007, 2009) is a simplified procedure designed for the treatment of periodontal osseous defects. The basic principle behind the SFA is the unilateral elevation of a limited mucoperiosteal flap to allow surgical access depending on the main, buccal or lingual, extension of the osseous defect leaving adjoining gingival tissues intact. To date, several studies reported considerable clinical improvements when the SFA was used as a stand-alone protocol (Trombelli et al. 2010, 2012) or in combination with regenerative technologies (Trombelli et al. 2007, 2009, 2010, Farina et al. 2013, Rizzi et al. 2013, Simonelli et al. 2013) for the treatment of deep periodontal intraosseous defects.

To date, the clinical improvements following a SFA/EMD combined approach have not been investigated in specifically designed studies. In addition, available studies do not provide evidence on patient and defect characteristics where the application of a combined EMD-graft approach rather than EMD alone should be indicated to optimize the treatment outcome of SFA. Therefore, the present study was performed to evaluate the clinical effectiveness of SFA with EMD either alone or in association with a xenograft in the treatment of periodontal intraosseous defects. A secondary aim was to evaluate which patient and defect/site characteristics influenced the clinician's decision on whether or not use the combined approach.

MATERIALS & METHODS

Experimental design

The study was designed as a pragmatic trial. Patients were screened and consecutively enrolled at Research Centre for the Study of Periodontal and Peri-Implant Diseases, University of Ferrara, Italy, and the Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy. All the clinical procedures were performed in accordance with the Declaration of Helsinki and the Good Clinical Practice Guidelines (GCPs). Each patient provided a written informed consent before participation.

Three experienced periodontal surgeons (L.T., L.M., G.R.) performed the clinical procedures, including surgical treatments and clinical recordings. In order to evaluate which patient/defect characteristics may influence the selection of the regenerative approach (i.e. EMD vs EMD+DBBM), the choice of using the additional xenograft to EMD treatment was left to the operator's judgement. Factors such as smoking habit as well as characteristics of the defect (depth of the intrabony component, residual bone walls, defect angle and width) that had been previously shown to affect the regenerative outcome were thus recorded for analysis. Clinical operators were kept blinded regarding the secondary aim of the study.

Patient and defect eligibility

Patients were included in the study if positive for each of the following inclusion criteria: (1) diagnosis of chronic or aggressive periodontitis; (2) no pregnancy or lactation; (3) no systemic diseases that contraindicated periodontal surgery; (4) no use of medications affecting periodontal status; (5) no assumption of anticoagulants, non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, or biological agents for the treatment of rheumatoid arthritis (e.g., TNF-α blockers, IL-1 blockers, IL-6 blockers); (6) presence of ≥ 1 interproximal intraosseous periodontal defect with probing pocket depth ≥ 5 mm and radiographic depth ≥ 3 mm; (7) limited to no extension of the defect on the lingual or palatal side as assessed by preoperative bone sounding; (8) full-mouth plaque score and full-mouth bleeding score < 20% at the time of the surgical procedure.

Third molars, teeth with degree III mobility, furcation involvement or inadequate endodontic treatment and/or restoration were excluded from the study.

Experimental protocol

Pre-surgery procedures

Each patient underwent a full-mouth session of scaling and root planing using mechanical and hand instrumentation and received personalized oral hygiene instructions. Temporary splinting and/or occlusal adjustment were performed for teeth with degree I or II mobility at re-evaluation following non-surgical instrumentation. The surgical phase was delayed until the achievement of minimal residual inflammation at the defect site.

Surgical procedures

All surgeries were performed using 2.5 magnifying loops. The site of surgery was anesthetized using mepivacaine-epinephrine 1:100,000. Transcrevicular probing (bone sounding) was always performed pre-surgery to determine the characteristics of the bony defect, including the defect morphology and extension, the probing bone level and the horizontal component of bone loss. The surgical access was performed by the elevation of a buccal mucoperiosteal flap according to previously detailed principles of the SFA (Trombelli et al. 2007, 2009, 2010, 2012, Farina et al. 2013, Rizzi et al. 2013, Simonelli et al. 2013). Briefly, a buccal envelope flap without vertical releasing incisions was performed, leaving the oral portion of the interdental supracrestal soft tissues undetached (Figures 1a-d, 2a-d). Root and defect debridement were performed using hand and ultrasonic instruments. The exposed root surface was conditioned with 24% EDTA gel for 2 minutes, and the defect was thoroughly rinsed with saline to remove gel remnants. Defects were then treated with EMD (Emdogain® gel; Straumann, Basel, Switzerland) alone (EMD group) or in association with DBBM (Bio-Oss® spongiosa granules 0.25-1.0 mm; Geistlich Pharma, AG, Wolhusen, Switzerland) (EMD+DBBM group). In EMD group, the EDTA-treated root surface and surrounding bony walls were conditioned with the amelogenin gel according to the manufacturer's instruction (Figures 1e-f). In EMD+DBBM group, a "sandwich" technique was adopted to treat the defect, i.e. apical layer of EMD, DBBM, coronal layer of EMD (Figures 2e-h) (Trombelli et al. 2006, Guida et al. 2007). The suturing technique was performed with Vycril® (Ethicon, Sommerville, NY) 5/0 or 6/0 according to the original description of the SFA (Trombelli et al. 2007, 2009, 2010, 2012, Farina et al. 2013, Rizzi et al. 2013, Simonelli et al. 2013), i.e. a horizontal internal mattress suture at the base of the papilla and a second internal mattress suture (vertical or horizontal) between the most coronal portion of the flap and the most coronal portion of the oral papilla. When needed (i.e. in case of a large, thick interdental

papilla), an interrupted suture was performed to ensure primary intention healing at the incision line. Primary flap closure was always obtained at suturing (Figures 1g, 2i).

Post-surgery procedures

Sutures were removed at 2 weeks post-surgery. The patients were asked to abstain from mechanical oral hygiene procedures in the surgical area for 4 weeks. A 0.12% chlorhexidine mouthrinse (10 mL BID/6 wks) was used to support local plaque control. Each patient was enrolled in a monthly recall program for 3 months and was reviewed according to personal needs thereafter. Each session included reinforcement of oral hygiene procedures and supragingival plaque removal. Subgingival scaling was performed following completion of the study at 6 month post-surgery (Figures 1h-i, 2j-k).

Recordings

Clinical recordings

Pre-surgery assessments

The following measurements were performed immediately before surgery using a manual pressure sensitive probe (UNC 15, Hu-Friedy, Chicago, IL, USA) with 1-mm increments at the site showing the greatest loss of clinical attachment:

- pocket probing depth (PPD), measured from the gingival margin to the bottom of the pocket;
- local bleeding score (BS): recorded as positive when bleeding on probing was present at the surgical site;
- clinical attachment level (CAL), measured from the cemento-enamel junction (CEJ) to the bottom of the pocket;
- gingival recession (REC), measured from the CEJ to the gingival margin.

On digital photographs showing the buccal aspect of the tooth presenting the intraosseous defect and taken as perpendicularly to the long axis of the tooth as possible, the width of the interdental papilla (pW) was measured in mm according to the method described by Farina (Fatina et al. 2013).

Intra-surgery assessments

At completion of the intra-surgical debridement, the following parameters were assessed (in mm) at the deepest interproximal point of the defect with a UNC 15 periodontal probe:

- distance between the CEJ and the bottom of the defect (CEJ-BD);
- distance between the CEJ and the most coronal extension of the interproximal bone crest (CEJ-BC);
- depth of the 1-, 2- and 3-wall intrabony component of the defect (1-WALL, 2-WALL, and 3-WALL, respectively).

Post-surgery assessments

At suture removal, performed at 2 weeks post-surgery, biomaterial exfoliation and other complications were assessed and recorded.

At 6 months after surgery, PPD, BS, CAL, and REC were re-assessed.

Radiographic measurements

On non-standardized periapical radiographs as taken before surgery, the following measurements were performed by a trained and calibrated examiner (A.S.) not involved in clinical procedures and blinded as to the clinical operator and type of treatment:

- defect angle: the defect angle was defined by two lines (Steffensen & Webert 1989). The first line was drawn connecting the most apical point of the defect and the CEJ on the side of the tooth presenting the intraosseous defect. The second line followed the defect surface from the most apical point of the defect and the point where the bone crest touched the neighboring tooth. In general, this second line corresponded well to the defect surface. However, in a few instances there were major discrepancies between the angulation of the upper and lower parts of this surface. Preference was then given to the lower part of the defect when constructing the defect angle;
- defect width: distance between the point where the bone crest touched the neighboring tooth and the root surface of the tooth presenting the defect.

Statistical analysis

Data were entered in a unique database file (STATISTICA® software version 7.1; StatSoft, Italia s.r.l., Vigonza, Italy). The patient was the statistical unit. CAL was regarded as the primary outcome variable, while PPD and REC were considered as the secondary outcome variables.

The depth of the intrabony component (INTRA) was calculated as (CEJ-BD - CEJ-BC). %1-WALL, %2-WALL, and %3-WALL were calculated as 1-WALL, 2-WALL, or 3-WALL, respectively, / INTRA * 100. 6-month changes in CAL, PPD, and REC were also calculated.

Data were expressed as mean ± standard deviation (SD). Within-group comparisons (pre-surgery vs. 6 months) were performed with Wilcoxon test. Inter-group comparisons were performed to test the superiority of EMD+DBBM compared to EMD, and Fisher's exact test, χ^2 test, and Mann-Whitney U test were used. To evaluate the interaction between defect morphology (as assessed in terms of %1-WALL, %2-WALL, and %3-WALL) and surgical treatment (EMD or EMD+DBBM), a 2-way permutation ANOVA (PERANOVA) (Anderson 2001) was performed.

The level of statistical significance was fixed at 0.05. A web-based software (http://www.dssresearch.com/KnowledgeCenter/toolkitcalculators/statisticalpowercalculators.aspx) was used for the calculation of the statistical power of the study. According to a sample size calculation performed with a a two-sided parametric test, a per-protocol study population of 24 patients (i.e., 12 patients per treatment group) was needed to detect a significant inter-group difference (at $p = 0.05$) with a statistical power of 71%, assuming a standard deviation in CAL of 1.0 mm and an expected inter-group difference in CAL gain of 0.9 mm. Data used for sample size calculation were based on the results of a recent meta-analysis reporting the weighted mean difference between EMD and EMD+DBBM outcomes (Koop et al. 2012).

RESULTS

Overall population

Twenty-four patients (mean age: 49.1 ± 9.6 years; 16 males; 16 non-smokers, 8 current smokers; mean daily cigarette consumption at the time of surgery: 11.3 ± 6.5 cigarettes/day) (Table 1) were included in the study, fully complied with the study protocol and completed the experimental phase. Patients contributed 24 intraosseous defects. CEJ-BD, CEJ-BC and INTRA amounted to 11.6 ± 2.3 mm, 5.8 ± 1.5 mm, 5.8 ± 1.9 mm. In all cases, buccal SFA ensured an adequate surgical access for root and defect instrumentation. Twelve defects were treated with EMD, whereas 12 defects were treated with EMD+DBBM.

At 6 months post-surgery, 100% tooth survival was observed. CAL varied from 9.9 ± 1.9 mm at baseline to 6.2 ± 1.9 mm at 6 months ($p< 0.001$; CAL gain: 3.6 ± 1.5 mm). PPD varied from 8.7 ± 1.5 mm at baseline to 3.7 ± 1.0 mm at 6 months ($p< 0.001$; PPD reduction: 5.0 ± 1.5 mm). These results were paralleled by a significant increase in REC from 1.2 ± 1.6 mm at baseline to 2.5 ± 2.0 mm at 6 months ($p< 0.001$; REC increase: 1.4 ± 1.2 mm). Also, a significant reduction of BS-positive sites from 15 to 7 was observed ($p< 0.001$).

EMD and EMD+DBBM groups

Patient, tooth and site characteristics in EMD and EMD+DBBM groups are shown in Tables 1 and 2. EMD group showed a higher prevalence of incisors and canines, while EMD+DBBM group showed a higher prevalence of premolars and molars ($p= 0.039$) (Table 2).

CEJ-BD, CEJ-BC and INTRA were not significantly different between groups (Table 2). The results of the PERANOVA showed that the interaction between the morphology of the intraosseous defect (in terms of %1-WALL, %2-WALL, and %3-WALL) and the surgical treatment was statistically significant ($p= 0.021$), with %1-WALL and %3-WALL being more prevalent in EMD+DBBM and EMD groups, respectively (Figure 3). Radiographic defect angle and width were higher in EMD+DBBM compared to EMD group, however, the difference did not reach the statistical significance (Table 2).

The clinical recordings in EMD and EMD+DBBM groups as assessed at baseline and 6 months are shown in (Table 3). A significantly greater REC was observed in the

EMD+DBBM group compared to the EMD group at baseline. At 6 months, both EMD and EMD+DBBM groups showed a statistically significant CAL gain and PPD reduction. A significant 6-month increase in REC was also observed in both groups. No significant difference in CAL gain, PPD reduction, and REC increase was observed between groups.

DISCUSSION

The present study was structured as a pragmatic trial. Unlike explanatory trials (eg, randomized controlled trials), pragmatic trials are primarily designed to evaluate the effectiveness of an intervention under real-life conditions (Schwartz et al. 1967, O'Mullane et al. 2012). When considering the aims of this study, the pragmatic trial appeared to be the most adequate methodologic option to maximize the external validity of the results and their applicability to the "usual" care setting, as well as to guide clinicians in the choice between different treatment options when approaching an intraosseous defect with an SFA/EMD strategy.

EMD with or without DBBM resulted in substantial CAL gain and PD reduction in deep periodontal intraosseous defects accessed with a buccal SFA, thus indicating that this simplified procedure to surgically access deep periodontal defects is efficacious in improving the clinical condition of the compromised tooth when used in conjunction with EMD. The magnitude of the clinical outcomes observed in our study are consistent with the treatment effects reported by previous meta-analyses on the use of EMD alone or in association with DBBM (Venezia et. al 2004, Tu et al. 2010).

In the present material, similar clinical outcomes were observed for EMD and EMD + DBBM treatments in defects accessed with the SFA. When evaluating pertinent literature on the use of EMD with or without DBBM, it seems evident that the adjunctive benefit of DBBM in EMD-based regenerative procedures is controversial. A meta-analysis reported lower CAL gain and PD reduction for intraosseous defects treated with EMD + DBBM compared with defects treated with EMD alone (Venezia et al. 2004). In contrast, a more recent meta-analysis showed an adjunctive CAL gain of 0.90 mm when DBBM was combined with EMD (Koop et al. 2012). A recent randomized controlled trial where a flap design similar to SFA was used to access deep intraosseous defects failed to find a difference in treatment effect between EMD and EMD + DBBM (Cortellini et al. 2011).

The composition of defects treated with EMD and EMD+DBBM was markedly different, with EMD-treated defects showing a dominant 3-wall component, while EMD+DBBM defects being predominantly 1-wall. In addition, defects in EMD+DBBM group showed a tendency to have a wider radiographic angle and mesio-distal space, although inter-group differences were only of borderline significance. The relevance of defect morphology in EMD-based regenerative procedures is still not clear. While some studies reported a correlation between the number of defect walls and the clinical outcomes of the procedure (Hijl 1997, Tonetti et al. 2002, Silvestri et al. 2003), other studies failed to demonstrate such effect (Heden 2002, Bratthall et al. 2001, Minabe et al. 2002). Defect angle was shown to be a prognostic factor for treatment outcomes of regenerative procedures based on EMD application, with narrow intraosseous defects being more prone to show substantial CAL gain at 1 year (Tsitoura et al. 2004). When interpreting our results in the light of these studies, it appears that deep intraosseous defects with an unfavorable morphology (i.e., mainly non self-contained due to a dominant 1-wall component, ample defect angle and width) treated with EMD+DBBM may respond similarly to defects with a more favorable morphology (i.e., mainly self-contained due to a dominant 3-wall component, narrow defect angle and width) treated with EMD only. Interestingly, when mainly 3-wall defects with a narrow defect angle were accessed with a flap design similar to SFA and treated with EMD with or without EMD+DBBM, no adjunctive benefit of DBBM over EMD was observed (Cortellini et al. 2011). Differently, when predominantly 1- or 2-wall defects were accessed with double flap papilla preservation techniques and treated with EMD or an association of EMD and an HA/β-TCP-based graft material, the combined regenerative approach showed a better clinical performance compared to EMD alone (De Leonardis et al. 2013).These observations suggest that the adjunctive use of a graft in a regenerative strategy based on SFA and including the application of EMD may be indicated in defects with an unfavorable morphology, i.e. defect configuration/features that may affect the endogenous regenerative potential.

In conclusion, the results of the present study indicate that both EMD and EMD+DBBM were clinically effective in the treatment of periodontal intraosseous defects accessed with a buccal SFA. The adjunctive use of DBBM in predominantly 1-wall defects located at posterior teeth seems to compensate, at least in part, the unfavorable osseous characteristics on the outcomes of the procedure.

Source of Founding: This study was entirely supported by the Research Centre for the Study of Periodontal and Peri-Implant Diseases, University of Ferrara.

Acknowledgements: The Authors wish to thank Prof. Chiara Scapoli and Dr. Alberto Carrieri, Department of Life Sciences and Biotechnologies, University of Ferrara, for their precious help in data analysis, and Dr. Stefano Faroni, Department of Biomedical, Surgical and Dental Sciences, University of Milan, for his help in data collection.

Conflict of interest: The Authors declare that they have no conflict of interest.

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TABLES

Table 1. EMD and EMD+DBBM groups: patient characteristics.

	EMD	**EMD + DBBM**	***p***
Patient characteristics			
age (years)	49.8 (± 9.0)	48.5 (± 10.5)	0.755
gender (males / females)	7 / 5	9 / 3	0.667
smoking status (never smoked or former smokers / current smokers)	10 / 2	6 / 6	0.193

Table 2. EMD and EMD+DBBM groups: tooth and site characteristics.

	EMD	**EMD + DBBM**	***p***
Tooth/site characteristics			
type (single-rooted / multi-rooted)	7 / 5	8 / 4	1
position (incisor or canine / premolar or molar)	9 / 3	3 / 9	0.039
interdental contact point (present / absent)	11 / 1	11 / 1	1
interdental soft tissue crater (present / absent)	2 / 10	2 / 10	1
pW (< 5 mm / ≥ 6 mm)	10 / 2	7 / 5	0.371
Defect characteristics			
CEJ-BD (mm)	11.1 (± 2.7)	12.2 (± 1.7)	0.160
CEJ-BC (mm)	5.5 (± 1.7)	6.1 (± 1.3)	0.242
INTRA (mm)	5.5 (± 2.2)	6.1 (± 1.7)	0.551
1-WALL (mm)	0.6 (± 1.4)	2.8 (± 2.9)	0.068
2-WALL (mm)	2.0 (± 3.0)	1.3 (± 1.4)	0.977
3-WALL (mm)	2.9 (± 2.5)	1.9 (± 2.4)	0.198
Radiographic defect angle (degrees)	31.0 (± 13.0)	37.0 (± 7.4)	0.068
Radiographic defect width (mm)	4.3 (± 1.3)	5.6 (± 1.7)	0.060

Table 3. EMD and EMD+DBBM groups: clinical outcomes.

	Clinical attachment level (CAL)				**Probing pocket depth (PPD)**				**Gingival recession (REC)**			
	pre-surg	**6 months**	***p***	**change***	**pre-surg**	**6 months**	***p***	**change***	**pre-surg**	**6 months**	***p***	**change***
EMD (n= 12)	9.3 (± 2.0)	5.4 (± 1.5)	0.002	3.8 (± 1.0)	8.8 (± 1.6)	3.8 (± 0.9)	0.002	4.9 (± 1.8)	0.5 (± 0.9)	1.7 (± 1.4)	0.018	1.2 (± 1.3)
EMD + DBBM (n= 12)	10.5 (± 1.7)	7.0 (± 2.0)	0.002	3.4 (± 1.9)	8.6 (± 1.4)	3.6 (± 1.1)	0.002	5.0 (± 1.2)	1.9 (± 2.0)	3.4 (± 2.1)	0.005	1.5 (± 1.1)
p value	0.143	0.052		0.291	0.755	0.630		0.671	0.033	0.039		0.410

* positive change values indicate CAL gain, PPD reduction, and REC increase.

FIGURES

Figure 1. EMD-based treatment of a periodontal intraosseous defect accessed with a buccal SFA. **a**) Pre-operative clinical attachment loss at the disto-buccal aspect of a maxillary central incisor. **b**) Pre-operative radiographic aspect of the lesion. **c**) An oblique, butt-joint incision is made at the level of the interdental papilla. **d**) The elevation of a buccal mucoperiosteal flap allows for proper root/defect debridement. Note the untouched interdental papilla. **e**) Intra-surgery assessment of the supra-osseous and intra-osseous component of the defect. The defect is predominantly 3-wall. **f**) The EDTA-treated root surface and surrounding bony walls are conditioned with the amelogenin gel. **g)** Wound closure is obtained with a horizontal internal mattress suture at the base of the papilla, first, and another internal mattress suture at the most coronal portion of the papilla, secondly. **h**) Soft tissue healing at 6 months following surgery. **i**) Radiographic aspect of the treated site at 6 months following surgery.

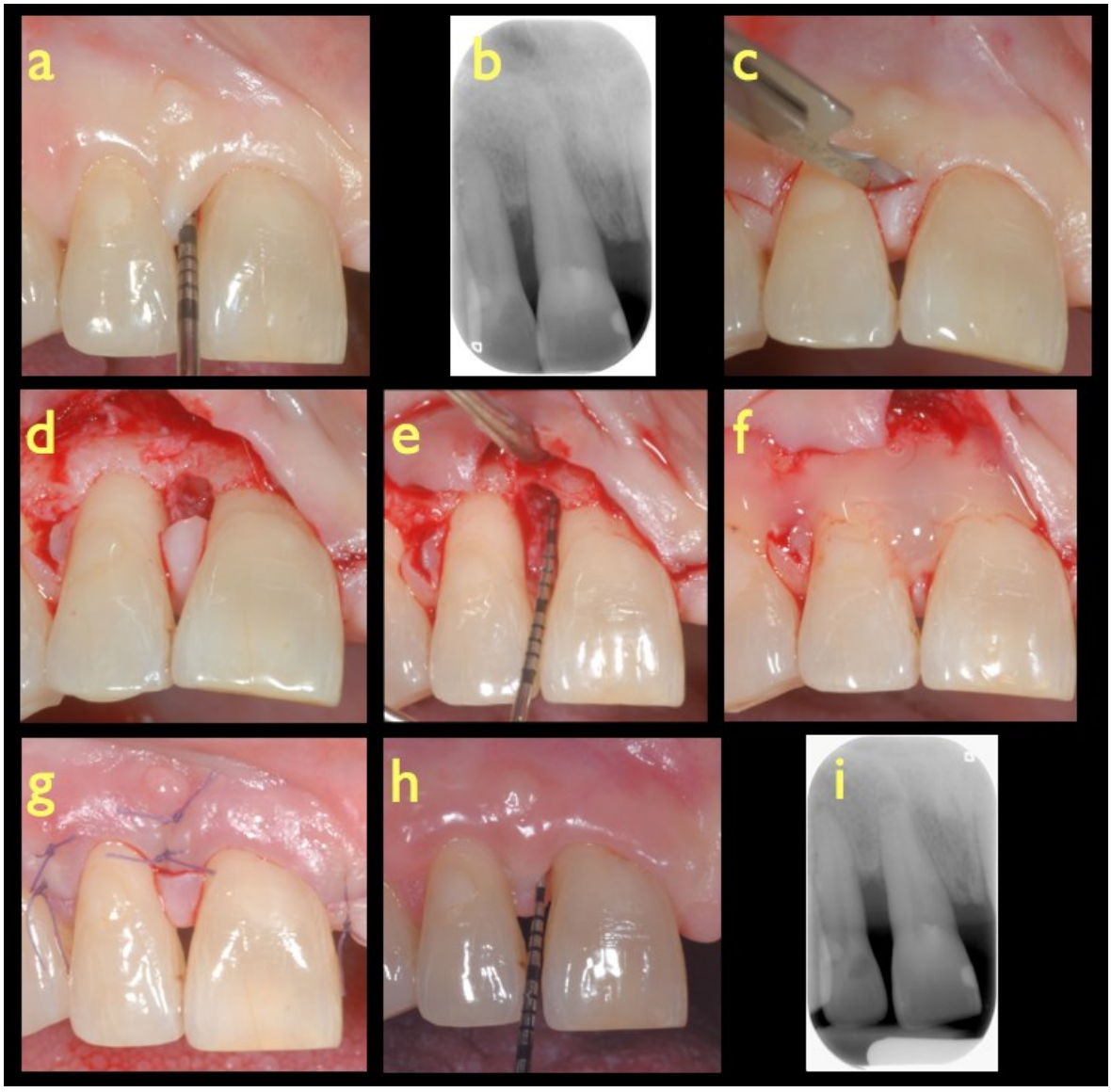


Figure 2. Treatment of a periodontal intraosseous defect accessed with a buccal SFA with a combination of EMD and DBBM. **a**) Pre-operative clinical attachment loss at the mesio-buccal aspect of a mandibular canine. **b**) Pre-operative radiographic aspect of the lesion. **c**) An oblique, butt-joint incision is made at the level of the interdental papilla. **d**) The elevation of a buccal mucoperiosteal flap allows for proper root/defect debridement. Note the untouched interdental papilla. **e**) Intra-surgery assessment of the supra-osseous and intra-osseous component of the defect. The defect is predominantly 1- and 2-wall. **f**) A first layer of EMD is injected to condition the bone defect and the most apical portion of the EDTA-treated root surface. **g**) DBBM is mixed with EMD and subsequently positioned to fill the intrabony component of the defect. **h**) A second layer of EMD is injected to cover the grafted DBBM particles and condition the portion of the root surface coronal to the bone crest. **i**) Wound closure is obtained with a horizontal internal mattress suture at the base of the papilla, first, and another internal mattress suture at the most coronal portion of the papilla, secondly. **j**) Soft tissue healing at 6 months following surgery. **k**) Radiographic aspect of the treated site at 6 months following surgery.

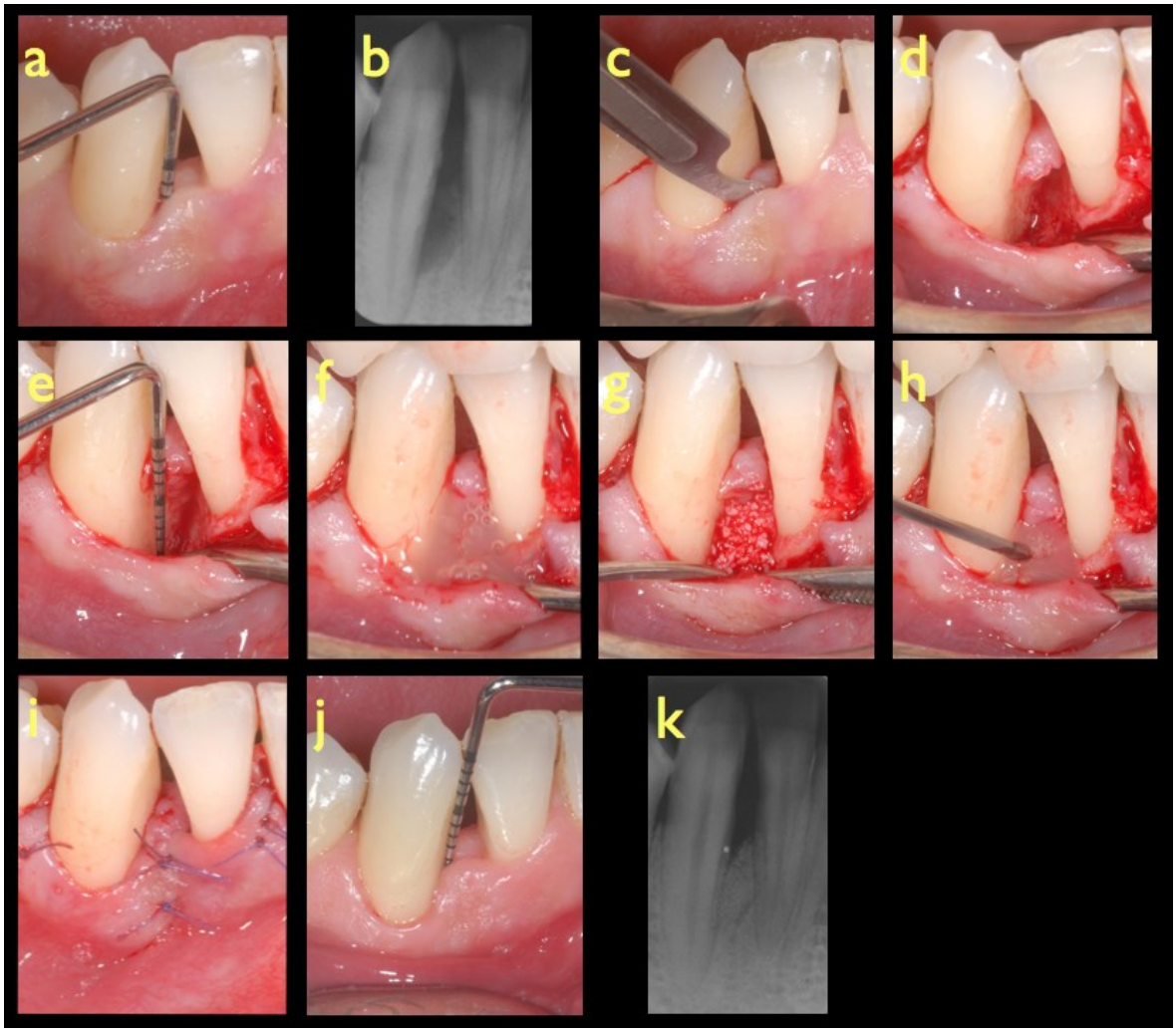
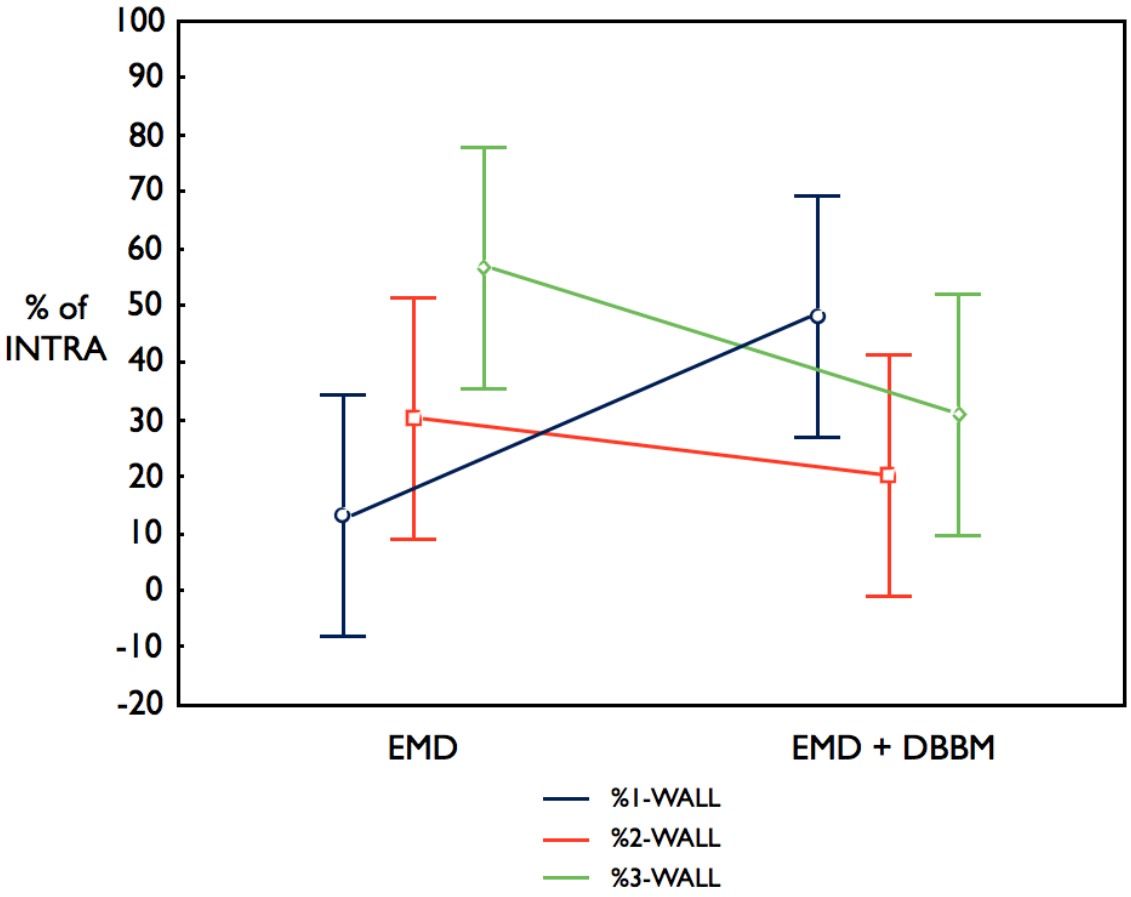


Figure 3. Interaction between the morphology of the intraosseous defect (in terms of %1-WALL, %2-WALL, and %3-WALL) and the surgical treatment (EMD or EMD+DBBM) as stemming from the permutation PERANOVA. Symbols indicate mean values, vertical bars indicate 95% confidence intervals.



CHAPTER 4

Single versus Double Flap Approach in Periodontal Regenerative Treatment

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Journal of Clinical Periodontology (2015) **42 (6)**, 557-66

Abstract

Aim: to compare outcomes of a regenerative strategy based on recombinant human platelet-derived growth factor–BB (rhPDGF-BB, 0.3 mg/ml) and *β*-tricalcium phosphate (*β*-TCP) in the treatment of intraosseous defects accessed with the Single Flap Approach (SFA) versus Double Flap Approach based on papilla preservation techniques (DFA).

Materials and Methods: Fifteen and 13 defects, randomly assigned to access with SFA or DFA, respectively, were grafted with rhPDGF-BB + *β*-TCP. The Early Wound Healing Index (EHI) was evaluated at 2 weeks post-surgery. Probing parameters were assessed before surgery and at 6 months post-surgery. Post-surgical pain (VAS_{pain}) was self-reported using a visual analog scale.

Results: Twelve sites in the SFA group and 6 sites in the DFA group showed complete flap closure at 2 weeks post-surgery. No significant differences in 6-month changes in probing parameters and radiographic defect fill were found between groups. Significantly lower VAS_{pain} was observed in SFA group compared to DFA group at day +1, +2 and +6. A significantly greater number of analgesics were consumed in the DFA group compared to the SFA group at day +1.

Conclusions: When combined with rhPDGF-BB and *β*-TCP, the SFA may result in similar clinical outcomes, better quality of early wound healing, and lower pain and consumption of analgesics during the first postoperative days compared to the DFA.

INTRODUCTION

The Single Flap Approach (SFA) is a simplified, minimally-invasive surgical approach to access intraosseous periodontal defects (Trombelli et al. 2007, 2009, 2010). The basic underlying principle of the SFA consists of the elevation of a limited mucoperiosteal flap to allow access to the defect from either the buccal or oral aspect only, depending on the main buccal/oral extension of the lesion, allowing the interproximal supracrestal gingival tissues to remain intact. The SFA represents a valuable reconstructive procedure *per se*, being at least as clinically effective as the elevation of a flap at both buccal and oral aspects according to the papilla preservation techniques (double flap approach, DFA) (Trombelli et al. 2012). In addition, the SFA or similar flap designs were effective when used in association with various reconstructive technologies, including graft materials, membranes and bioactive agents (Cortellini & Tonetti 2009, Trombelli et al. 2010, Farina et al. 2014).

It is well established that currently available regenerative technologies may enhance the clinical performance of access flap protocols (Trombelli et al. 2002; Needleman et al. 2006; Esposito et al. 2009). In particular, the association of recombinant human platelet derived growth factor (rhPDGF-BB) with graft materials in the treatment of periodontal defects has been evaluated *in vivo* (Camelo et al. 2003, Nevins et al. 2003, 2005, McGuire et al. 2006, Rosen et al. 2011, Thakare & Deo 2012, Nevins et al. 2013; see Trombelli & Farina 2008, Kaigler et al. 2011 for review). When the combination of two different doses of rhPDGF-BB (0.3 and 1.0 mg/ml) with β-tricalcium phosphate (β-TCP) were compared with β-TCP alone in the treatment of deep intra-osseous defects, the rate of gain in clinical attachment was shown to be more rapid in the low-dose rhPDGF-BB + β-TCP group when compared to the control group at 3 months post-surgery. Importantly, both rhPDGF-BB formulations were significantly more effective than the control group (β-TCP + buffer) in the improvement of linear bone growth and percentage of bone defect fill at 6 months (Nevins et al. 2005).

The objective of the present investigation was to compare the clinical, radiographic, and patient-centered outcomes of a regenerative strategy based on the use of rhPDGF-BB + β-TCP in deep intraosseous periodontal defects accessed with SFA *versus* DFA.

MATERIALS & METHODS

Ethical aspects

The study protocol was approved from the Internal Review Board of the University of Connecticut, Farmington, Connecticut (US) (protocol number: #12-098-2; date of approval: 18/1/2012). All the clinical procedures were performed in accordance with the Declaration of Helsinki and the Good Clinical Practice Guidelines (GCPs). Each patient signed an informed consent form before participation.

Study design

The study was designed as a single center, parallel-arm, double-blind, randomized controlled trial. Except for the soft tissue management (SFA or DFA), the clinical procedures for both groups were identical.

The Research Centre for the Study of Periodontal and Peri-implant Diseases, University of Ferrara, Italy, was the Coordinating Center and was responsible for protocol preparation, treatment allocation and radiographic measurements. The Division of Periodontology, School of Dental Medicine, University of Connecticut Health Center, Farmington, Connecticut (US), was the Clinical Center and was responsible for patient recruitment, treatment and collection of pertinent documentation.

Visit schema and procedures for each visit are reported in Appendix 1.

Screening procedures

Patients were recruited among those diagnosed with chronic or aggressive periodontitis in the post-graduate periodontology clinic at University of Connecticut Health Center. An initial evaluation, including medical and dental history, clinical examination, and radiographic examination, was conducted to determine patient eligibility for the study.

Inclusion and exclusion criteria are reported in Appendix 2. Briefly, patients with at least one intraosseous periodontal defect associated with probing depth ≥ 6mm were included in the study.

Pre-surgical procedures

Each patient had full-mouth sessions of scaling and root planing using mechanical and hand instrumentation and received personalized oral hygiene instructions. The surgical phase was delayed until the patient achieved a minimal residual inflammation and optimal soft tissue conditions at the defect site. Patients did not enter the surgical phase of the trial until full-mouth plaque score (O'Leary et al. 1972) and full-mouth bleeding score were lower than 20%.

Allocation and allocation concealment

Each eligible patient was given a subject randomization number. An independent investigator, not involved in clinical procedures, generated the randomization list for treatment allocation using a freeware (http://www.graphpad.com/quickcalcs/randomize1.cfm). This information was concealed in sealed envelopes, which were opened before the surgical treatment. The surgeon was not aware of the group assignment (SFA or DFA) until the day of surgery. The examiners responsible for clinical (E.H., A.S.) and radiographic (A.S.) measurements, as well as the patient, remained blinded with respect to treatment allocation.

Surgical procedures

The same experienced operator (G.P.S.) performed all surgeries using 4.0 magnifying loops. The site of surgery was anesthetized using lidocaine-epinephrine 1:100,000. Transcrevicular probing (bone sounding) was performed pre-surgery to determine the characteristics of the bony defect, such as defect morphology and extension, probing bone level, and horizontal component of bone loss.

In the SFA group, the surgical access was obtained through the elevation of a buccal or oral mucoperiosteal flap for defects with a prevalent extension (as assessed by pre-operative bone sounding) on the buccal or oral side, respectively, as previously detailed (Trombelli et al. 2007, 2009) (Fig. 1). SFA consisted of an envelope flap. The mesio-distal extension of the flap was kept as limited as possible while ensuring proper access for defect debridement and graft positioning and stabilization. Sulcular incisions were performed on the buccal or oral side (for defects with a prevalent extension on the buccal or oral side, respectively) following the gingival margin of the teeth included in the surgical area. In the interproximal area (i.e., at the level of the inter-dental papilla) overlying the intraosseous defect, an oblique or horizontal incision was made following the

profile of the underlying bone crest. The distance between the tip of the papilla and the apico-coronal level of the inter-dental incision was based on the apico-coronal dimension of the supracrestal soft tissues. The greater the distance from the tip of the papilla to the underlying bone crest (as assessed by pre-operative probing), the more apical (i.e., close to the base of the papilla) the incision in the inter-dental area. This was done to provide an adequate amount of untouched supracrestal soft tissue connected to the undetached papilla on the opposite side to ensure flap adaptation and suturing and to warrant proper access to the intraosseous defect for debridement and graft positioning. All defects were approached by elevating a flap only on the buccal or oral side and leaving the opposite portion of the inter-dental supracrestal soft tissues undetached. The fullthickness elevation of the marginal portion of the flap was performed with a microsurgical periosteal elevator.

In the DFA group, the defectassociated inter-dental tissue was approached with surgical techniques for the preservation of the inter-dental papilla, namely the simplified papilla preservation flap (SPPF) (Cortellini et al. 1999) or the modified papilla preservation technique (MPPT) (Cortellini et al. 1995) based on the anatomical characteristics of the surgical site (Cortellini & Tonetti 2005) (Fig. 2). Mesio-distal extension of the buccal and oral incisions was influenced by defect morphology and severity. In other words, the flap could involve the teeth adjacent to the tooth presenting the defect to ensure proper root and defect debridement. Releasing incisions were never performed. Full-thickness flap reflection was performed on both buccal and oral aspects to provide adequate visibility of the bone crest on both buccal and oral aspects. Root and defect debridement were performed using hand and ultrasonic instruments. After surgical debridement, defects were grafted with rhPDGF-BB + b-TCP (GEM 21S®; Osteohealth Company, Shirley, NY, USA). β-TCP was combined with rhPDGF-BB (0.3 mg/ml) and allowed to sit for ~10 min. to permit binding of the rhPDGF-BB protein to the β -TCP before being placed into the defect. Wound closure was obtained according to the original suturing technique of either SFA (Trombelli et al. 2007, 2009) (Fig. 1) or MPPT (Cortellini et al. 1995) and SPPF (Cortellini et al. 1999) (Fig. 2) with a non-resorbable monofilament suture (Monosof™ 6.0; Covidien, Mansfield, MA, USA).

Post-surgery procedures

At the end of each session, patients were prescribed a rescue analgesic (Ibuprofen 600 mg) to be used as needed. Sutures were removed at 2 weeks post-surgery. The patients were asked to abstain from mechanical oral hygiene procedures in the surgical area for 4 weeks.

A 0.12% chlorhexidine mouth rinse (10 mL BID/2 wks) was used to support local plaque control. Each patient was inserted into a monthly recall program for 3 months and was reviewed according to personal needs thereafter. Each session included reinforcement of oral hygiene procedures and supragingival plaque removal. Sub-gingival scaling was performed following completion of the study at 6 months post-surgery.

Examiners' calibration

Before the study initiation, a calibration session was performed to evaluate (i) the intra-examiner agreement in the assessment of clinical recordings (Cohen's coefficient k= 0.86) and (ii) the intra-examiner agreement in the assessment of radiographic measurements (Kendall τ coefficient for intra-examiner agreement: 0.89).

Clinical parameters

Immediately before surgery and at 6 months post-surgery, studied parameters were recorded at 6 sites (mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual, disto-lingual) of the tooth exhibiting the intraosseous defect using a manual pressure sensitive probe (UNC 15, Hu-Friedy, Chicago, IL, USA) with 1-mm increments and applying approximately 0.3-N force. Measurements were rounded to the nearest mm. The following clinical measurements were performed by the same examiner (E.H): pocket probing depth (PPD), clinical attachment level (CAL), and gingival recession (REC). In addition, local bleeding score (BS) was recorded as positive when bleeding on probing was present at the surgical site.

At the completion of the intra-surgical debridement, the distance between the CEJ and the base of the defect as well as the depth of the intrabony component (measured as the distance between the deepest point of the defect and the most coronal point of the alveolar crest at the adjacent tooth) were assessed with a UNC 15 periodontal probe. The configuration of the defect with respect to the number of bony walls was also recorded.

Using digital photographs taken at 2 weeks post-surgery, wound healing was evaluated using the Early Healing Index (EHI) (Wachtel et al. 2003) by an examiner (A.S.) involved in previous trials that included the assessment of EHI (Farina et al. 2013). Kendall τ coefficient for intra-examiner agreement for EHI was 0.97.

Radiographic parameters

Periapical radiographs were obtained immediately before surgery and 6 months after surgery. The films were digitized, and the following linear radiographic measurements were performed by the same examiner (A.S) using dedicated software (NIS Elements™; Nikon Instruments S.P.A. Campi Bisenzio, Firenze, Italy):

- CEJ-base of the defect (CEJ-BD): distance (in mm) between the CEJ and the most apical extension of the defect (i.e., where the periodontal ligament space was considered having a normal width);
- CEJ-bone crest (CEJ-BC): distance (in mm) between the CEJ and the bone crest of the adjacent tooth;
- ANGLE (Steffensen & Webert 1989): defect angle (in degrees) defined by the line connecting the most apical point of the defect and the CEJ of the tooth presenting the intraosseous defect and the line connecting the most apical point of the defect and the point where the bone crest touched the neighboring tooth.

For each patient, linear defect fill (lDF) was calculated as the difference between pre-surgery CEJ-BD and 6-month CEJ-BD.

Patient-centered outcomes

A visual analog scale (VAS, 100 mm) was used to assess the patient's self-perceived pain (VAS_{pain}). Self-recordings of VAS_{pain} were performed immediately after surgery, at 8 a.m., 1 p.m. and 8 p.m. on each postoperative day up to the 3rd day, and at 8 p.m. on the 4th, 5th and 6th, 7th and 14th postoperative day.

Patients were also asked to record the postoperative consumption (timing, dosage) of the rescue analgesic.

Statistical analysis

Statistical software (Statistica v8.0; Tulsa, OK, US) was used for data analysis. A *per protocol* analysis was conducted with the patient being regarded as the statistical unit. The aspect of the tooth topographically related to the intraosseous defect presenting the largest CAL value at pre-surgery was used for comparisons and statistical analysis of outcome variables. Data was expressed as mean ± standard deviation (SD).

Intra-group and inter-group comparisons were performed with the Wilcoxon signed-rank test and the Mann Whitney rank-sum test, respectively. For nominal and ordinal data the Chi-square test and Mann-Whitney rank-sum test were used, respectively. Two-way Friedman's ANOVA was used to evaluate the effect of time and treatment on VAS_{pain}. The level of significance was set at 5% for all statistical tests.

A post-hoc calculation of the statistical power of the study, performed assuming a standard deviation of CAL change of 1 mm and using the *per protocol* size of each treatment group, revealed that the study had a power of 82.7% to detect a inter-group difference in CAL change of 1.1 mm (as previously reported by Trombelli et al. 2012) using a parametric test with a 0.05 two-sided significance level.

RESULTS

Study population

Twenty-nine patients (15 in SFA group, 14 in DFA group), each contributing 1 defect, were included. The experimental period was comprised between July 2012 (date of first surgery) and August 2014 (last follow-up visit). In the SFA group, 13 defects were accessed with a buccal SFA, while 2 defects were accessed with an oral SFA. Data on the mesio-distal extension of the flap in SFA and DFA groups are reported in Table 1 as the frequency distribution of patients in each group according to the number of teeth and papillae involved in surgery. In particular, a mean of 2.7 ± 0.7 papillae were involved in each SFA procedure, while 2.5 ± 0.5 buccal papillae and 2.2 ± 0.4 oral papillae were involved in each DFA procedure (Table 1). None of the patients in the SFA group was excluded from the study because of insufficient surgical access or an extension of the defect morphology preventing adequate root and defect instrumentation. One patient in the DFA group exited the study due to root fracture before the 6-month visit. All 28 patients who completed the study fully complied with the study procedures. Patient and defect characteristics in SFA and DFA groups are reported in Table 2. No significant differences were observed between groups in terms of age, gender and smoking status as well as defect location and severity. Patient distribution according to defect morphology significantly differed between groups ($p< 0.05$), with 1-wall defects more prevalent in DFA group while 2- and 3-wall defects more prevalent in the SFA group (Table 2).

Clinical parameters

EHI is reported in Table 3. A significant difference in patient distribution according to EHI was observed between groups (p= 0.025). In particular, 12 sites in the SFA group and 6 sites in the DFA group showed complete flap closure (i.e., EHI= 1, 2 or 3). The frequency of sites showing optimal wound healing (i.e., EHI= 1) was 8 and 3 in the SFA and DFA group, respectively.

Pre-surgery and 6-month post-surgery values of the clinical measurements as well as their 6-month changes are reported in Table 4. Pre-surgery, no significant inter-group differences in CAL, PPD, REC and prevalence of BS+ sites were observed. Both treatments resulted in significant 6-month CAL gain and PPD reduction, with no significant increase in REC. At 6 months, no significant differences in CAL, PPD and REC were found between groups (Table 4). At 6 months, the prevalence of BS+ sites remained unvaried compared to pre-surgery in both groups.

Radiographic parameters

In two patients in the DFA group, 6-month radiographs were not suitable for radiographic measurements. These patients were excluded from radiographic analysis. The pre-surgery and 6-month radiographic measurements in SFA and DFA groups are reported in Table 4.

Pre-surgery, no significant differences in CEJ-BD, CEJ-BC, and ANGLE were observed between groups.

At 6 months, both treatment groups showed a significant reduction in CEJ-BD. lDF was 2.0 ± 2.3 mm and 2.0 ± 1.3 mm in the SFA and DFA group, respectively. No significant changes in CEJ-BC were observed in both groups at 6 months compared to pre-surgery. When groups were compared in terms of 6-month CEJ-BD, CEJ-BC (Table 4) and lDF, no significant differences were found.

Patient-Centered Outcomes

VAS_{pain} in SFA and DFA groups throughout the first 14 postoperative days is illustrated in Figure 3.

Time and treatment showed a significant effect on VAS pain (p< 0.001). Significantly lower values of VAS_{pain} were observed in SFA group compared to DFA group at day 1 (8 a.m., 1 p.m., 8 p.m.), day 2 (1 p.m., 8 p.m.) and day 6 (Figure 3).

The mean total dose number of analgesics during the first 2 postoperative weeks was 2.73 ± 5.04 in the SFA group, and 8.69 ± 11.6 in the DFA group. A significantly greater number of analgesics was used in the DFA group compared to the SFA group (3.2 ± 2.9 vs 1.1 ± 2.2, respectively) at day +1 (p= 0.019) (Table 5).

DISCUSSION

The regenerative potential of the combination of rh-PDGF-BB with graft materials in general (Trombelli & Farina 2008, Kaigler et al. 2011), and β-TCP in particular, (Thakare & Deo 2012), in the treatment of human periodontal defects accessed with conventional double flap designs is well established. The present study was designed to evaluate whether and to what extent the clinical outcomes of a procedure based on a rh-PDGF-BB / β-TCP combination are similar when combined to either DFA or SFA. This study paralleled a previous RCT where SFA was compared to DFA when used to access 2–3 walled intraosseous defects in absence of any regenerative technology (Trombelli et al. 2012). The purpose of these two ompanion studies was, therefore, to assess whether and to what extent the SFA (with or without a regenerative technology) may be regarded as a suitable option for the surgical debridement of an intraosseous lesion. Clinical and patient-reported outcomes were considered to test the null hypothesis. The fact that the investigated technology is not commercially available in several countries (although limiting the generalizability of the procedure) seems not to influence the scientific validity of the study design and the robustness of the results. In the SFA group, the mean 6- month CAL gain was 4.0 mm, exceeding the mean improvements in CAL (3.2 mm) observed in the DFA group (although the difference did not reach statistical significance) and those reported in previous studies applying the same technology in conjunction with conventional, double flap designs (Nevins et al. 2005, 2013, Thakare & Deo 2012). Similarly, when deep intraosseous defects received surgical debridement without additional use of reconstructive devices (i.e., graft materials or membranes) or bioactive agents, SFA favored 1-mm greater CAL gain and PPD reductions compared to access with DFA (Trombelli et al. 2012), largely exceeding CAL gain values reported for conventional access flaps (Graziani et al. 2012). In the light of these findings and the minimal postoperative increase in REC (0.1 mm) observed in the SFA group, the surgical access obtained according to the SFA principles seems to optimize the regenerative outcomes as well as minimize the aesthetic impairment of the patient following regenerative treatment

of intraosseous defects with a combination of rh-PDGF-BB and β-TCP. In the SFA group, the tendency to show better reconstructive outcomes compared to DFA may in part be due to the better quality of early wound healing at the incision margin (as assessed at 2 weeks with the EHI). Consistently, SFA either alone or in combination with a reconstructive technology led to a consistently higher incidence of complete flap closure at 2 weeks postsurgery (Farina et al. 2013). Overall, these data indicate that the SFA may promote proper conditions for wound stability compared to conventional DFA, impacting positively on the clinical effectiveness of the procedure. Patients in SFA group reported lower postoperative pain and dose of rescue analgesics compared to the DFA group. Differences between groups may be due to the different invasiveness of the investigated procedures (Table 1). This consideration is corroborated further by the results of a previous study on the clinical outcomes of another technique (i.e., minimally invasive surgical technique, MIST) based on the elevation of a double flap and characterized by a more limited mesio-distal extension (in terms of inter-dental spaces/ papillae involved) compared to the DFA as adopted in this study. On average, the VAS pain scores reported by Cortellini & Tonetti (2007) for MIST within the first 2–3 postoperative days (19 ± 10) were intermediate between those recorded for DFA and SFA in this study (Fig. 3). Moreover, it can be speculated that the operative time, which was previously demonstrated to influence the severity of postoperative pain following periodontal surgical procedures (Griffin et al. 2006, Tan et al. 2014), may have been longer for DFA than SFA. The prescription of analgesics was not standardized with the intention to consider the self-administration of ibuprofen as an indirect outcome of the level of postoperative pain. Although this may have introduced a potential bias for VAS validity, when VAS and dosage of analgesics spontaneously taken by the patient were jointly considered, data indicate that SFA may result in a more tolerable post-operative course when compared to DFA. The different distribution of patients observed in SFA and DFA groups according to defect morphology may represent a potential source of bias since it has been reported that defect morphology is associated with varying regenerative potential (Selvig et al. 1993, Tonetti et al. 1996, 2002, Silvestri et al. 2003, Cortellini et al. 2009). However, when considering the relationship between defect configuration and the clinical outcome of intraosseous defects treated with a combination of biological agents and graft biomaterial, the evidence is limited and not conclusive (Kao et al. 2015). In particular, previous studies based on the use of rhPDGF-BB and β-TCP seem to indicate that defect morphology has a limited impact on the outcomes of periodontal regenerative procedures. One- to two-wall intraosseous defects showed similar bone fill compared to 3-wall/circumferential defects at

either 6 (Nevins et al. 2005) or 36 months (Nevins et al. 2013) post-surgery. In conclusion, the current results indicate that: (i) deep intraosseous periodontal defects, accessed with the SFA or conventional papilla preservation techniques, may be effectively treated with careful debridement and root planing in combination with a composite graft of rhPDGF-BB (0.3 mg/ml) and β- TCP; and (ii) when used in combination with rhPDGF-BB/β-TCP technology, surgical access performed in accordance with SFA principles may result in better quality of early wound healing, lower pain and consumption of analgesics during the first postoperative days compared to the use of traditional papilla preservation techniques.

Source of Founding: The present study was supported by Osteohealth Company, Shirley, NY, US; the Research Centre for the Study of Periodontal and Peri-implant Diseases, University of Ferrara, Italy; and the Division of Periodontology, School of Dental Medicine, University of Connecticut Health Center, Farmington, Connecticut (US).

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TABLES

Table 1. Frequency distribution of patients in Single Flap Approach and Double Flap Approach groups according to the number of teeth and papillae involved in surgery.

N° OF TEETH INVOLVED IN SURGERY	**SFA (N=15) N° of patients**	**DFA (N=15) N° of patients**
2	1	1
3	8	8
4	5	4
5	1	0

N° OF PAPILLAE INVOLVED IN SURGERY	**N° of patients**	**N° patients (buccal aspect)**	**N° patients (oral aspect)**
2	6	7	10
3	7	6	3
4	2	0	0

Table 2. Patient and defect characteristics in Single Flap Approach and Double Flap Approach groups.

	SFA (n = 15)	**DFA (n = 13)**	*p*
Patient characteristics			
Gender (males/females)	9/6	8/5	1
Age (years) (mean ± SD)	50.1 ± 14.8	46.7 ± 15.4	0.821
Smokers (yes/no)	3/12	0/13	0.226
Defect characteristics			
Dental arch (maxillary/mandibular)	7/8	7/6	0.708
Tooth type (incisors/canines/premolars/molars)	3/2/5/5	3/2/4/4	1
CEJ - base of the defect (mm, as assessed during surgery) (mean ± SD)	10.3 ± 2.4	8.8 ± 1.5	0.142
Intrabony component (mm, as assessed during surgery) (mean ± SD)	7.7 ± 2.6	5.8 ± 1.5	0.058
Defect configuration (bony walls) as assessed during surgery (n° of defects)			
mainly 1-wall mainly 2-wall mainly 3-wall	1 6 8	7 2 4	0.032

Table 3. Distribution of patients in Single Flap Approach and Double Flap Approach groups according to the Early Healing Index (as assessed at defect sites 2 weeks following surgery).

	SFA (n = 15)	**DFA (n = 13)**	*p*
EARLY HEALING INDEX			
score 1 (complete flap closure – no fibrin line in the inter-proximal area)	8	3	
score 2 (complete flap closure – fine fibrin line in the inter-proximal area)	3	3	
score 3 (complete flap closure – fibrin clot in the inter-proximal area)	1	0	0.025
score 4 (incomplete flap closure – partial necrosis of the inter-proximal tissue)	3	5	
score 5 (incomplete flap closure – complete necrosis of the interproximal tissue)	0	2	

Table 4. Clinical recordings and radiographic measurements in Single Flap Approach and Double Flap Approach groups.

* Negative value for REC indicates an increase.

	pre-surgery	**6 months**	*p*	**6-month change***
Clinical recordings				
CAL (mm)				
SFA	9.7 ± 2.5	5.7 ± 2.6	<0.001	4.0 ± 1.9
DFA	8.5 ± 1.6	5.2 ± 1.6	0.001	3.2 ± 1.4
p	0.339	1		0.316
PPD (mm)				
SFA	8.7 ± 2.0	4.5 ± 1.6	<0.001	4.1 ± 1.7
DFA	7.7 ± 1.5	4.1 ± 1.2	0.001	3.6 ± 1.1
p	0.254	0.496		0.413
REC (mm)				
SFA	1.1 ± 1.3	1.2 ± 1.5	0.529	-0.1 ± 0.7
DFA	0.8 ± 1.3	1.2 ± 1.6	0.343	-0.4 ± 1.3
p	0.363	0.363		0.618
BS (positive/negative)				
SFA	8/7	8/7	1	-
DFA	7/6	2/11	0.097	
p	1	0.055		
Radiographic measurements				
ANGLE (degrees)				
SFA	31.9 ± 11.6			
DFA	33.6 ± 9.9			
p	0.495			
CEJ-BD (mm)				
SFA	8.1 ± 3.4	6.1 ± 2.3	0.003	-
DFA	8.0 ± 2.2	5.9 ± 2.2	0.005	
p	0.683	0.838		
CEJ-BC (mm)				
SFA	3.2 ± 1.7	2.9 ± 1.6	0.268	-
DFA	3.2 ± 1.7	2.7 ± 1.3	0.333	
p	0.891	0.646		

Table 5. Self-reported dose (expressed as mean ± SD) of rescue analgesics assumed during the first two postoperative weeks in Single Flap Approach and Double Flap Approach groups.

	Post-operative day													
	+1	**+2**	**+3**	**+4**	**+5**	**+6**	**+7**	**+8**	**+9**	**+10**	**+11**	**+12**	**+13**	**+14**
SFA	1.1 (± 2.2)	0.9 (± 1.7)	0.3 (± 0.7)	0.2 (± 0.4)	0.1 (± 0.4)	0	0.1 (± 0.3)	0	0	0	0	0	0	0.1 (± 0.3)
DFA	3.2 (± 2.9)	1.8 (± 2.7)	1.5 (± 2.7)	0.5 (± 1.1)	0.5 (± 1.1)	0.5 (± 1.1)	0.3 (± 0.9)	0	0	0	0	0	0	0.2 (± 0.8)
p	0.019	0.277	0.217	0.751	0.586	0.316	0.683	1	1	1	1	1	1	0.964

APPENDICES

Appendix 1. Visit schema.

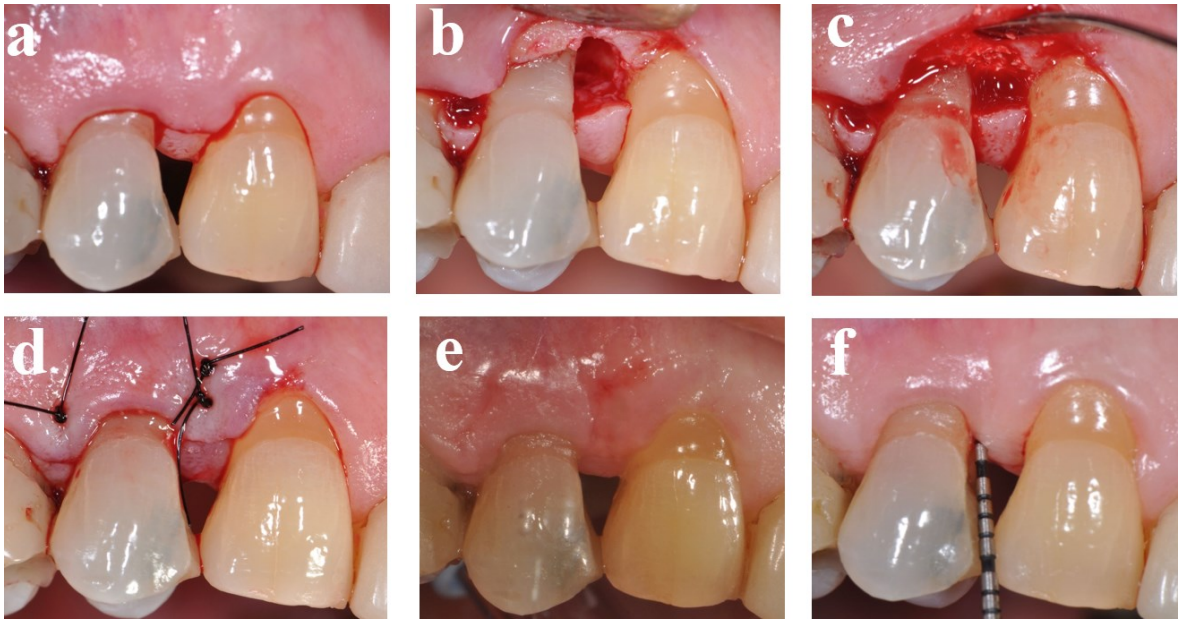
	Screening	*Initial therapy*	*Surgery*	*Suture removal*	*Follow-up*	*Follow-up*
		day -28	**day 0**	**day +14**	**day +36/ +56 / +84**	**day +180**
Informed consent	X					
Subject demographics	X					
Medical and dental history	X					
Inclusion / exclusion criteria	X					
Randomization			X			
Periapical radiographs			X			X
Clinical recordings (CAL, PPD, REC, BS)			X			X
VAS questionnaire			X			
Suture removal				X		
Scaling and root planing		X				X
Professional prophylaxis / OHI		X			X	X
Clinical photography			X	X	X	X

Appendix 2. Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
• ≥ 18 years of age • provision of informed consent • diagnosis of chronic or aggressive periodontitis • presence of at least one intraosseous defect (as detected on periapical radiographs) associated with pocket probing depth ≥ 6mm • Full Mouth Plaque Score (O'Leary et al., 1972) and Full Mouth Bleeding Score < 20% at the time of the surgical procedure	*Conditions that prevented study participation:* • time constrain that prevented returning to follow up visit • inability to follow investigator's instruction • no compliance with the study requirements • simultaneous participation in other studies *Systemic conditions:* • conditions requiring chronic routine use of antibiotics or requiring prolonged use of steroids • long-term use of bisphosphonate (≥ 3 years) • history of leukocyte dysfunction or deficiencies, bleeding disorders, neoplastic disease requiring radiation or chemotherapy, metabolic bone disorder, uncontrolled endocrine disorders, HIV infection • use of investigational drugs or devices within 30 days of study period • alcoholism or drug abuse • smoking >10 cigarettes per day *Local conditions (experimental tooth):* • inadequate restoration • endodontic lesions • inadequate endodontic treatment • untreated carious lesion • third molars were excluded

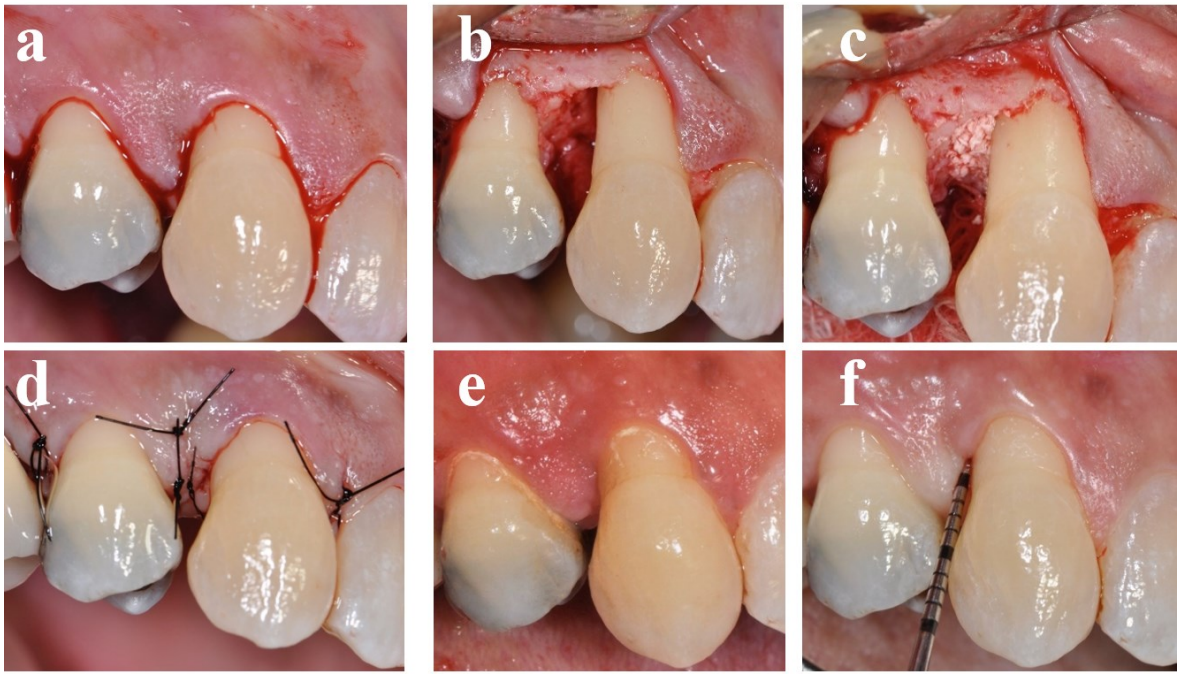
FIGURES

Figure 1. Treatment of a periodontal intraosseous defect with a buccal Single Flap Approach and rh-PDGF-BB plus *β*-TCP.



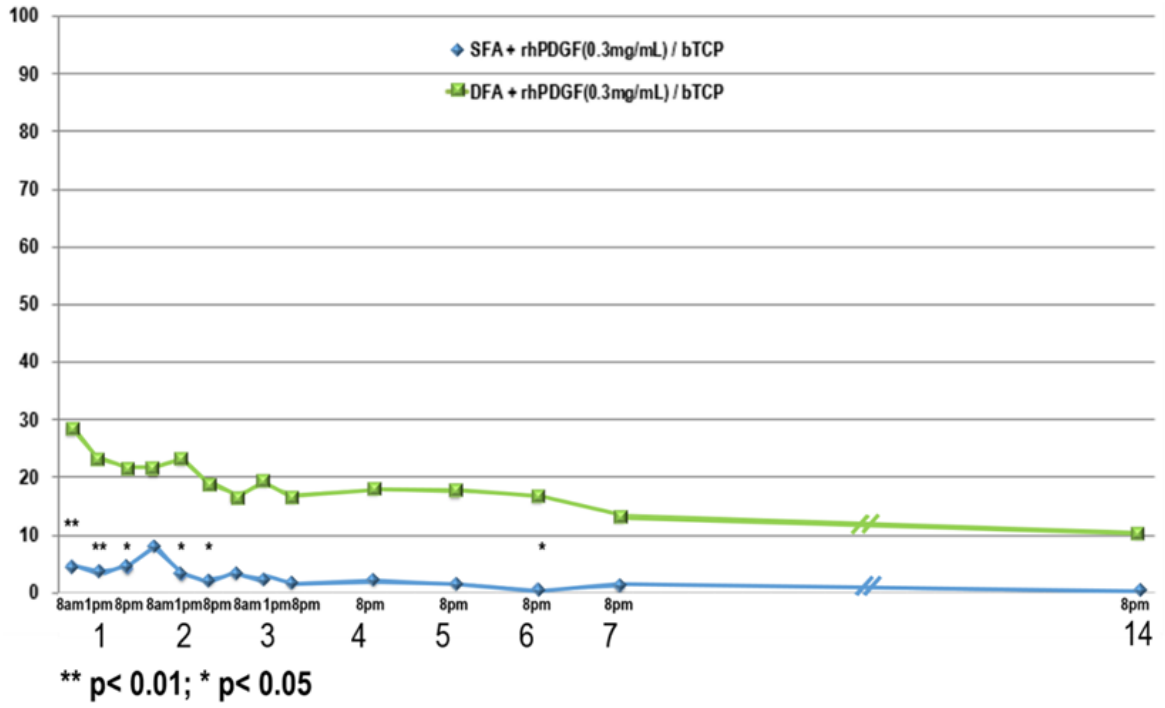
(**a**) An envelope flap without vertical releasing incisions is performed. Sulcular incisions are made following the gingival margin of the teeth included in the surgical area. The mesio-distal extension of the flap is kept limited while ensuring access for defect debridement. An oblique or horizontal, butt-joint incision is made at the buccal aspect of the inter-dental papilla overlying the intraosseous defect. An adequate amount of supracrestal soft tissue remains connected to the undetached papilla to ensure subsequent flap adaptation and suturing. (**b**) A microsurgical periosteal elevator is used to raise a flap only on one side (buccal or oral), leaving the other portion of the inter-dental supracrestal soft tissues undetached. (**c**) The intraosseous component of the defect is filled with b-TCP mixed with rh-PDGF-BB. (**d**) For wound closure, a horizontal internal mattress suture is placed between the flap and the base of the attached papilla to ensure repositioning of flap. A second internal mattress suture (vertical or horizontal) is placed between the most coronal portion of the flap and the most coronal portion of the papilla as needed. (**e**) Clinical aspect at suture removal (2 weeks post-surgery). (**f**) At 6 months post-surgery, pocket probing depth amounts to 4 mm.

Figure 2. Treatment of a periodontal intraosseous defect with a Double Flap Approach (simplified papilla preservation flap, SPPF; Cortellini et al. 1999) and rh-PDGF-BB plus β-TCP.



(**a**) An envelope flap without vertical releasing incisions is performed. Sulcular incisions are made following the gingival margin of the teeth included in the surgical area. The mesio-distal extension of the flap is kept limited while ensuring access for defect debridement. An incision is made at the buccal aspect of the inter-dental papilla overlying the intraosseous defect according to the SPPF (Cortellini et al. 1999). (**b**) A microsurgical periosteal elevator is used to raise a flap on both buccal and oral sides. (**c**) The intraosseous component of the defect is filled with β-TCP mixed with rh-PDGF-BB. (**d**) Primary closure is achieved according to the suturing technique of the SPPF (Cortellini et al. 1999). First, a horizontal, "offset" internal mattress suture is placed in the defect associated inter-dental space. The inter-dental tissue above the defect is then closed with two interrupted sutures. (**e**) Clinical aspect at suture removal (2 weeks post-surgery). (**f**) At 6 months post-surgery, pocket probing depth amounts to 3 mm.

Figure 3. Mean VAS_{pain} in Single Flap Approach and Double Flap Approach groups.



CHAPTER 5

Change in the gingival margin profile after the *Single Flap Approach* in periodontal intraosseous defects

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Journal of Periodontology (2015) **86**, 1038-1046

Abstract

Background: to evaluate the association of patient-related and site-specific factors as well as the adopted treatment modality with the change in buccal and interdental recession (bREC and iREC, respectively) observed at 6 months following treatment of periodontal intraosseous defects with the Single Flap Approach (SFA).

Materials and Methods: sixty-six patients contributing 74 intraosseous defects accessed with a buccal SFA and treated with different modalities were retrospectively selected. A multivariate 2-level (patient, site) model was constructed, with the 6-month changes in bREC and iREC being regarded as the dependent variables.

Results: (i) significant 6-month increases in bREC (-0.6 ± 0.7 mm) and iREC (-0.9 ± 1.1 mm) were observed; (ii) bREC change was significantly predicted by pre-surgery probing depth (PD) and depth of osseous dehiscence at the buccal aspect; (iii) iREC change was significantly predicted by pre-surgery PD and the treatment modality, with defects treated with SFA in combination with a graft material and a bioactive agent being less prone to iREC increase compared to defects treated with SFA alone.

Conclusions: following buccal SFA, greater post-surgery increase in buccal gingival recession must be expected for deep intraosseous defects associated with a buccal dehiscence. The combination of a graft material and a bioactive agent in adjunct to the SFA may limit the postoperative increase in interdental gingival recession.

INTRODUCTION

When a surgical procedure is performed to access a periodontal intraosseous defect, post-surgery occurrence or increase of gingival recession (GR) is a highly prevalent, undesired side effect of treatment (Graziani et al. 2012), being mainly due to the combined effect of the surgical trauma, the reduction in tissue edema and the post-surgery remodeling of supracrestal tissues during wound healing (Trombelli & Farina 2012). While maximizing the reconstructive outcome, therefore, modern strategies for the management of intraosseous defects aim at minimizing post-surgery increase in GR. In this context, a recent systematic review showed that the extent of post-surgery GR increase may vary according to the type of surgical flap used to access an intraosseous defect, with defects accessed with papilla preservation techniques showing lower GR increase compared to defects accessed with less conservative flap designs (Graziani et al. 2012). Since previous studies reported that GR significantly affects the oral-health related quality of life (Needleman et al. 2004, Ng et al. 2006, Patel et al. 2008), the adoption of surgical procedures that may limit post-surgery GR increase while maximizing treatment outcomes in terms of clinical attachment level (CAL) gain and probing depth (PD) reduction should be encouraged.

In 2007, we proposed the Single Flap Approach (SFA), a minimally invasive surgical procedure designed for the reconstructive therapy of intraosseous defects (Trombelli et al. 2007, 2009). The basic principle behind the SFA is the unilateral elevation of a limited mucoperiosteal flap to allow surgical access. Interproximal gingival tissues (i.e. the interdental papilla) are kept intact, allowing for an easy repositioning of the flap in the pre-operatory position with primary wound closure. To date, a series of studies have demonstrated the clinical efficacy of the SFA either as a stand-alone protocol or in combination with different regenerative technologies (Trombelli et al. 2007, 2009, 2010, 2012, Farina et al. 2013, 2014, Rizzi et al. 2013, Simonelli et al. 2013, Schincaglia et al. 2015). The SFA was shown to be associated with low mean postoperative GR increases, with 5 over 6 prospective clinical trials reporting mean variations within 1 mm at 6 months following surgery (Trombelli et al. 2007, 2009, 2010, 2012, Farina et al. 2013, 2014, Schincaglia et al. 2015). Also, encouraging results were reported by two randomized controlled trials where limited post-surgery GR increase was observed for sites treated with SFA compared to sites accessed with papilla preservation techniques (Trombelli et al. 2012, Schincaglia et al. 2015). Similarly, low GR increase was reported when intraosseous defects were accessed with a flap design similar to the SFA and treated with different

modalities (Cortellini & Tonetti 2011). Data dispersion as reported in the available studies on the SFA (Trombelli et al. 2007, 2009, 2010, 2012, Farina et al. 2013, 2014, Schincaglia et al. 2015), however, suggests that patient-related and/or local factors as well as the adopted treatment strategy may influence the predictability of the SFA in terms of post-surgery GR change.

Therefore, the present study was performed to evaluate the influence of patient-related and site-specific factors as well as the adopted reconstructive strategy on the change in GR at 6 months following the treatment of periodontal intraosseous defects with the SFA.

MATERIALS & METHODS

Experimental design and ethical aspects

The present study is a retrospective analysis of series of consecutively treated cases. The study was approved by the Local Ethical Committee of Ferrara, Italy (date of approval: July 10, 2014). All the clinical procedures had been performed in accordance with the Declaration of Helsinki and the Good Clinical Practice Guidelines (GCPs). Each patient had provided a written informed consent to surgical treatment.

Study population

Data were retrospectively derived from the record charts of periodontal patients seeking care at the Research Centre for the Study of Periodontal and Peri-Implant Diseases, University of Ferrara, Italy; the Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy; the Division of Periodontology, School of Dental Medicine, University of Connecticut Health Center, Farmington, CT; and 1 private dental office (Ferrara, Italy), in the period comprised between October, 2006 and April, 2014. Five experienced periodontal surgeons (L.T., R.F., G.R., G.P.S., and L.M.) specifically trained in previous SFA studies (Trombelli et al. 2007, 2009, 2010, 2012, Farina et al. 2013, 2014, Schincaglia et al. 2015) had performed the clinical procedures, including surgical treatments and clinical recordings.

Patients were included in the analysis if positive for each of the following inclusion criteria: 1) diagnosis of generalized chronic or aggressive periodontitis (Armitage 1999); 2) presence of at least one isolated interproximal periodontal intraosseous defect (as detected

on periapical radiographs) associated with a PD≥ 5 mm; 3) surgical treatment with a buccal SFA (due to limited to no extension of the defect on the lingual or palatal side as assessed by preoperative bone sounding) either per se or in association with graft materials, membrane devices, bioactive agents, or combinations; 4) full-mouth plaque score (O'Leary 1972) and full-mouth bleeding score (Cortellini et al. 1993) < 20% at the time of the surgical procedure; 5) compliance with the scheduled post-surgical recall sessions for professional plaque removal. Patients were excluded from the analysis if positive for at least one of the following criteria: 1) use of medications affecting periodontal status (including bisphosphonates, cyclosporine, phenytoin, nifedipine and other calcium channel blockers, corticosteroids and non steroidal anti-inflammatory drugs); 2) furcation involvement, 3) degree III mobility (Miller 1950) or 4) inadequate endodontic treatment and/or restoration of the tooth presenting the intraosseous defect. Third molars were also excluded from the analysis.

Clinical procedures

Pre-surgery procedures

Each patient had undergone a full-mouth session of scaling and root planing using mechanical and hand instrumentation and had received personalized oral hygiene instructions. Temporary splinting and/or occlusal adjustment had been performed for teeth with degree I or II mobility (Miller 1950) at re-evaluation following non-surgical instrumentation. The surgical phase had been delayed until the achievement of minimal residual inflammation at the defect site.

Surgical procedures

Defects had been treated according to the principles of the buccal SFA (Trombelli et al. 2007, 2009) (Figure 1). Briefly, a buccal envelope flap without vertical releasing incisions had been performed. Sulcular incisions had been made following the gingival margin of the teeth included in the surgical area. An oblique or horizontal butt-joint incision had been made at the level of the interdental papilla overlying the intraosseous defect; the greater the distance from the tip of the papilla to the underlying bone crest, the more apical (i.e., close to the base of the papilla) the buccal incision in the interdental area. A flap had been raised only on the buccal side, leaving the oral portion of the interdental supracrestal soft tissues undetached. Root and defect debridement had been performed using hand and ultrasonic instruments. At the operator's judgement, defects had been treated with one of the

following modalities: spontaneous healing; enamel matrix derivative (EMD) alone or in combination with deproteinized bovine bone mineral (DBBM); recombinant human platelet-derived growth factor BB (rh-PDGF-BB) with β-tricalcium phosphate (β-TCP); or resorbable collagen membranes in combination with DBBM or synthetic hydroxyapatite in a collagen matrix (S-HA) (Table 1). The suturing technique had been performed according to the original description of the SFA (Trombelli et al. 2007, 2009, 2010, 2012, Farina et al. 2013, 2014, Schincaglia et al. 2015) i.e. a horizontal internal mattress suture at the base of the papilla and a second internal mattress suture (vertical or horizontal) between the most coronal portion of the flap and the most coronal portion of the oral papilla. When needed (i.e. in case of a large, thick interdental papilla), an interrupted suture had been performed to ensure primary intention healing at the incision line. Primary flap closure had always been obtained at suturing.

Post-surgery procedures

Sutures had been removed at 2 weeks post-surgery. The patients had been asked to abstain from mechanical oral hygiene procedures in the surgical area for 4 weeks. A 0.12% chlorhexidine mouthrinse (10 mL BID/6 wks) had been used to support local plaque control. Each patient had been enrolled in a monthly recall program for 3 months and had been reviewed according to personal needs thereafter. Each session had included reinforcement of oral hygiene procedures and professional plaque removal. Subgingival scaling had been performed at 6 month post-surgery.

Study parameters

Patient-related parameters (related to the day of surgery)

Data related to age (in years), gender, and smoking status (recorded as current smoker or non-smoker) were extracted from the patient record charts.

Site-related parameters

At pre-surgery and 6-month visit, each periodontal surgeon had assessed the following parameters at the interproximal aspect of the defect showing the most severe pre-surgery attachment loss using a manual pressure sensitive probe (UNC-15, Hu-Fready, Chicago, IL, USA) with 1-mm increments:

- probing depth (PD), measured in mm from the gingival margin to the bottom of the pocket;

- clinical attachment level (CAL) measured in mm from the cemento-enamel junction (CEJ) or the apical margin of a restoration to the bottom of the pocket;

- interdental gingival recession (iREC), measured from the CEJ or the apical margin of a restoration to the gingival margin;

- interdental keratinized tissue width (iKT), measured from the tip of the interdental papilla to the mucogingival junction.

On digital photographs taken as much perpendicular as possible to the long axis of the tooth presenting the intraosseous defect at pre-surgery, an examiner not involved in clinical procedures (A.S.) assessed the following parameters using a software for image analysis (NIS Elements® v4.2; Nikon Instruments, Campi Bisenzio, Firenze, Italy):

- gingival biotype: the transparency of a periodontal probe through the gingival tissues when probing the buccal aspect of the tooth was evaluated according to a previously described method (De Rouck et al. 2009). If the probe was visible through the gingival tissues, the biotype was defined as “thin”, otherwise it was defined as “thick”;

- buccal gingival recession (bREC), measured from the CEJ to the gingival margin;

- buccal keratinized tissue width (bKT), measured from the gingival margin to the mucogingival junction.

The examiner had been previously involved in a calibration session (intra-class correlation coefficient for the single measure: 0.989) due to the involvement in previous studies including linear measurements on digital photographs (Farina et al. 2013, 2014). To account for photographic magnification, bREC and bKT were referred to the increments of the UNC 15 probe as depicted in the same photograph. Measurements were roundest to the nearest 0.1 mm. bREC and bKT measurements were repeated on the photographs taken at the 6-month visit.

Immediately after the completion of root and defect debridement, each surgeon had assessed the following defect-related characteristics (in mm) using a UNC 15 periodontal probe:

- depth of the intrabony component (IBD), measured as the distance between the most coronal point of the alveolar crest and the bottom of the defect;

- distance between the CEJ and the bottom of the defect (CEJ-BD);

- distance between the CEJ and the most coronal extension of the interproximal bone crest (iCEJ-BC).

- distance between the CEJ and the most coronal extension of the buccal bone crest (bCEJ-BC);

The morphology of the intraosseous defect (i.e. number of bony walls), defined as: mainly 1-wall, combined 1-2 walls, mainly 2-walls, combined 2-3 walls, mainly 3-wall, was also recorded.

Statistical analysis

Data were entered in a unique database file (STATISTICA® software version 8.0; StatSoft s.r.l., Vigonza, Italy). Data were expressed as mean ± standard deviation (SD). Within-group comparisons (pre-surgery vs. 6 months) were performed with Wilcoxon test and χ^2 test or Fisher's exact test.

All data were transferred into a statistical software specifically designed for multilevel analysis (MLWin 2.28, Centre for Multilevel Modelling, Bristol University, UK) and used for the construction of a multivariate 2-level model (patient and defect), with 6-month changes in bREC and iREC being regarded as the dependent variables and the following parameters being investigated as predictors:

- patient-related factors: age; gender; smoking status;

- site-specific factors: defect location in terms of dental arch (maxillary/mandibular) and tooth type (incisor/canine/premolar/molar); pre-surgery parameters related to the buccal aspect (i.e., thickness of the buccal gingival tissue, bREC, bKT, bCEJ-BC) and to the interproximal aspect of the defect showing the most severe pre-surgery attachment loss (i.e., iREC, PD, CAL, iKT; IBD; iCEJ-BC; CEJ-BD); defect morphology;

- treatment modality (SFA alone; SFA + EMD; SFA + EMD + DBBM; SFA + rhPDGF-BB + β-TCP; SFA + hydroxyapatite-based graft material + resorbable membrane).

Due to an unbalanced distribution of defects among operators, it was not possible to include the operator as a predictor in the multivariate model. Since the variance at patient level was not significantly different from 0 when a 2-level model was tested, the site was regarded as the statistical unit.

A parsimonious model (namely, the "final model"), including the predictors that had a statistically significant impact ($p< 0.05$) on one or both dependent variables, was built. The coefficients were estimated using iterative generalized least squares (IGLS) and the significance of each covariate was tested using a Wald test. Nested models were tested for significant improvements in model fit by comparing the reduction in -2LL (-2 log likelihood) with a chi-squared distribution.

RESULTS

Study population

Sixty-six patients contributing 74 intraosseous defects were included in the study. The characteristics of the study population according to patient- and site-related factors as well as treatment modality are reported in Tables 1 and 2. Defects were left filled with blood clot (n= 24) or treated with different treatment modalities (n= 50). All patients complied with the recall program until the re-evaluation. All treated teeth were suitable for experimental assessments at the 6-month visit.

Clinical outcomes

Baseline and 6-month probing parameters as well as their changes are reported in Table 2. The procedure resulted in significant CAL gain (3.7 ± 0.2 mm; $p< 0.001$) and PD reduction (4.5 ± 2.0; $p< 0.001$), which were paralleled by a significant increase in bREC (-0.6 ± 0.7 mm, range: -2; 1; $p< 0.001$) and iREC (-0.9 ± 1.1 mm, range: -3; 3; $p< 0.001$).

At 6 months, bREC remained stable or decreased in 29 sites, and showed an increase within 1 mm or above 1 mm in 33 sites and 12 sites, respectively. iREC remained stable or decreased in 26 sites, and showed an increase within 1 mm or above 1 mm in 28 sites and 20 sites, respectively.

Predictors of 6-month changes in bREC and iREC

When the 6-month changes in bREC and iREC were entered as outcome variables into a multivariate model, none of the investigated patient-related factors showed a significant effect on changes in either bREC or iREC.

bREC change was significantly predicted by pre-surgery interproximal PD ($p< 0.001$) and CEJ-BC ($p< 0.001$) (Figure 2). A stable or improved bREC should be expected only for defects with a PD of 6.0 mm or lower and CEJ-BC of 2.0 mm. The predicted bREC increases by 0.10 mm for each 1-mm increment in pre-surgery PD over 6 mm and by 0.13 mm for each 1-mm increment in CEJ-BC over 2 mm.

iREC change was significantly predicted by pre-surgery interproximal PD ($p< 0.001$) and the treatment modality ($p< 0.001$) (Figure 3). Defects where the SFA was combined with EMD+DBBM or rh-PDGF-BB+β -TCP were less prone to iREC increase compared to defects treated with SFA alone ($p< 0.001$ for both comparisons) (Table 3, Figure 3). For example, for a defect with pre-surgery PD= 7 mm, the predicted increase in iREC was 1.16 mm for SFA alone, 0.98 mm for SFA+EMD, 0.53 mm for SFA + hydroxyapatite based graft material and resorbable membrane, 0.13 mm for SFA+EMD+DBBM, and 0.03 mm for SFA+rh-PDGF-BB+ β -TCP.

Defect location in terms of dental arch (maxillary/mandibular) and tooth type (incisor/canine/premolar/molar), the thickness of the buccal gingival tissue, the pre-surgery values of bREC, iREC, CAL, KT, iKT as well as several of the bone-related measurements (i.e., IBD; iCEJ-BC; CEJ-BD; defect morphology) did not show a significant effect on the 6-month changes in either bREC or iREC.

The inclusion of all significant predictors in the multivariate model led to a significant improvement of the model ($p< 0.001$) as assessed from the reduction in -2*loglikelihood compared to a chi-squared distribution with 7 degrees of freedom.

The multivariate model identified a significant, positive correlation between the 6-month changes in bREC and iREC at the site level (correlation coefficient= 0.48, $p= 0.004$) (Table 3).

DISCUSSION

The present study was performed to evaluate the influence of patient-related and site-specific factors as well as the adopted treatment modality on the change in bREC and iREC at 6 months following the treatment of periodontal intraosseous defects with the SFA. Sixty-six patients contributing 74 intraosseous defects were retrospectively selected and included for analysis. Defects had been accessed with a buccal SFA and, according to the operator's discretion, had been treated with various treatment modalities. Data were used for the construction of a multivariate model, with 6-month changes in bREC and iREC being regarded as the primary outcome variables. The 6-month results of the study indicated that: (1) the substantial CAL gain and PD reduction following SFA were paralleled by a limited, although significant, increase in bREC (-0.6 ± 0.7 mm) and iREC (-0.9 ± 1.1 mm); (2) bREC change was significantly predicted by pre-surgery interproximal PD and bCEJ-BC; (3) iREC change was significantly predicted by pre-surgery interproximal PD and the treatment modality, with defects treated with SFA in combination with a graft material and a bioactive agent being less prone to iREC increase compared to defects treated with SFA alone.

Systematic reviews indicate that the surgical treatment of periodontal intraosseous defects is frequently paralleled by occurrence or increase of REC when performed by open flap debridement either alone (Graziani et al. 2012, Duane 2012) or in association with reconstructive technologies (Needleman et al 2006, Li et al. 2012). The management of interdental supracrestal soft tissues according to the principles of the SFA, which include the preservation of the integrity of supracrestal tissues and the repositioning of the buccal flap at the pre-surgery level, was demonstrated to provide the conditions for primary closure and optimal quality of early wound healing (Farina et al. 2013, Schincaglia et al. 2015), substantial CAL gain (Trombelli et al. 2007, 2009, 2010, 2012, Farina et al. 2013, 2014, Rizzi et al. 2013, Simonelli et al. 2013, Schincaglia et al. 2015) as well as low postoperative morbidity and discomfort (Schincaglia et al. 2015). In our cohort, about one third of the treated defects showed the postoperative stability of the buccal gingival margin or a decrease in bREC. Similarly, one third of the treated defects showed stable or improved iREC. These results demonstrate that the SFA has the potential to minimize the postoperative shrinkage of the gingival tissues, suggesting that this effect may be favored (as observed in the present study) by some local, site-specific factors and some adjunctive treatments. Overall, these data reinforce the need for an accurate pre-surgery evaluation of the area to be treated with the SFA as well as implementation of future studies aimed at

identifying the factors explaining the residual variability in REC following SFA (e.g., clinical operator and his/her training).

A limited REC increase was observed in the present and previous clinical trials evaluating the performance of the SFA in different patient cohorts and clinical conditions (Trombelli et al. 2009, 2010, 2012, Farina et al. 2013, 2014, Schincaglia et al. 2015). These findings seem to indicate that the increased REC results from the tissue remodeling that occurs following the debridement of a deep intraosseous defects. This consideration is corroborated further by the fact that even a non-surgical debridement of intraosseous defects is associated with a certain amount of REC increase (Nibali et al. 2011, Ribeiro et al. 2011). On the other hand, a flap design similar to the SFA at periodontally healthy sites is associated with no post-surgery REC change (Taschieri et al. 2014). Data from the present multivariate model showed that an increase in bREC following SFA may be expected when a pre-surgery interproximal PD of > 5.0 mm and buccal osseous dehiscence > 2.0 mm are present (Figure 2). On the basis of these findings, it seems recommended to combine the SFA with specific additional procedures/technologies aimed at controlling the post-surgery increase in bREC whenever a limited to null post-surgery shrinkage of the gingival margin is of paramount importance (e.g. esthetic sensitive areas). In this respect, different Authors have proposed the coronal advancement of the SFA (Checchi et al. 2008) or the combination of the SFA with an autologous soft tissue graft (Trombelli et al. 2008, Zucchelli et al. 2014) or a tridimensional collagen matrix (Rizzi et al. 2013). The efficacy of these procedures in controlling post-operative bREC increase when compared to SFA alone, however, needs to be further evaluated. Previous systematic reviews showed that the combined use of EMD and DBBM may significantly temper postoperative REC increase compared to EMD alone in the treatment of periodontal intraosseous defects (Li et al. 2012, Trombelli & Farina 2008, Esposito et al. 2009). Consistently, we observed that defects treated with a graft material and a bioactive agent (including EMD+DBBM) were less prone to show an increase in iREC following SFA - as predicted in the present model - than defects treated with open flap debridement with or without EMD (Table 3). Overall, the present data support the additional use of a graft material when intraosseous defects located at esthetic-sensitive areas are treated with the SFA alone or in combination with a non-space making technology such as EMD. In the present analysis, patient-related factors and site-related factors which were previously shown to have a significant effect on REC changes following the surgical treatment of intraosseous defects were included as potential predictors of REC change following SFA. More specifically, previous Authors reported a

significant impact of smoking (Zucchelli et al. 2002) and gingival thickness (Cosyn et al. 2012) on the magnitude of postoperative REC change. Also, a "nonsupporting" defect anatomy was identified as relevant risk factors for REC increase (Cosyn et al. 2012). In contrast with findings from these studies, REC change following SFA was not dependent on any of the investigated patient-related predictors and defect-related bone measurements (e.g., severity of bone loss, interdental intrabony component and suprabony components, defect morphology).

CONCLUSIONS

In conclusion, the results of the present study showed that following buccal SFA greater post-surgery increase in buccal gingival recession must be expected for deep intraosseous defects associated with a buccal dehiscence. The combination of a graft material and a bioactive agent in adjunct to the SFA seemed to limit the postoperative increase in interdental gingival recession.

Source of Founding: This study was entirely supported by the Research Centre for the Study of Periodontal and Peri-Implant Diseases, University of Ferrara. Prof. Trombelli has received research grants and honoraria from Geistlich Pharma (Wolhusen, Switzerland), Institute Straumann (Basel, Switzerland), and OSTEOHEALTH (Shirley, New York). Dr. Rasperini has received research grants and honoraria from Geistlich Pharma and Institute Straumann. Dr. Tomasi has received honoraria from Geistlich Pharma and Institute Straumann. Dr.Minenna has received honoraria from Geistlich Pharma. The other authors report no conflicts of interest related to this study.

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TABLES

Table 1. Patient-related and site-specific characteristics as well as patient distribution according to treatment modality.

Patient-related characteristics	**age (years)** (mean ± SD)	51.2 ± 10.5
	gender males (n) females (n)	 48 18
	smoking status non-smokers (n) current smokers (n)	 50 16
Site-specific characteristics	**gingival biotype** thin (n) thick (n)	 20 54
	dental arch maxillary (n) mandibular (n)	 41 33
	tooth type incisors (n) canines (n) premolars (n) molars (n)	 19 19 23 13
	IBD (mm) (mean ± SD; range min-max)	 5.8 ± 2.7 (1-14)
	CEJ-BD (mm) (mean ± SD; range min-max)	 10.1 ± 3.0 (4-20)
	iCEJ-BC (mm) (mean ± SD; range min-max)	 4.3 ± 1.6 (1.1-9.0)
	bCEJ-BC (mm) (mean ± SD; range min-max)	 5.0 ± 2.5 (0.9-12)
	defect configuration mainly 1 wall (n) combined 1-2 walls (n) mainly 2 wall (n) combined 2-3 walls (n) mainly 3 wall (n)	 17 11 4 21 21
Treatment modality	SFA alone (n) SFA + EMD* (n) SFA + rhPDGF-BB + β-TCP† (n) SFA + EMD* + DBBM‡ (n) SFA + HA§ + resorbable membrane‖ (n)	24 10 13 20 7

* Emdogain® gel; Straumann, Basel, Switzerland
† GEM 21S®; Osteohealth Company, Shirley, NY, US
‡ Bio-Oss® spongiosa granules 0.25-1.0 mm, Geistlich Pharma, AG, Wolhusen, Switzerland
§ Bio-Oss® spongiosa granules 0.25-1.0 mm, Geistlich Pharma, AG, Wolhusen, Switzerland; or Biostite, GABA Vebas, S. Giuliano Milanese, Milan, Italy
‖ Bio-Gide, Geistlich Pharma, AG, Wolhusen, Switzerland; or Paroguide, GABA Vebas, S. Giuliano Milanese, Milan, Italy

Table 2. Baseline and 6-month probing parameters.

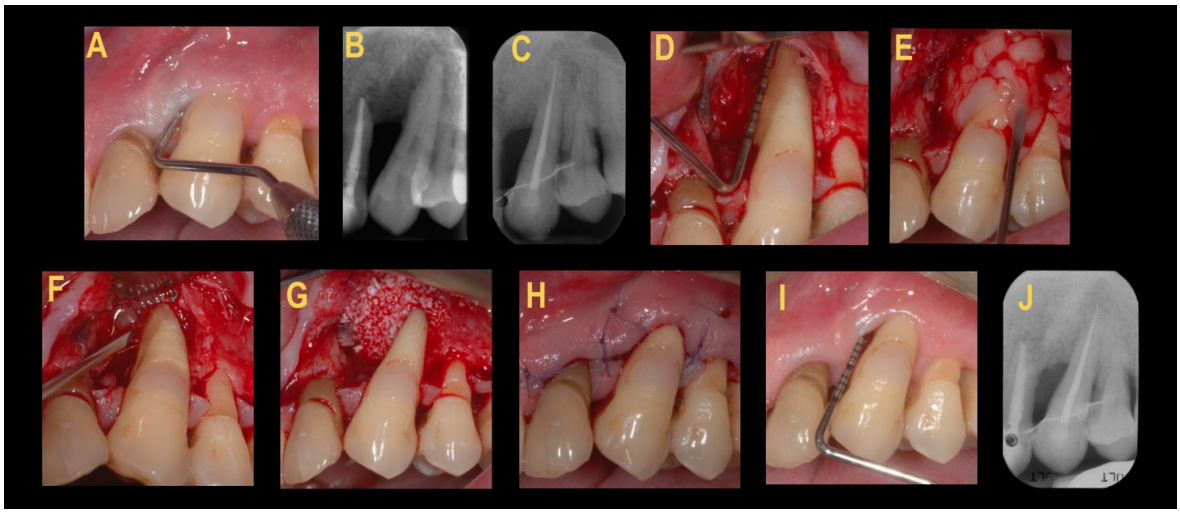
	pre-surgery	6 months	p value	6-month change (pre-surgery - 6 months)
PD (mm) (mean ± SD; range min-max)	8.3 ± 2.1 (5 - 15)	3.8 ± 1.2 (2 - 8)	< 0.001	4.5 ± 2.0 (1 - 11)
CAL (mm) (mean ± SD; range min-max)	9.9 ± 2.4 (6 - 17)	6.2 ± 2.0 (2 - 11)	< 0.001	3.7 ± 0.2 (0 - 10)
bREC (mm) (mean ± SD; range min-max)	1.3 ± 1.3 (0 - 5)	1.8 ± 1.4 (0 - 6)	< 0.001	-0.6 ± 0.7 (-2 - 1)
iREC (mm) (mean ± SD; range min-max)	1.6 ± 1.6 (0 - 8)	2.5 ± 1.6 (0 - 6)	< 0.001	-0.9 ± 1.1 (-3 - 3)
bKT (mm) (mean ± SD; range min-max)	4.2 ± 1.7 (0 - 9)	4.0 ± 1.9 (0 - 10)	0.013	0.2 ±1.2 (-5 - 2.1)
iKT (mm) (mean ± SD; range min-max)	7.8 ± 2.1 (2 - 13)	6.8 ± 2.0 (2 - 11)	< 0.001	0.9 ±1.6 (-3 - 6)

Table 3. Multivariate multilevel model on changes in buccal and interdental recession (bREC and iREC, respectively).

		empty model	standard error	final model	standard error	significance
	Fixed Part iREC					
	Intercept	-0.83	0.14	-1.41	0.21	
	pre-surgery PD			-0.20	0.05	*p<0.001*
	Treatment modality (reference: SFA alone)					
	SFA+HA+GTR			0.58	0.41	*p=0.16*
	SFA+EMD			0.12	0.35	*p=0.72*
	SFA+EMD+DBBM			1.13	0.30	*p<0.001*
	SFA+rhPDGF-BB+β-TCP			1.10	0.33	*p<0.001*
	Fixed Part bREC	empty model	standard error	final model	standard error	significance
	Intercept	-0.59	0.08	-0.58	0.07	
	pre-surgery PD			-0.10	0.03	*p<0.001*
	bCEJBC			-0.13	0.03	*p<0.001*
	Random Part	empty model	standard error	final model	standard error	correlation cofficient
Patient level	Variance iREC	0.36	0.36	0.44	0.21	
	Covariance iREC/bREC	-0.15	0.18	0.00	0.00	0.00
	Variance bREC	0.07	0.15	0.00	0.00	
Site level	Variance iREC	0.92	0.36	0.49	0.19	
	Covariance iREC/bREC	0.45	0.20	0.20	0.07	0.48
	Variance bREC	0.43	0.16	0.36	0.06	
	2*loglikelihood:	371.97		325.53		

FIGURES

Figure 1. Treatment of a periodontal intraosseous defect according to the principles of a buccal Single Flap Approach (Trombelli et al. 2007, 2009) in combination with enamel matrix derivative and a xenograft.



a, **b**. Periodontal intraosseous defect associated with a deep pocket probing depth. **c**. Clinical aspect at the completion of open flap debridement. A buccal envelope flap without vertical releasing incisions has been performed and raised only on the buccal side. The oral portion of the interdental supracrestal soft tissues has been left undetached. **d**. Root conditioning with ethylene diamine tetraacetic acid. **e**. Application of enamel matrix derivative. **f**. The intraosseous component of the defect has been filled with deproteinized bovine bone mineral. **g**. Clinical aspect immediately after suturing. Note a horizontal internal mattress suture at the base of the papilla and a second vertical internal mattress suture between the most coronal portion of the flap and the most coronal portion of the oral papilla. **h**. Clinical aspect at 6 months following surgery. **i**. Periapical radiograph as taken 6 months following surgery.

Figure 2. 6-month increase in buccal gingival recession (bREC) following SFA as predicted on the basis of pre-surgery probing depth (PD) and buccal osseous dehiscence (bCEJ-BC).

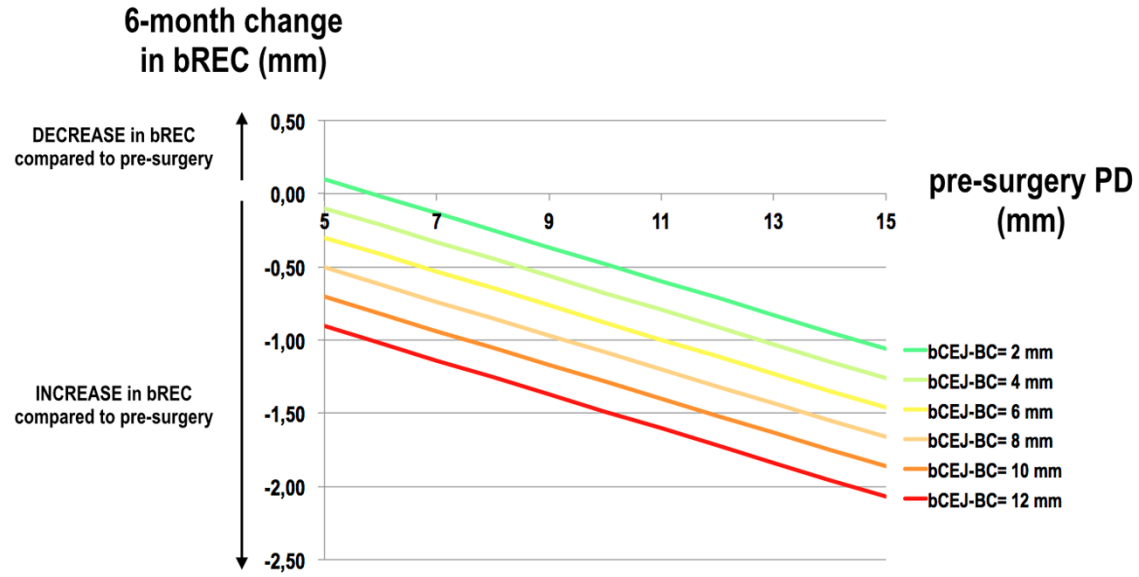
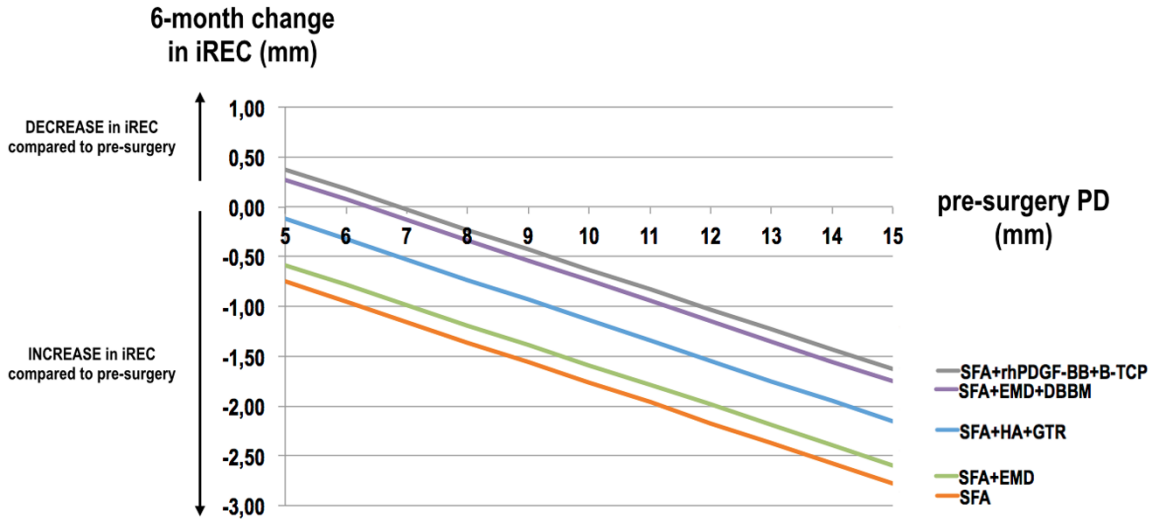


Figure 3. 6-month increase in interdental gingival recession (iREC) following SFA as predicted on the basis of pre-surgery probing depth (PD) and treatment modality.



CHAPTER 6

Treatment of an intraosseous periodontal defect associated with a *cemental tear* with the *Single Flap Approach*

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Dental Cadmos (2013) **81**, 365-373 (article in italian)

Abstract

Background: The Cemental Tear (CT) is an unfrequent root fracture characterized by the separation of a cementum fragment from the underlying root surface along the cemento-dentinal interface. CT can affect tooth prognosis leading to rapid and progressive attachment loss. The aim of the present study is to illustrate the treatment of an intraosseous periodontal defect associated with a CT with a minimally invasive surgical technique (Single Flap Approach, SFA) in combination with reconstructive devices.

Materials and Methods: A severe and localized periodontal attachment loss associated with bleeding and suppuration upon probing was identified at tooth 1.1. Radiographic examination revealed the presence of a CT on the mesial aspect of the root surface. A buccal SFA was performed, the CT was removed and the defect was debrided with mechanical and manual instruments. Then, the defect was filled with deproteinized bovine bone mineral and covered with a resorbable collagen membrane.

Results: At 6 months following surgery, a clinical attachment gain of 10 mm and 7 mm and a probing depth reduction of 12 mm and 8 mm were observed at the mesio-buccal and buccal aspect, respectively, of 1.1. Radiographic examination confirmed the absence of the CT.

Conclusions: The present case report indicates that buccal SFA in combination with a HA-based biomaterial and resorbable membrane may be an effective strategy for the treatment of deep intraosseous defects associated with CT.

INTRODUCTION

A Cemental Tear (CT) is a specific type of root surface fracture infrequently seen in clinical dental practice. It is defined as a complete or incomplete separation of cementum within the root surface along the cemento-dentinal interface (Leknes et al. 1996, Tulkki et al. 2006, Tai et al. 2007, Chou et al. 2004, Ishikawa et al. 1996, Lin et al. 2010, Muller 1990). The mechanism by which CT develop is currently not fully clear, but several etiologic factors have been reported: among these age, trauma, occlusal overloading and previous periodontal treatment (Ishikawa et al. 1996, Lin et al. 2010, Muller 1990, Moskow 1969). An additional factor that may predispose to CT include an imperfect attachment of the cementum to the underlying dentin, condition that predisposes these tissues to separate under normal occlusal loading (Tulkki et al. 2006, Dastmalchi et al. 1990, Yamamoto et al. 1990, Arola et al. 2005, Lyons et al. 2005).

The presence of a CT may indirectly affect the tooth prognosis. CTs are categorized as localized tooth-related factors that modify or predispose to plaque-induced gingival diseases and/or periodontitis (Armitage 1999, Blieden 1999). In this context, the periodontal involvement associated with CTs typically presents as localized attachment loss due to asymptomatic periodontal pocketing of sudden onset and rapid progression, accompanied by radiographic evidence of concomitant alveolar bone loss. If undiagnosed or left untreated, this condition may lead to a periodontal breakdown and, in particular, to tooth loss.

When a CT is solely an occasional finding with no accompanying signs or symptoms, intervention is not necessary but only a periodic monitoring can be sufficient (Ishikawa et al. 1996). On the other hand, different strategies for the treatment of periodontal defects associated with CT have been proposed: from scaling and root planning (Ishikawa et al. 1996) to open flap debridement alone or in association with regenerative procedures (Chou et al. 2004, Ishikawa et al. 1996, Harrel et al. 2000). A common feature of all these treatments is the removal of the detached root fragment, responsible of the clinical attachment loss and bone loss (Chou et al. 2004). Among regenerative treatments of periodontal defects associated with CT, Harrel proposed a minimally invasive surgical approach characterized by the elevation of a double flap –on the vestibular and oral side- to access the periodontal defect and to regenerate the intrabony component. In this context, after surgical debridement, a graft material and a resorbable membrane were used to fill the intrabony defect obtaining a complete resolution of the defect 6-months after surgery

(Harrel et al. 2000). According to some data reported in literature, furthermore, the long-term stability after periodontal surgical treatment of a defect associated with a CT is possible. In particular, Chou et al. illustrated the 7-year follow-up of a periodontal intraosseous defect associated with CT treated with only open flap debridement and root instrumentation (Chou et al. 2004).

The *Single Flap Approach* (SFA) is a minimally invasive surgical approach specifically design for the regenerative treatment of periodontal intraosseous defects. The basic principle behind the SFA is the elevation of a limited mucoperiosteal flap to allow surgical access from either the buccal or oral aspect only, depending on the main buccal or oral extension of the lesion, and leaving the interproximal supracrestal gingival tissues intact (Trombelli et al. 2007, 2008, 2009, 2010, 2012). Considering the site-specificity of the periodontal lesion associated to a CT, it could be reasonable to consider the SFA an adequate surgical approach, able to properly access these type of lesions and remove the CT.

The present case report illustrate the surgical treatment of a deep periodontal intraosseous defect associated with a CT. The surgical access for defect debridement and CT removal was performed in accordance with the SFA principles. After surgical debridement, the reconstructive treatment of the defect was performed using idroxapatite-based material and a resorbable membrane.

MATERIALS & METHODS

Case Description

A Caucasian, 75-year-old male patient was referred to the Research Centre for the Study of Periodontal and Peri-Implant Diseases of the University of Ferrara by his general dentist for the suspected fracture of teeth 1.1. The patient was a heavy smoker (15 sig/day) and was affected by a chronic renal insufficiency. His chief complaint was gingival swelling of the maxillary anterior region and pain coming from the same area. The patient experienced a similar event the previous year with a complete and spontaneous remission. No dental treatments during the previous 12 months were reported; moreover, the patient denied any history of trauma involving the maxillary anterior region and, in particular, tooth 11.

Dental examination revealed a complete edentulism of the maxillary premolar-molar areas and of the mandibular molar areas, restored with a removable prosthesis that the patient rarely used. Occlusal examination did not reveal any occlusal discrepancies or overloading.

A diagnosis of moderate chronic periodontitis was expressed after periodontal examination and charting. Periodontal examination revealed probing depths ≥ 5mm on tooth 1.1 and generalized 1 to 3 mm probing depths throughout the remaining dentition. The bleeding on probing index (BoP%) of the patient was 56%.

The clinical attachment level (CAL) of 1.1 was 16 mm, 12mm and 10 mm at the mesio-vestibular, vestibular and mesio-palatal aspects, respectively. The corresponding probing probing depth (PPD) parameters were 14 mm, 11mm and 9 mm (Fig.1). These sites were positive to bleeding upon probing and suppuration upon probing. A fistula was noted on the buccal gingiva, 6 mm apically from the gingival margin (Fig.2). The tooth presented a moderate mobility (Miller Class I) and resulted positive at the vitality test.

The radiographic evaluation showed a radio-transparent area in the alveolar bone, on the mesial aspect of tooth 1.1. This lesion extended 8 mm in corono-apical direction and 3 mm in mesio-distal direction. The radiograph also indicated a 5 mm long prickle-like radiopaque structure on the mesial aspect of tooth 1.1 (Fig. 3). This lesion was completely separated from the root structured.

All the clinical procedures described in the present report have been performed in full accordance with the Declaration of Helsinki and the Good Clinical Practice (GCPs) guidelines. The patient signed a written informed consent before undergoing clinical procedures.

Non-surgical treatment

The patient underwent a full-mouth session of scaling and root planning with ultrasonic (Piezosteril 5; Castellini S.p.A., Castel Maggiore, Bologna, Italy) and manual instruments (Hirschfeld file # 9/10 Man #6 anterior; Hu-Friedy, Milano, Italy). At teeth 1.1, a selective root planning was performed under local anesthesia using manual instruments with the aim to remove the CT and regulate the radicular surface. At the end of this session, oral hygiene instructions were provided (use of a soft-bristle toothbrush, modified Bass' technique).

At re-evaluation visit, performed 3 weeks after initial therapy, no significant changes at teeth 1.1were observed. PPD values remained unchanged and bleeding and suppuration upon probing were still evident. A new radiograph revealed the persistence of the CT lesion. A surgery was programmed with the aim to remove the CT, debride and reconstruct the defect CT-associated.

Surgical treatment

Three days before surgery the patient was placed on amoxicillin + clavulanic acid, 1gr every 12 hours for 6 days. The surgical procedure was performed using 2.5x magnifying loops. The site of surgery was anesthetized using mepivacaine-epinephrine 1:100,000 and transcrevicular probing (bone sounding) was performed to determine the characteristics of the bony defect that had a prevalent extension on the vestibular and interproximal side. The surgical access was performed by the elevation of a buccal mucoperiosteal flap, from the distal aspect of 1.2 to the distal aspect of 2.1, according to previously detailed principles (Trombelli 2007, 2008, 2009). In particular, an intra-sulcular incision was made following the gingival margin of the teeth included in the surgical area and an oblique butt-joint incision was made at the level of the interdental papilla overlying the intraosseous defect. A microsurgical periosteal elevator (P-TROM periosteal elevator, Hu-Friedy, Milan, Italy) was used to raise a flap on the buccal side only, leaving the oral portion of the interdental supracrestal soft tissues undetached.

Following initial debridement, performed with manual (Hirschfeld file # 9/10 Man #6 anterior; Hu-Friedy, Milano, Italy) and ultrasonic instruments (Piezosteril 5; Castellini S.p.A., Castel Maggiore, Bologna, Italy), a partially detached peace of tooth fracture was noted mesial aspect of tooth 1.1 (Fig. 4). This hard tissue fragment, the CT, was removed intact from the root surface with the use of a curette. The examination of the mesial aspect of the root surface of tooth 1.1, reveled a cervical enamel projection a cervical enamel projection, a radicular aberration characterized from the ectopic development of enamel apical to the CEJ (Fig. 5). Part of the cervical enamel projection was located under the CT fragment. Following removal of the CT, the root surface was planned smooth with specific high-speed burs (Perio Set Intensiv; Pearson, Sylmar, Los Angeles, CA, USA) (Fig. 6).

At the completion of the intra-surgical debridement, the intraosseous defect CT-associated was examined: the defect was a 2-3 wall defect, with a prevalent extension on the interproximal and vestibular side. The intrabony component of the defect was 13 mm deep and the supra-crestal component was 5 mm deep. The defect was filled with deprotenized

bovine bone mineral (Bio-Oss; Geistlich Biomaterials, Switzerland) (Fig. 7a) and fitted with a resorbable collagen membrane (Bio-Gide, Geistlich Biomaterials, Switzerland) to cover the defect (Fig. 7b). The interproximal portion of the membrane was adapted under the supracrestal soft tissues of the undetached oral papilla.

Wound closure was obtained according to the original suturing technique of the SFA (Trombelli 2007, 2009): a horizontal internal mattress suture was placed between the buccal flap and the base of the attached oral papilla to ensure the repositioning of the buccal flap. A wound closure was achieved by means of a second internal mattress suture that was placed between the most coronal portion of the flap and the most coronal portion of the oral papilla (Fig. 8). Finally, an occlusal adjustment of the tooth 1.1 was performed in order to minimize the occlusal overload in the post-operative period.

Post-surgery procedures

Sutures were removed 2 weeks after surgery. The patient was asked to abstain from mechanical oral

hygiene procedures in the surgical area for 4 weeks. A 0.12% chlorhexidine mouthrinse (10 mL twice daily for 6 weeks) was used to support local plaque control. The patient was enrolled in a monthly recall program for 3 months and was reviewed according to personal needs thereafter. Each session included reinforcement of oral hygiene procedures and supragingival plaque removal. Subgingival scaling was performed following completion of the study at 6 months post-surgery.

RESULTS

At the re-evaluation visit, performed 6-months after surgery, the element 1.1 resulted positive to the vitality test and no clinically detectable mobility was noted. Clinical evaluation revealed that the fistula was no longer present.

Periodontal examination revealed a CAL of 6mm and 5 mm at the mesio-vestibular and vestibular aspects of 1.1, respectively. All aspects of 1.1 presented PPD within 3mm; in particular PPD of 2mm and 3mm were registered at the mesio-vestibular and vestibular aspects, respectively (Fig. 9a,b). Both sites resulted negative to bleeding and suppuration on probing. PPD reduction was paralled by an increase in gingival recession of 1 mm on

the vestibular aspect and 2 mm on the mesio-vestibular aspect. A periapical radiograph revealed the complete fill of the defect and no evidence of CT was find (Fig. 10).

DISCUSSION

The present case report illustrated the treatment of a deep periodontal intraosseous defect associated with a CT, a quite infrequent clinical condition characterized by the separation of cementum within the root surface along the cemento-dentinal interface (Leknes et al. 1996, Tulkki et al. 2006, Tai et al. 2007, Chou et al. 2004, Ishikawa et al. 1996, Lin et al. 2010, Muller 1990). A minimally invasive surgical approach (i.e. Single Flap Approach, SFA) allowed the CT removal and, at the same time, the regenerative treatment of the intraosseous defect -caused by the CT- with a combination of regenerative devices (i.e. idroxiapatite-based biomaterial) and a resorbable membrane. The 6-months clinical and radiographic examination showed a complete resolution of the pre-operatory signs of acute infection as well as the complete reconstruction of the intrabony component of the defect. No evidence of CT was identified.

In the present case report, the CT, partially separated from the root surface, was observed in a tooth under evident occlusal trauma, in a 75-yaer old patient. All these observations are consistent with data reported from literature. Several reports showed that CT frequently occurred in man with age >50 years-old (Lin et al. 2011) and can be caused by occlusal trauma (Ishikawa et al. 1996, Lin et al. 2010, Muller 1990, Moskow 1969) or other type of injuries (Tulkki et al. 2006, Ishikawa et al. 1996) and weakness of the cement-dentinal interface (Leknes et al. 1996, Tulkki et al. 2006, Tai et al. 2007, Chou et al. 2004, Ishikawa et al. 1996, Stewart et al. 2006). Moreover, other predisposing factors seems justify the presence of the CT in this specific case. First, the presence of a cervical enamel projection, a radicular aberration characterized from the ectopic development of enamel apical to the CEJ, may have determined an incomplete adhesion of the cementum to the underlying radicular dentin. This condition may have favored the partial separation of the CT from the root surface under occlusal load. Moreover, we can also hypothesize an overload of the anterior elements due to a complete edentulism of the posterior elements.

In the present case report, the periapical radiograph revealed a radiopaque fragment that oriented the final diagnosis of CT. The radiographic exam also revealed the morphology of the intrabony defect associated with the CT and, in particular, its corono-apical and mesio-

distal extension. When considering the low incidence of the CT and the poor specificity of the clinical symptoms, the radiographic exam seems to be the instrument of choice in the diagnosis of CT and in the definition of the periodontal lesion CT-associated. Furthermore, the radiographic exam can exclude the presence of root fractures or endodontic lesions in the determinism of the periodontal lesion (Chou et al. 2004, Stewart et al. 2006).

The continuity between the CT and the periodontal intraosseous defect - first observed radiographically and then confirmed with the surgical access- together with the complete absence of other periodontal lesions, suggest that the CT and the cervical enamel projection may represent the local risk factors in the development and progression of the intraosseous defect (Armitage 1999, Blieden 1999). In fact, the presence of a cervical enamel projection may impair the functional attachment of the periodontal ligament: this condition represents a *locus minori resistentiae* for the progression of the sub-gingival biofilm that can lead to the formation of a periodontal intraosseous defect similar to the one observed in this case report. The surgical access to the periodontal intraoseous defect was performed in accordance with the SFA, a minimally invasive technique, specifically design for the treatment of intraosseous defect (Trombelli et al. 2007, 2008, 2009, 2010, 2012, Farina et al. 2013). This surgical technique, elaborated for periodontal reconstructive procedures, is based on the minimization of the surgical trauma through the elevation of a single flap (only on the vestibular or oral side), leaving undetached the opposite side. The SFA may pose several clinical advantages. First, it may facilitate flap repositioning and suturing; the flap can easily be stabilized to the undetached papilla, thus optimizing wound closure for primary intention healing (Trombelli et al. 2010, Farina et al. 2013). Second, by limiting the surgical trauma on the vascular supply of the interproximal supracrestal soft tissues due to a limited flap elevation, a faster wound-healing process, particularly at the level of the incision line, is promoted. Wound stabilization and preservation of an intact interdental papilla may also minimize the post-surgery shrinkage of gingival tissues and, therefore, limit the esthetic impairment of the patient. To date, several studies reported considerable clinical improvements when the SFA was used as a stand-alone protocol (Trombelli et al. 2010, 2012) or in combination with regenerative technologies (Trombelli et al. 2007, 2008, 2009, 2010) for the treatment of deep periodontal intraosseous defects.

After CT removal and surgical debridement of the intraosseous defect and root surface, the defecet was treatred with deprotenized bovine bone mineral and a resorbable collagen membrane. At the re-evaluation visit, 6-months after surgery, a CAL gain of 10 mm and 7 mm and a PPD reduction of 12 mm and 8 mm was identified at the mesio-vestibular and

vestibular aspect of tooth 1.1, respectively. A recent sistematyc review showed that, the use of membranes in the surgical treatment of periodontal intraosseous defecs hesitate in additional clinical attachment gain of 1.22 mm at 12 months, when compared with the open flap debridement (Needleman et al. 2005). Furthermore, the adjunctive effect of a biomaterial seems to increase the GTR efficacy in terms of PPD reduction and post-surgical REC increase, compare to GTR alone (Needleman et al. 2005). Consistently with our results, previous studies evaluated the association of hidroxiapatite-based biomaterial and resorbable membrane with SFA in the regenerative treatment of deep intraosseous defects reporting substantial CAL gain and PPD reduction (Trombelli et al 2010).

CONCLUSIONS

The CT is an unfrequent root fracture characterized by the separation of a cementum fragment from the underlying root surface along the cemento-dentinal interface. These structural alterations are often associated with alveolar bone loss and with periodontal intraosseous defects. According to the results of the present case report, the SFA may represent an appropriate surgical approach to: i) access a periodontal intraosseous defect associated with a CT; ii) have an adequate surgical access to remove the CT; iii) treat the intraosseous defect with a combination of hidroxiapatite-based biomaterial and a resorbable collagen membrane.

Source of Founding: This study was entirely supported by the Research Centre for the Study of Periodontal and Peri-Implant Diseases, University of Ferrara.

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FIGURES

Fig. 1: Clinical attachment level (CAL) and pocket probing depth (PPD) at the vestibular, mesio-vestibular and mesio-palatal aspects of element 1.1. (**A**) Vestibular aspect: CAL=12mm, PPD=11mm; (**B**) mesio-vestibular aspect: CAL=16mm, PPD=14mm; (**C**) mesio-palatal aspect: CAL=10mm, PPD=9mm.

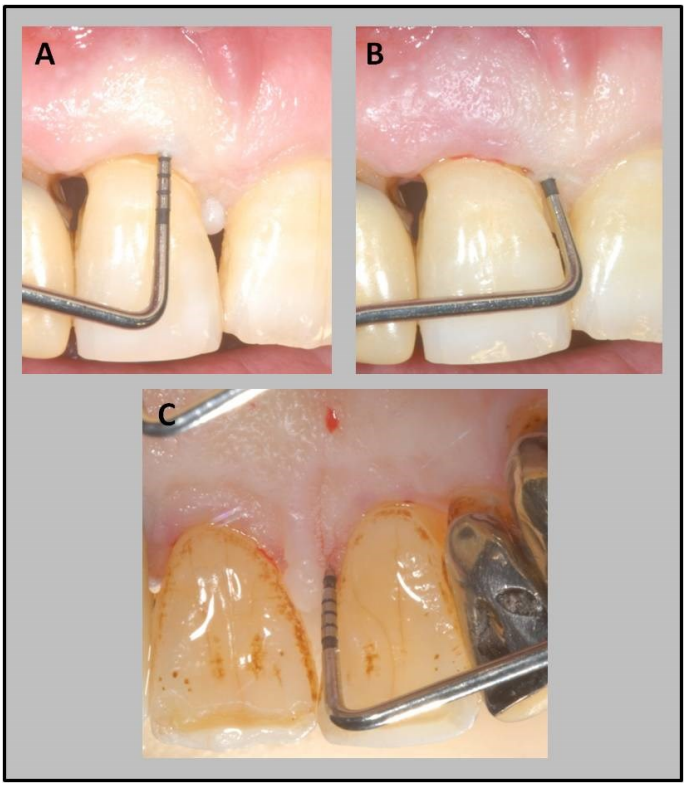


Fig. 2: Fistula on the buccal gingiva, located 6 mm apically from the gingival margin.

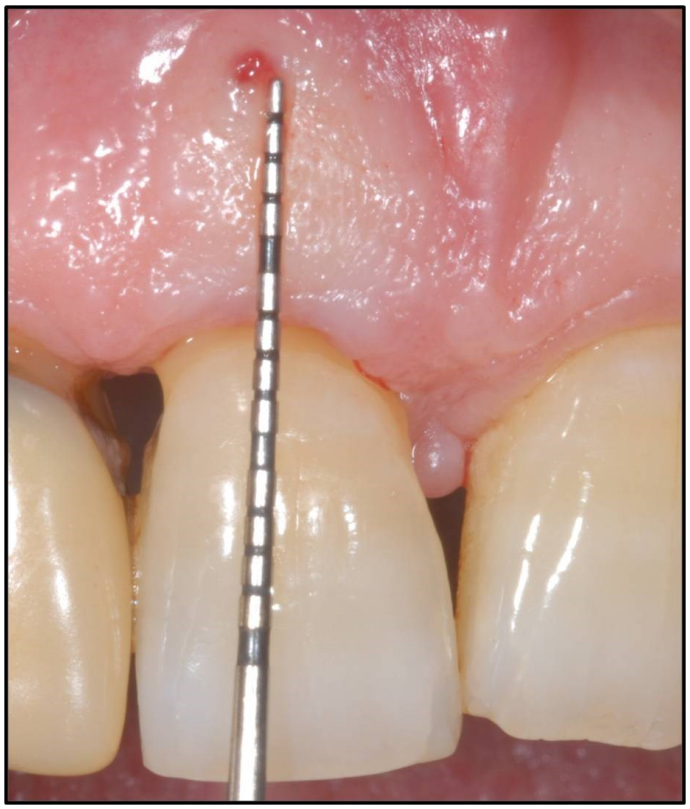


Fig. 3: Pre-operatory radiograph showing a radio-transparent area on the radicular mesial aspect of tooth 1.1. Inside the radio-transparent area, a radio opaque fragment representing the CT is visible.

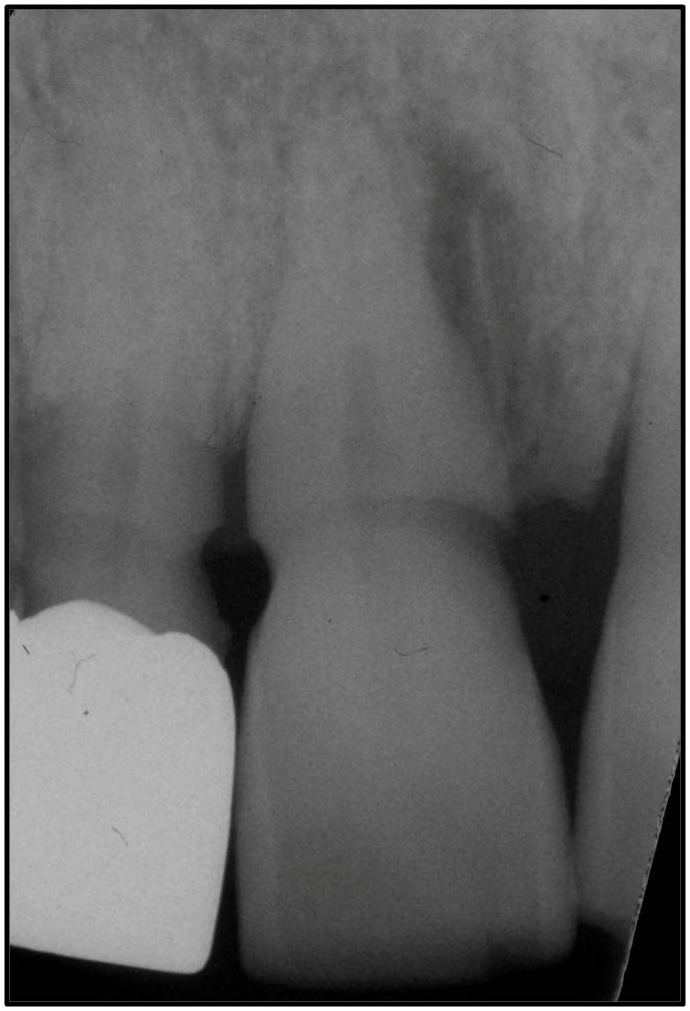


Fig. 4: Identification of the CT on the mesial aspect of the root surface of 1.1.

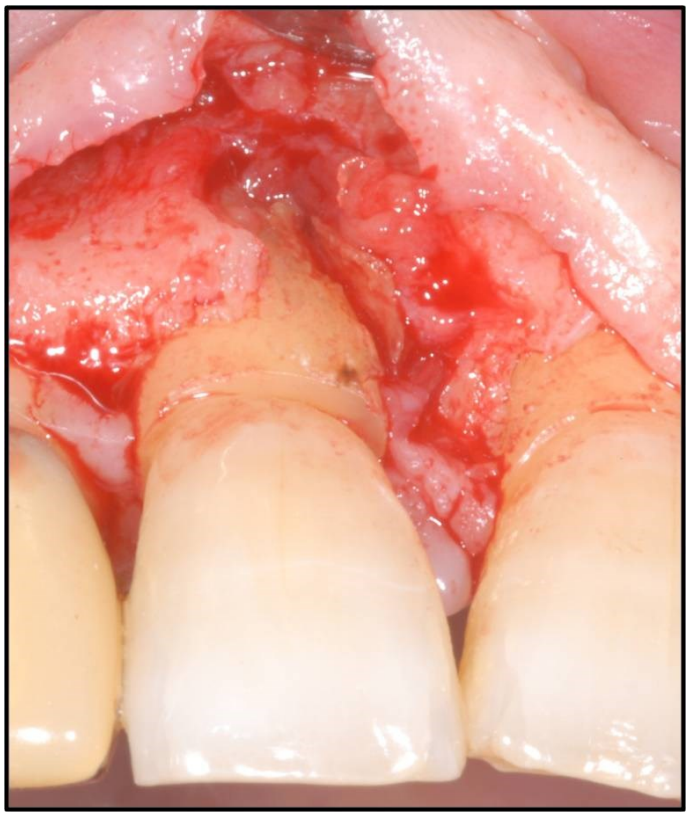


Fig. 5: Cervical enamel projection located on the mesial radicular surface of tooth 1.1, This radicular aberration was located under the CT fragment.

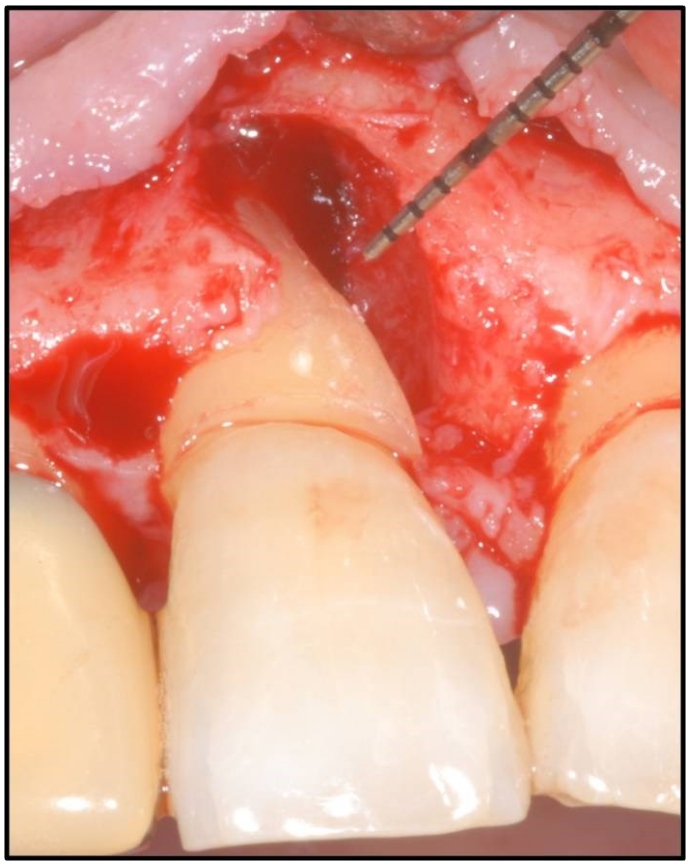


Fig. 6: Elimination of the cervical enamel projection using a dedicated set of burs.

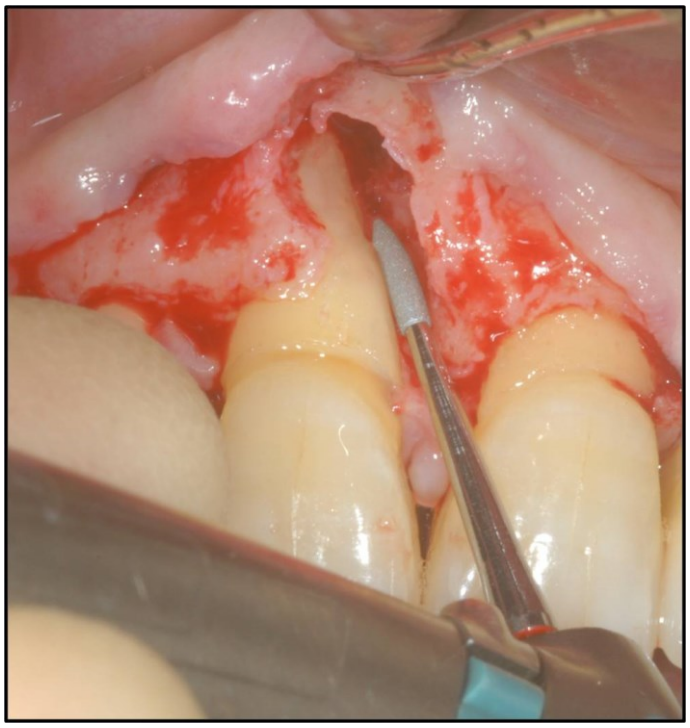


Fig. 7: (**A**) The intraosseous component of the defect is filled with an HA-based biomaterial (Bioss, Geistlich Biomaterials, Switzerland). (**B**) A resorbable collagen membrane is used to cover the defect.

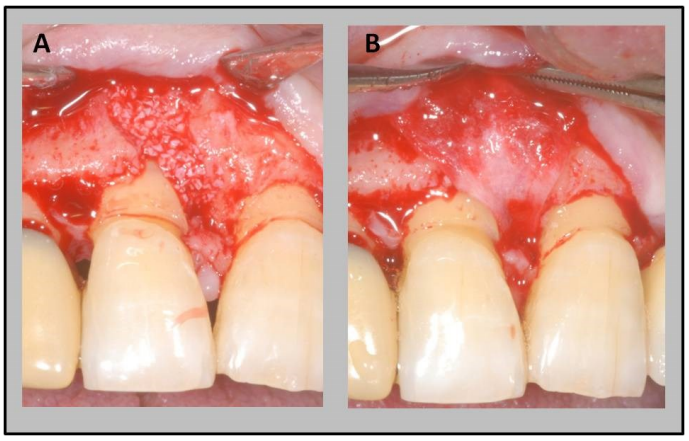


Fig. 8: For wound closure, a horizontal internal mattress suture is placed between the flap and the base of the attached papilla to ensure repositioning of flap. A second internal mattress suture is placed between the most coronal portion of the flap and the most coronal portion of the papilla as needed.

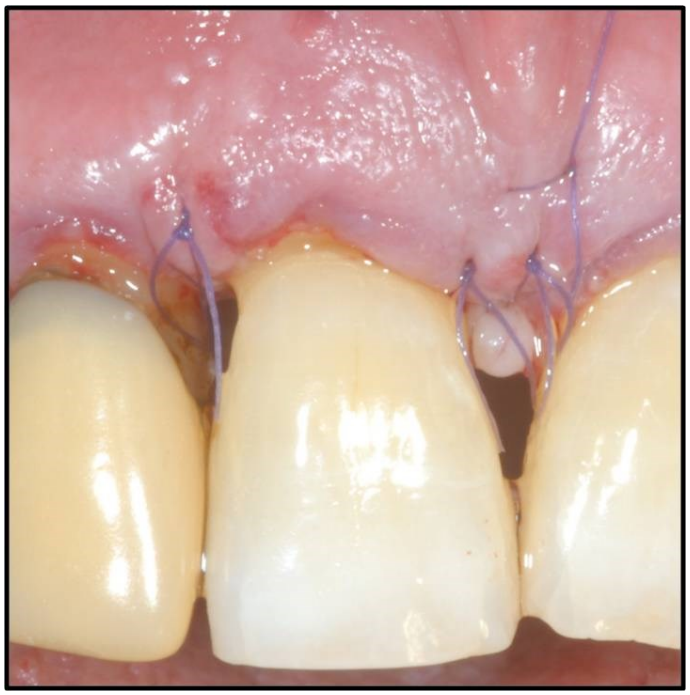


Fig. 9: Clinical attachment level (CAL) and pocket probing depth (PPD) 6-months after surgery. (**A**) Vestibular aspect: CAL=5mm, PPD= 3mm; (**B**) Mesio-vestibular aspect: CAL= 6mm, PPD= 2mm.

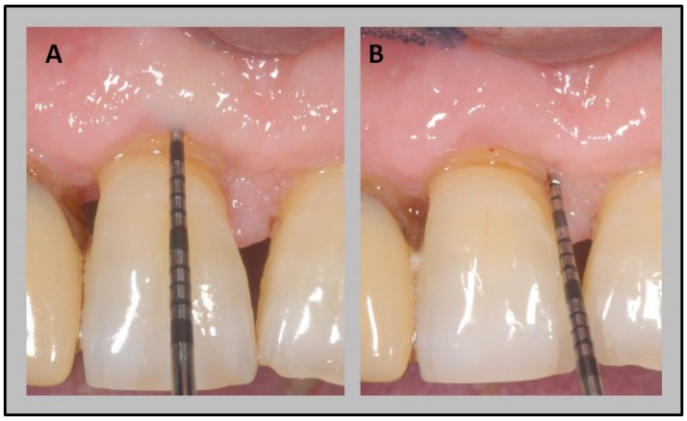
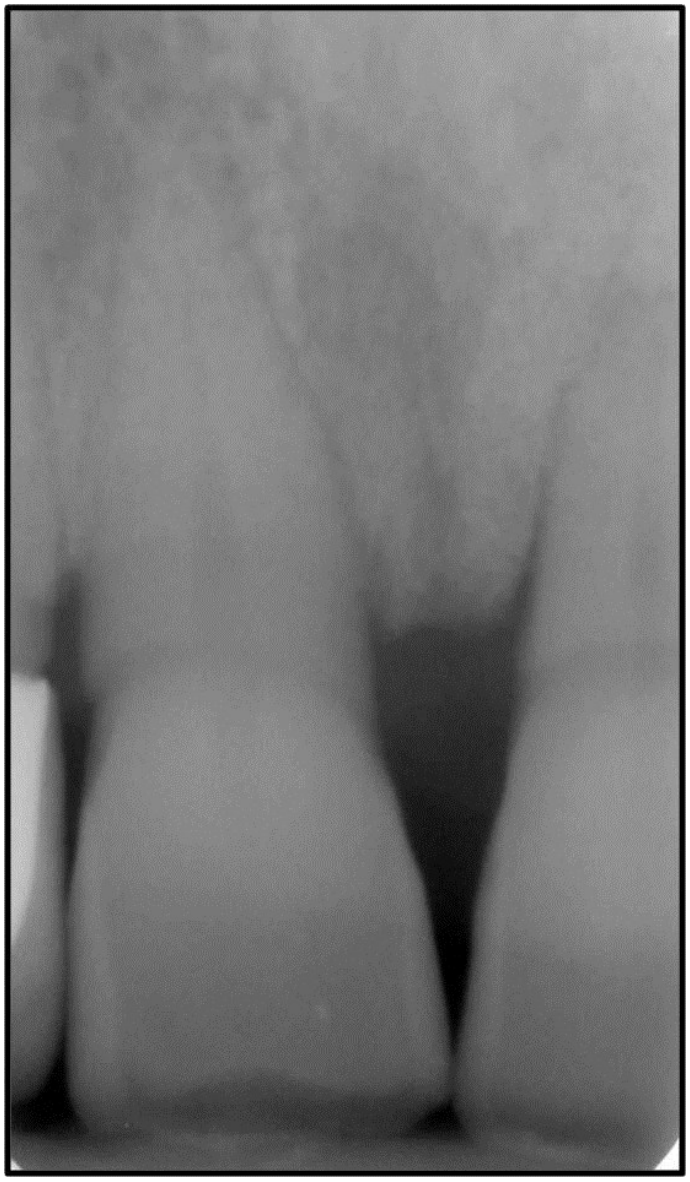


Fig. 10: Radiographic aspect of the treated site at 6 months following surgery. A complete fill of the bony defect is evident.



CHAPTER 7

Single flap approach in combination with different reconstructive technologies in the treatment of an intraosseous defect associated with a buccal dehiscence

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Dental Cadmos (2013) **81**, 299-307 (article in italian)

Abstract

Background: the present study describes the treatment of a intraosseous periodontal defect associated with a buccal dehiscence with (i) an access flap performed according to the principles of
the Single Flap Approach (SFA) and (ii) a combination of β-tricalcium phosphate (β-TCP), recombinant human platelet-derived growth factor (rhPDGF-BB), and a three-dimensional collagen matrix.

Materials & Methods: The defect was accessed with a buccal SFA, the defect extension being prevalent on the buccal side (as assessed by pre-operative bone sounding). Briefly, a full-thickness buccal flap was performed keeping the oral and interdental tissues intact. After defect degranulation
and root debridement, a β-TCP graft was mixed with rhPDGF-BB and placed to fill the intraosseous
component of the defect and to cover the buccal dehiscence. A resorbable collagen matrix was placed at the buccal aspect and fixed with interrupted sutures.

Results: At 8 months following surgery, pocket probing depht showed a reduction from 6 mm to 1 mm at the buccal aspect and from 6 mm to 2 mm at the disto-buccal aspect. Clinical attachment gain was 5 mm at both sites. No reduction in the amount of keratinized tissue was observed, and 100% root coverage was maintained.

Conclusion: The present case report demonstrated that a reconstructive approach based on (i) access
flap performed in accordance with the principles of the SFA and (ii) a combination of β-TCP, rhPDGFBB and a three-dimensional collagen matrix may be clinically effective in the treatment of
intraosseous periodontal defects associated with a buccal dehiscence.

INTRODUCTION

Periodontal intraosseous defects (infrabony defects) have been associated with a higher risk of periodontal progression and eventually tooth loss (Papapanou & Wennstrom 1991). In particular, the deeper the intrabony component of the defect, the greater the possibility to lose the tooth. When approaching an intraosseous defect, the main treatment goal is the reduction of the intrabony component of the defect and, secondly, the correction of the anatomical aberrations of soft tissues (i.e. pocket probing depth).

To date the traditional surgical approach to treat periodontal intraosseous defects is the Open Flap Debridement (OFD). The OFD consists in the surgical access of the defect in order to remove the granulation tissue and debride the root surface. A recent systematic review showed that the treatment of intraosseous defects with OFD results in mean clinical attachment gain of 1.65 mm and pocket probing depth reduction of 2.8 mm, 12 months after surgery (Graziani et al 2012). Nevertheless, histological studies on human biopsies reveal that the regenerative potential of this type of approach seems to be lower (Bowers et al 1989).

The use of adjunctive technologies such as biomaterials, membranes and biological agents in addition to the OFD, may enhance the final clinical outcomes of reconstructive procedures (Trombelli et al. 2002, Needleman et al.2006, Esposito et al.2009) and promote a partial regeneration of periodontal tissues (Nyman 1987, Sculean 2008). Moreover, better clinical outcomes were reported for associations of different reconstructive devices compared to the use of single devices alone (Trombelli e Farina 2008). In particular, the platelet-derived growth factor (PDGF), potent chemotactic agent and mitogen, involved in the processes of ostheogenesis and angiogenesis, is able to improve the reconstructive performance of different biomaterials (Trombelli e Farina 2008). In this context, an histological study reported the regeneration of new alveolar bone, new periodontal ligament and new cementum when recombinant-human platelet-derived growth factor (rhPDGF-BB), was associated with β-tricalcium phosphate and a resorbable collagen membrane in the regenerative treatment of periodontal intraosseous defects (McGuire et al. 2009).

In 2007, the Single Flap Approach (SFA), a minimally invasive surgical procedure designed for the reconstructive therapy of intraosseous defects, was proposed. The basic principle behind the SFA is the unilateral elevation of a limited mucoperiosteal flap to allow surgical access. Interproximal gingival tissues (i.e., the interdental papilla) are kept

intact, allowing for an easy repositioning of the flap in the pre-operatory position with primary wound closure (Trombelli et al. 2007, 2008, 2009,2010, 2012, Trombelli & Farina 2012, Farina et al. 2013). To date, a series of studies have demonstrated the clinical efficacy of the SFA either as a stand-alone protocol (Trombelli et al. 2010, 2012) or in combination with different regenerative technologies, such as bone substitutes and membranes (Trombelli et al. 2007, 2008, 2009, 2010).

The present case report describes the treatment of a periodontal intraosseous defect associated with a buccal deishence with an access flap performed according to the principles of the SFA and a combination of rhPDGF-BB, β-TCP and a three-dimensional collagen matrix.

MATERIALS & METHODS

Case Description

The patient, a 47-year old healthy white male, presented with a complaint of recurrent abscess on the maxillary right canine (Fig.1). His medical history was unremarkable. Periodontal examination revealed a moderate generalized chronic periodontitis. According to the periodontal risk assessment method elaborated from the Research Centre for the Study of Periodontal and Peri-implant diseases, University of Ferrara (Farina et al. 2007, Trombelli et al. 2009), the patient has a middle-elevated risk of progression of periodontitis.

An ulcerative lesion at the gingival margin of tooth 1.3 was evident. Moreover, spontaneous bleeding and supra-gingival plaque were detected. The vestibular and disto-vestibular aspects of tooth 1.3 showed pocket probing depths of 6mm and were both positive at bleeding and suppuration upon probing.

Pre-surgical procedures

The patient underwent four sessions of quadrant scaling and root planning with ultrasonic (Piezo Smart; Mectron s.p.a., Carasco, Genova) and manual instruments (curette mini-five; Hu-Friedy Chicago, Illinois, USA). At the end of each session, oral hygiene instructions were reinforced (modified Bass' technique for tooth brushing, interproximal floss and interproximal brush for anterior and posterior sextants, respectively).

At the re-evaluation visit, performed 4 weeks after the last session of quadrant scaling and root planning, no significant changes at teeth 1.3 were observed: PPDs of 6 mm were still present at vestibular and disto-vestibular aspects. These sites were positive at bleeding and suppuration upon probing (Fig. 3). Transcrevicular probing, performed under local anesthesia, revealed a periodontal intraosseous defect at the distal and disto-vestibular aspects of tooth 1.3. Moreover, a bone dehiscence was detected on the buccal side.

In order to maximize the outcomes of non-surgical therapy and improve the soft-tissue quality in preparation to the surgical phase, a further session of scaling and root planning was performed at teeth 1.3. Moreover, the patient was placed on amoxicillin + clavulanic acid, 1gr every 12 hours for 6 days (Augmentin; GlaxoSmithKline S.p.A., Verona, Italy).

Surgical procedure

Following three days of antibiotic therapy, tooth 1.3 underwent reconstructive periodontal treatment. The surgery was conducted in accordance with the Single Flap Approch principles (Trombelli et al. 2007, 2009): the incision line was performed only at the buccal side, leaving undetached the interproximal and oral tissues (fig. 4). Intrasulcular incisions were performed along the buccal gingival margin of teeth included in the surgical area while butt-joint incisions were performed at the interdental papillae. The mesio-distal extension of the incision was kept limited to the teeth adjacent 1.3. At the distal aspect of the incision line, a releasing incision beyond the muco-gingival line was performed.

A microsurgical periosteal elevator (P-TROM periosteal elevator, Hu-Friedy, Milan, Italy) was used to raise a flap only on the buccal side, leaving the oral portion of the interdental supracrestal soft tissues undetached. Root and defect debridement were performed using hand (curette mini-five e lime di Hirshfeld; Hu-Friedy Chicago, Illinois, USA) and ultrasonic instruments (Piezo Smart; Mectron s.p.a., Carasco, Genova). The 1-2 walls intraosseous defect has an intrabony component of 5 mm and a prevalent extension on the buccal side. A cementum aberration 2 mm long was identified on the root surface, next to the buccal bone dehiscence (Fig. 6). The aberration was revoved with specific high-speed burs (Perio Set Intensiv; Pearson, Sylmar, Los Angeles, CA, USA) (Fig. 7). After surgical debridement, the intraosseous component of the defect and the buccal bone dehiscence were grafted with rhPDGF-BB + β-TCP (GEM 21S®; Osteohealth, Shirley, NY, USA), prepared in accordance with manufacture's instructions (Fig. 8). The defect was fitted with a resorbable collagen membrane (Mucograft®; Geistlich, Wolhusen, Switzerland) to cover the buccal dehiscence and the interproximal areas. Interrupted sutures in Vycril® 6/0

(Johnson & Johnson, New Brunswick, New Jersey, USA) were used for membrane fixation (Fig.9).

Wound closure was obtained according to the original suturing technique of SFA (Trombelli et al. 2007, 2009) with interrupted sutures in Vycril® 6/0. At mesial and distal papilla of 1.3, primary wound closure was obtained with two vertical internal mattress sutures: the first was placed at the base of the interproximal papilla while the second was placed more coronally. At the distal papilla, an additional suture was performed to ensure the primary intention healing. The releasing incision was sutured with interrupted sling sutures (Fig. 10, 11).

At the completion of the surgical procedure, a periapical radiograph was performed (Fig. 12).

Post-surgery procedures

The patient was asked to abstain from mechanical oral hygiene procedures in the surgical area for 4 weeks. A 0.12% chlorhexidine mouthrinse (10 mL twice daily for 6 weeks) was used to support local plaque control. The antibiotic therapy was continued for the three days following surgery. Sutures were removed 2 weeks after surgery.

The patient was enrolled in a monthly recall program for 3 months and was reviewed according to personal needs thereafter. Each session included reinforcement of oral hygiene procedures and supragingival plaque removal. Subgingival scaling was performed following completion of the study at 6 months post-surgery.

RESULTS

Following non-surgical periodontal treatment, all pocket depths were lower than 5 mm and bleeding on probing was lower than 20%. According to the periodontal risk assessment method elaborated from the Research Centre for the Study of Periodontal and Peri-implant diseases, University of Ferrara (Farina et al. 2007, Trombelli et al. 2009), at the end of non-surgical periodontal therapy, patient showed a middle-low risk of progression of periodontitis.

In the post-surgical period, no uneventful events happened. 8-months after surgery a substantial improvement of the soft tissues quality was evident together with the complete

resolution of ulcerations and spontaneous bleeding. The keratinized tissue high was entirely preserved and a complete root coverage (Fig. 13) as well as the soft tissue buccal-lingual width were maintained (Fig.14). Periodontal examination performed 8 months after surgery revealed a CAL gain of 5 mm at both vestibular and disto-vestibular aspects of 1.3. Moreover, PPD of 1mm and 2mm were registered at the vestibular (Fig. 13) and disto-vestibular aspects of 1.3, respectively. Both sites resulted negative to bleeding and suppuration on probing. The 8-months follow-up periapical radiograph showed the integrity of the peri-radicular and crestal lamina dura, expression of periodontal stability (Fig. 15).

DISCUSSION

The present case report describes the reconstructive treatment of a periodontal intraosseous defect associated with a buccal dehiscence, located in an aesthetic area. After multiple session of scaling and root planning, the persistence of pocket probing depths of 6mm associated with bleeding and suppuration upon probing were evident at tooth 1.3. Transcrevicular probing revealed a periodontal intraosseous defect at the disto-vestibular aspect of 1.3 associated with a buccal dehiscence. The peculiarities of the treatment performed were: (i) the surgical technique used to access the periodontal intraosseous defect (*Single Flap Approach*; Trombelli et al. 2007, 2008, 2009, 2010, Trombelli & Farina 2012, Farina et al. 2012), and (ii) the association of different reconstructive devices for the regenerative treatment of hard (combination of PDGF-BB and β-TCP) and soft tissues (resorbable collagen membrane). The morphology of the periodontal lesion described in this case report can be probably attributed to the progression of periodontitis in two sites with different anatomic characteristics. From one hand, the intraosseous periodontal defect located at the disto-vestibular aspects of tooth 1.3 is the result of a vertical bone reabsorption in a thick alveolar crest. On the other hand, the buccal bone dehiscence is the result of a thin buccal alveolar crest bone reabsorption, caused from the cementum aberration. When considering the morphological characteristics of this type of defect, the aims of the reconstructive therapy were (i) to reconstruct the intrabony component of the intraosseous defect in order to improve the tooth prognosis and (ii) to restore the integrity of the buccal alveolar crest and increase the thickness of the above soft tissues.

The surgical access to the defect was performed in accordance with the principles of Single Flap Approach (Trombelli et al. 2007, 2008, 2009, 2010, 2012, Trombelli & Farina 2012, Farina et al. 2012). The SFA may pose several clinical advantages. First, it may facilitate flap repositioning and suturing; the flap can easily be stabilized to the undetached papilla, thus optimizing wound closure for primary intention healing (Trombelli et al. 2010, Farina et al. 2012). Second, by limiting the surgical trauma on the vascular supply of the interproximal supracrestal soft tissues due to a limited flap elevation, a faster wound-healing process, particularly at the level of the incision line, is promoted. Wound stabilization and preservation of an intact interdental papilla may also minimize the post-surgery shrinkage of gingival tissues and, therefore, limit the esthetic impairment of the patient.

In the present case report was used a reconstructive technology based on the combination of rhPDGF-BB, β-TCP and a tridimensional collagen membrane. The non-containing morphology of the treated intraosseous defect was determinant in the selection of a tridimensional scaffold biomaterial to sustain the maturation of the blood clot in a new mineralized tissue. Moreover, the osteogenetic process was implemented by the local administration of rhPDGF-BB, a potent chemotactic of undifferentiated osteoprogenitive cells, able to improve the reconstructive outcomes of scaffold biomaterials (Trombelli & Farina 2008). In addition, a tridimensional collagen membrane was used to maintain an adequate soft tissues thickness at the buccal aspect of 1.3. Recently, tridimensional collagen membranes have been proposed as substitutes of autologous connective tissues in muco-gingival plastic surgery (Sanz 2009, McGuire 2010, Cardaropoli 2012). The resorbable collagen matrix acts as space-maker during the blood clot maturation at the interface between the flap and radicular interface. Preliminary data showed that the use of tridimensional collagen membranes in association with coronally advanced flaps in the treatment of gingival recessions may result in a mean recession reduction of 2.62 mm, 6-months after surgery. Moreover, a root coverage of 88.5% and an increase of keratinized tissue of 1.34 mm (McGuire 2010) were also evident. In a recent study (Sanz 2009) the use of a collagen matrix resulted in a mean keratinized tissue width of 2.5 mm in tooth elements with pre-operatory keratinized tissue < 2mm. At the re-evaluation visit, performed 8-months after surgery, the disto-vestibular and vestibular aspects of 1.3 showed probing depth reductions of 4 mm and 5 mm, respectively. Simultaneously, a clinical attachment gain of 5 mm was observed in both sites. The clinical results are consistent with previous studies on similar surgical approaches. A multicenter randomized

clinical trial reported a mean clinical attachment gain of 3.8 mm after periodontal treatment of intraosseous defects with rhPDGF-BB + β-TCP (Nevins et al. 2005). More recently, deep intraosseous defects were treated with a buccal Single Flap Approach in combination with hydroxyapatite-based biomaterial and a resorbable collagen membrane. The 6-months clinical outcomes revealed a mean clinical attachment gain of 4.8 mm (Trombelli et al. 2009) and 4.7 mm (Trombelli et al. 2010). At the buccal aspect of 1.3, no post-operative gingival recession occurred. A 100% root coverage and an adequate keratinized tissue dimension were maintained. These data are consistent with the existent literature. Recently, an increase in gingival thickness from 0.82 mm to 1.82 mm was reported after treatment of recession defects with a collagen membrane and coronally advanced flap (Cardaropoli et al. 2012). Moreover, when the collagen matrix was used in association with rhPDGF-BB around dental implants in aesthetic areas, an increase in soft tissue thickness was observed 4-months after surgery (Simion 2012). The clinical results obtained with the reconstructive procedure described in this case report are not still supported by an histologic evidence. However, histological studies reported a complete regeneration of the periodontal attachment apparatus after treatment of intraossoeus defects and bone dehiscences with rhPDGF-BB. In this context, 4 over 6 intraosseous defects and 4 over 4 class II furcation defects presented a complete regeneration of the periodontal tissues after treatment (Nevins 2003). Furthermore, a recent histologic study showed the regeneration of cementum, periodontal ligament and alveolar bone after treatment of bone dehiscences with and association of β-TCP, rh-PDGF-BB and a collagen dressing (McGuire 2009). Therefore, on the base of these data, it is possible to speculate that in the present case report a regeneration of the periodontal attachment apparatus occurred.

CONCLUSIONS

The present case report demonstrated that a reconstructive approach based on (i) access flap performed in accordance with the principles of the SFA and (ii) a combination of β-TCP, rhPDGFBB and a three-dimensional collagen matrix may be clinically effective in the treatment of intraosseous periodontal defects associated with a buccal dehiscence. Further investigations are needed to evaluate the adjunctive effect of this procedure compared to the conventional approach (i.e. open flap debridement) and to identify the specific indications in order to maximize the final clinical outcomes.

Source of Founding: This study was entirely supported by the Research Centre for the Study of Periodontal and Peri-Implant Diseases, University of Ferrara.

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FIGURES

Fig. 1: Clinical aspect of the soft tissues around 1.3 at first visit. Spontaneous bleeding and supra-gingival plaque are evident.

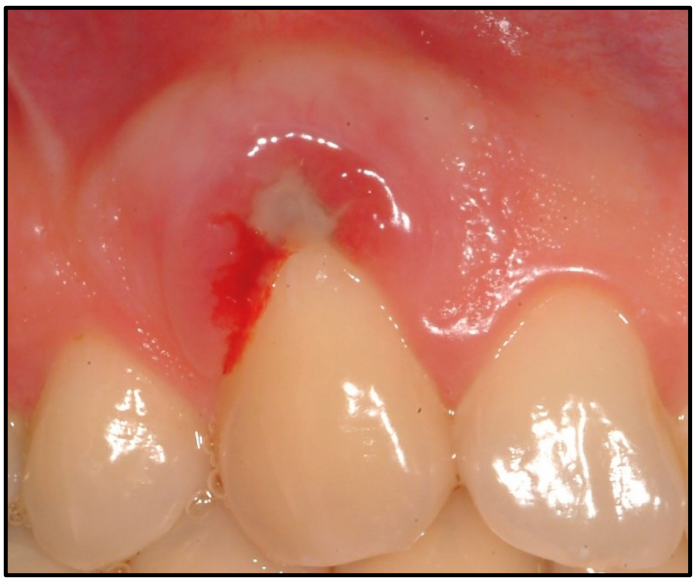


Fig.2: Preoperative radiograph of tooth 1.3.

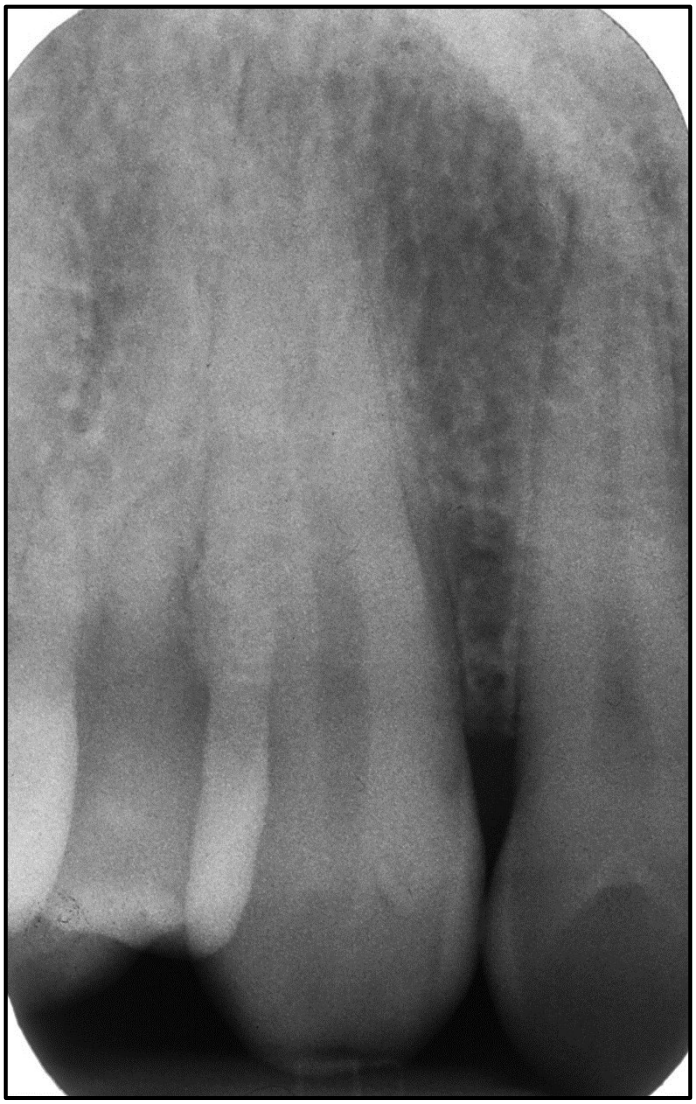


Fig.3: Clinical aspect of tooth 1.3 after non-surgical periodontal therapy. A pocket probing depth (PPD) of 6mm associated with suppuration upon probing is evident.

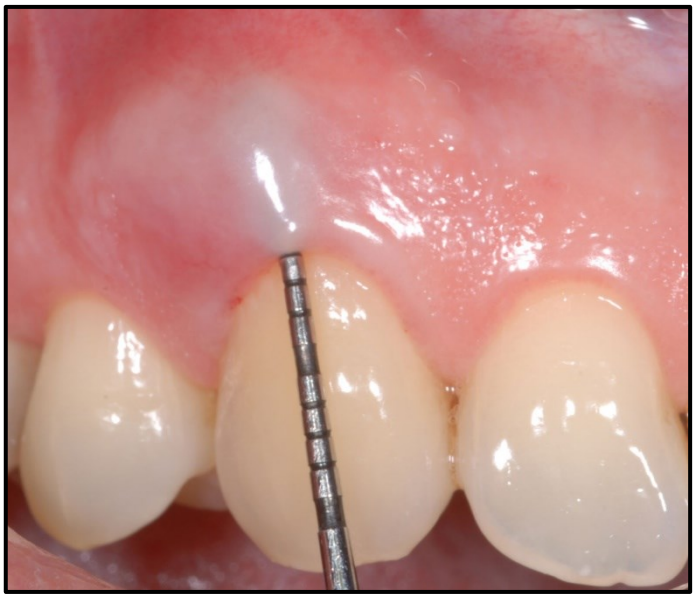


Fig. 4: Buccal Single Flap Approach: the incision line is performed only at the buccal side, leaving undetached the interproximal and oral tissues.

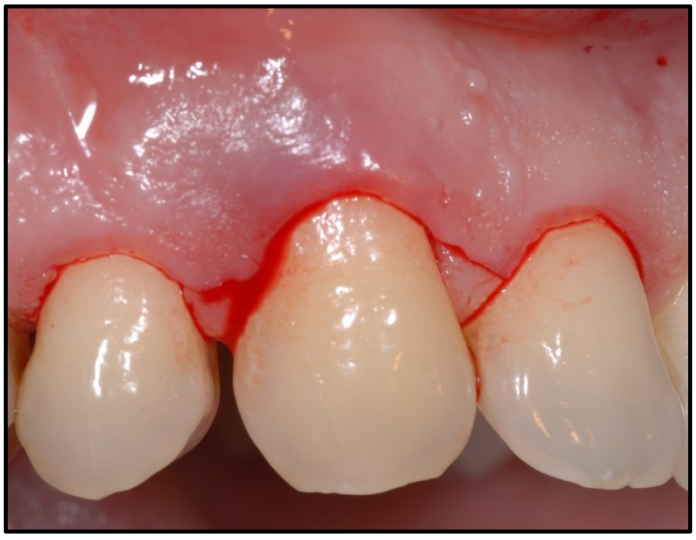


Fig. 5: Elevation of the buccal Single Flap Approach. the 1-2 walls intraosseous defect has an intrabony component of 5 mm and a prevalent extension on the buccal side.

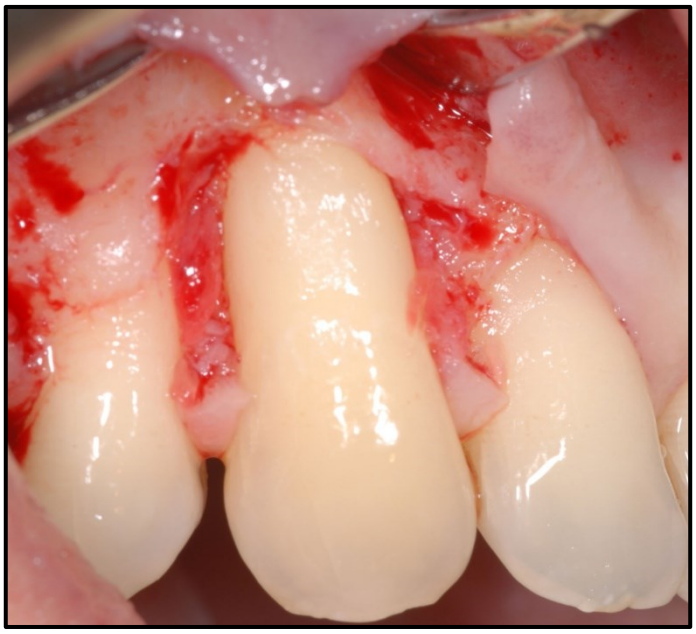


Fig. 6: Identification of a 2 mm long cementum aberration on the root surface, next to the buccal bone dehiscence.

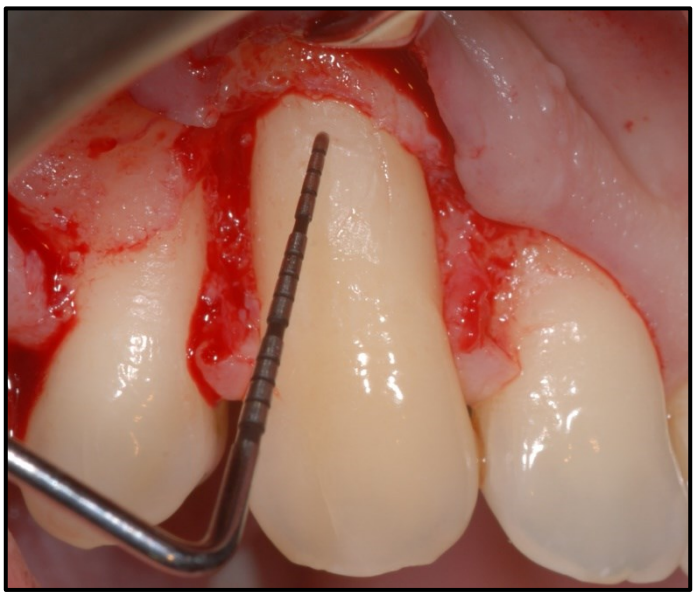


Fig. 7: Elimination of the cementum aberration using a dedicated set of burs.

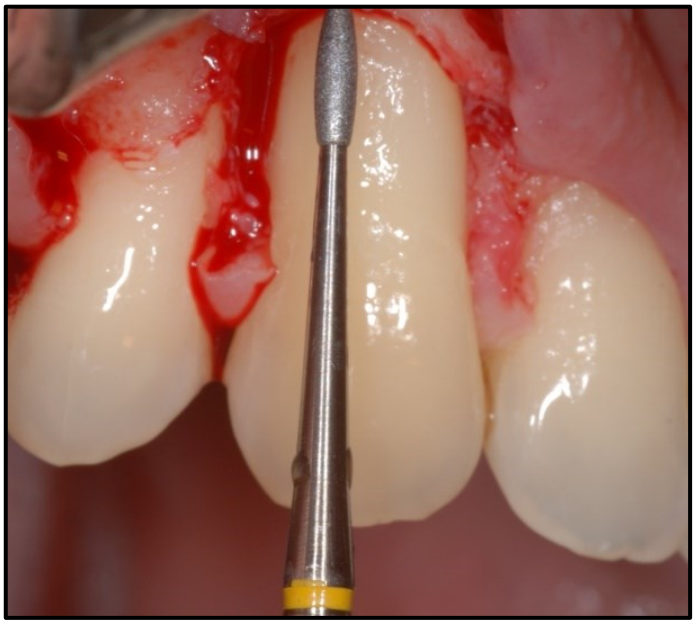


Fig. 8: The intraosseous component of the defect and the vestibular aspect of the root surface are filled with β-TCP mixed with rhPDGF-BB.

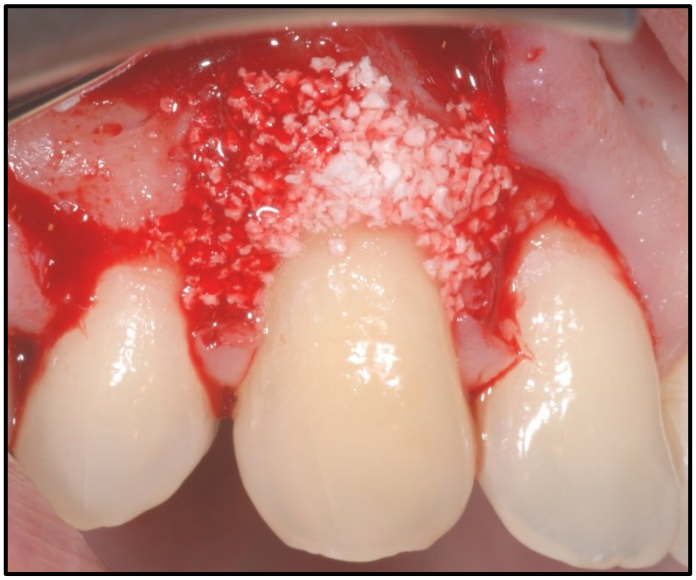


Fig. 9: A tridimensional collagen membrane is adapted on the vestibular and interproximal aspects of tooth 1.3. Interrupted sutured are used for membrane fixation at interproximal papillae.

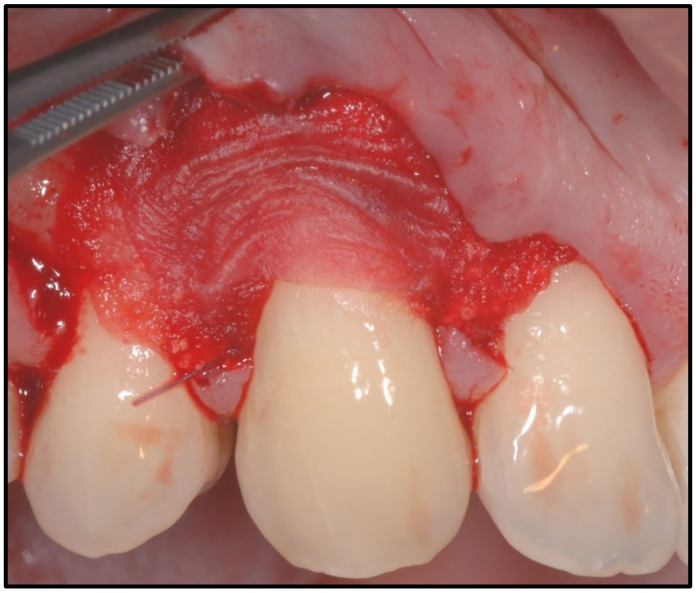


Fig. 10: Suture of the flap (frontal view). For wound closure, horizontal internal mattress suture are placed between the flap and the attached papilla to ensure repositioning of flap. The releasing incision is sutured with interrupted sling sutures.

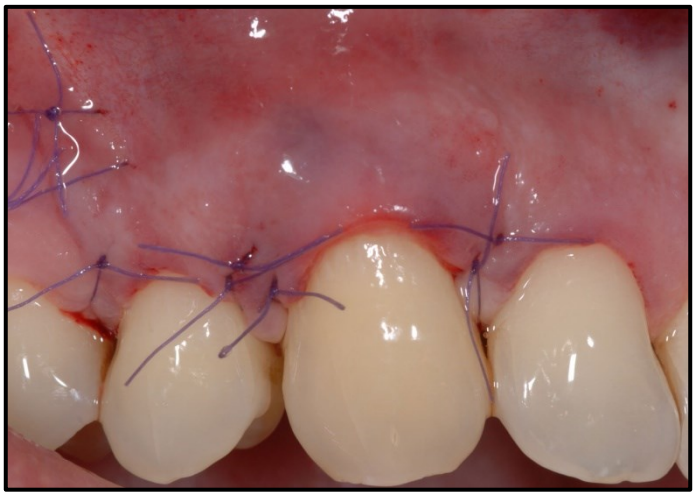


Fig. 11: Suture of the flap (occlusal view). An increase of gingival tissues width on the buccal aspect is evident.

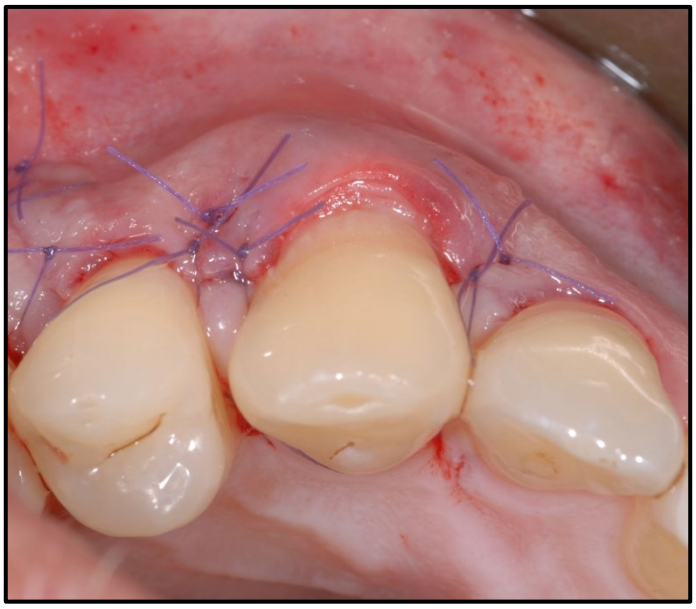


Fig. 12: Postoperative radiograph of tooth 1.3.

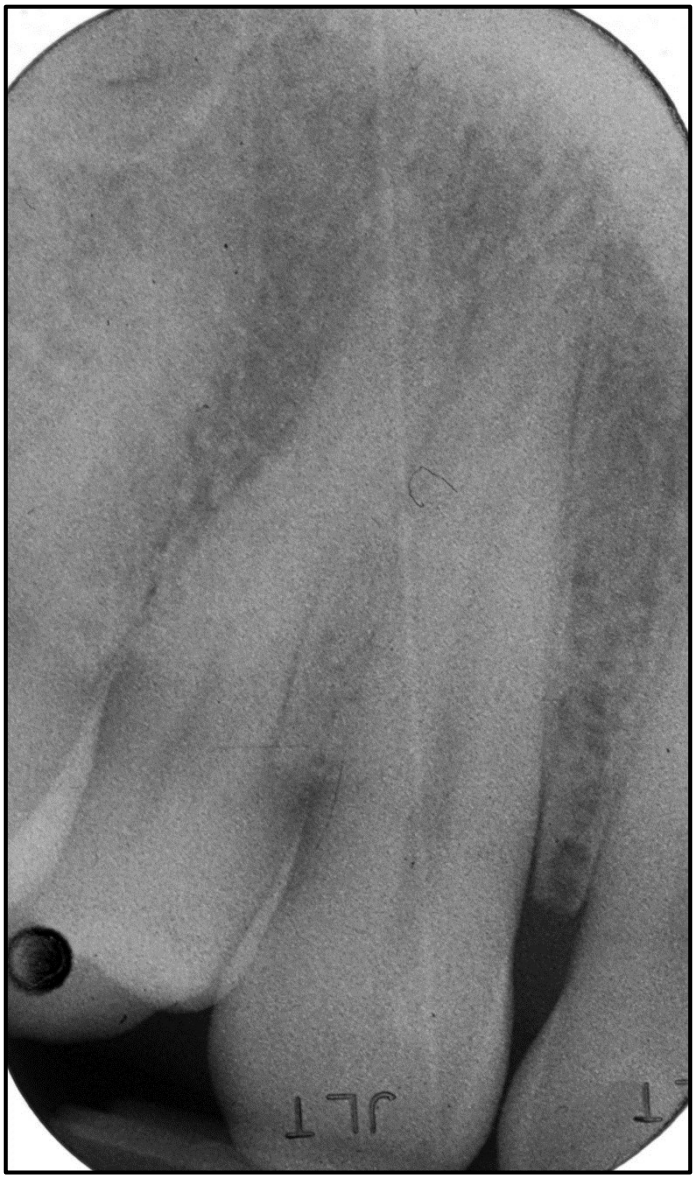


Fig. 13: Clinical aspect of soft tissues 8-months after surgery (frontal view). Soft tissues around 1.3 are healthy. A PPD= 1mm can be observed at the buccal aspect of 1.3. This site was negative to bleeding and supporation upon probing. The keratinized tissue width is entirely preserved.

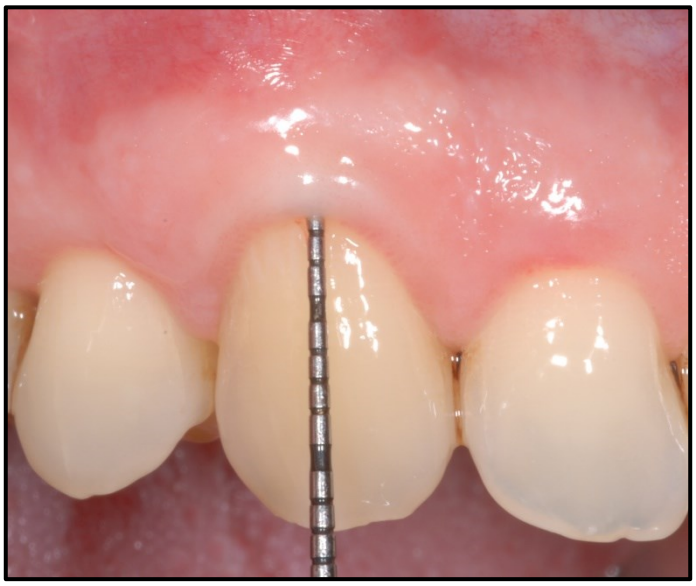


Fig. 14: Clinical aspect of soft tissues 8-months after surgery (occlusal view). The soft tissue buccal-lingual width is maintained.

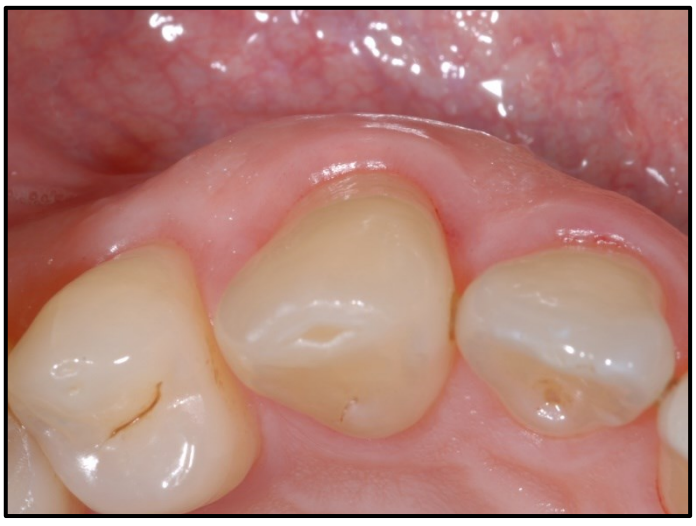
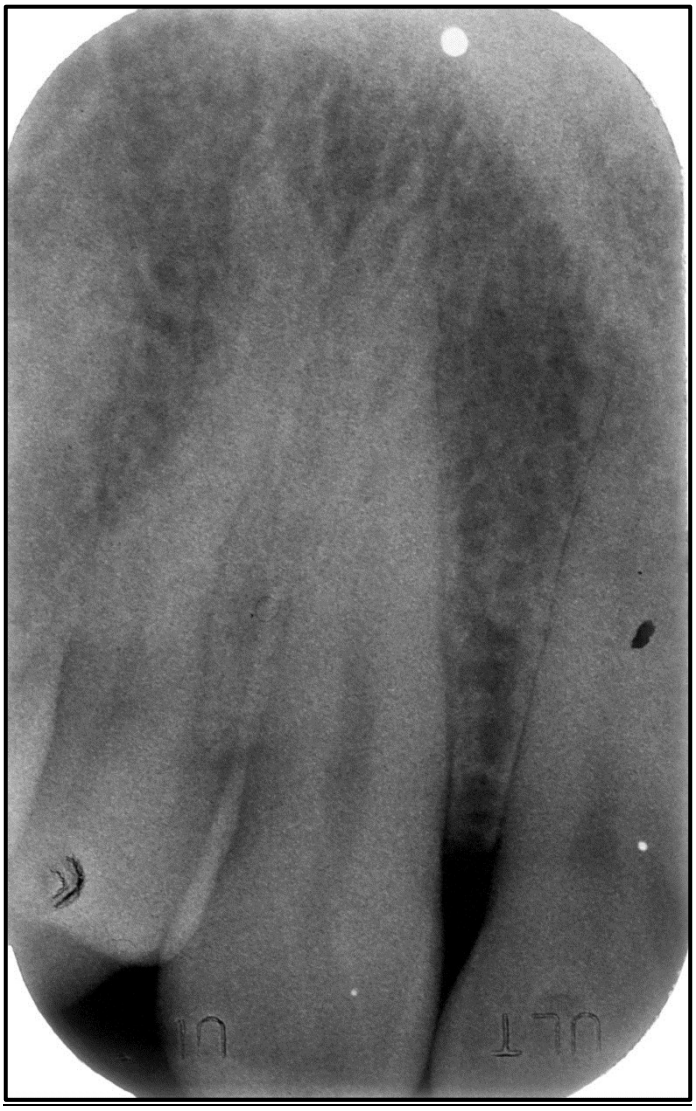


Fig. 15: Radiographic aspect of tooth 1.3 8 months after surgery.



CHAPTER 8

General discussion

In the current series of studies, the effectiveness of different bioactive agents (BAs) used alone or in combination with scaffold biomaterials in the regenerative treatment of periodontal intraosseous defects accessed with a simplified surgical procedure (i.e., the *Single Flap Approach, SFA*; Trombelli et al. 2007) was evaluated. Findings from these studies will be critically discussed in this chapter, mainly focusing on the following clinical issues:

1. What is the clinical effectiveness of BAs when used in combination with the SFA versus traditional double flap approaches in the treatment of periodontal intraosseous defects?

2. When treating an intraosseous defect with BA, does the SFA improve postoperative morbidity of the intervention compared to traditional double flap approaches?

3. What is the quality of early post-operative healing at the incision margin following regenerative treatment of periodontal intraosseous defects performed with the SFA with/without reconstructive devices (including bioactive agents)?

4. Which factors (either related to the patient or the treated site) may influence the quality of early wound healing following regenerative treatment of periodontal intraosseous defects with BAs in combination with the SFA?

5. What is the rationale for combining BAs and graft materials when approaching an intraosseous defect with the SFA?

What is the clinical effectiveness of BAs when used in combination with the SFA versus traditional double flap approaches in the treatment of periodontal intraosseous defects?

Regenerative therapy represents a proven method to improve clinical parameters, periodontal prognosis, and tooth retention. In this contest, the use of BAs in association with simplified surgical procedures represent a new and viable method for the regenerative treatment of periodontal intraosseous defect (Kao et al. 2015). In the present thesis, EMD and rhPDGF-BB were used in combination with the SFA in the treatment of deep intraosseous defects (Rizzi et al. 2013, Farina et al. 2014, Schincaglia et al. 2015). Overall, findings from these studies demonstrate substantial clinical outcomes at 6-months after

surgery, expressed by significant CAL gain and PD reduction. In particular, when deep intraosseous defects were treated with the SFA+EMD and SFA+EMD+DBBM a mean CAL gain of 3.8 ± 1.0 mm and 3.4 ± 1.9 mm, respectively, was observed. These results were parallel by a PD reduction of 4.9 ± 1.8 mm in the SFA+EMD group and 5.0 ± 2.0 mm in the SFA+EMD+DBBM group. Similarly, the combination of SFA and rhPDGF-BB+β-TCP resulted in a mean CAL gain of 4.0 ± 1.9 mm and mean PD reduction of 4.1 ± 1.7 mm. All these clinical results largely exceed the CAL gain and PD reduction values reported for conventional access flap (Graziani et al. 2012).

To maximize the regenerative potential of the Bas, a proper root and defect instrumentation is of paramount importance. In this respect, the selection of an adequate surgical access plays a crucial role. The SFA is a simplified surgical procedure to access periodontal intraosseous defects (Trombelli et al. 2007, 2009). The basic principle of the SFA consists of the elevation of a limited mucoperiosteal flap to allow access to the defect from either the buccal or oral aspect only, depending on the main buccal/oral extension of the lesion, preserving the integrity of the interproximal supracrestal gingival tissues. When considering the SFA characteristics, it is evident that this kind of surgical approach can be only applied in specific type of defects characterized by a prevalent extension on the interproximal and vestibular/oral side. In this respect, a proper pre-operatory diagnosis with bone sounding is of paramount importance.

In order to assess whether the SFA may be regarded as a suitable option for the surgical debridement of an intraosseous lesion, a recent study (Schincaglia et al. 2015) was performed to compare the SFA with the gold standard technique, characterized by the elevation of a double flap at both buccal and oral aspects, according to the papilla preservation techniques (Double Flap Approach, DFA, Cortellini et al. 1995, 1999). In this study, both surgical procedures were combined with the use of rhPDGF-BB+β-TCP. The clinical results showed that in the SFA group, the mean 6-months CAL gain was 4.0 mm, exceeding the mean improvements in CAL gain (3.2 mm) observed in the DFA group (although the difference did not reach statistical significance) and those reported in previous studies applying the same technology in conjunction with conventional, double flap designs (Nevins et al. 2005, 2013, Thakare & Deo 2012). The effectiveness of the SFA procedure is also confirmed by a previous companion study were 2-3 walled intraosseous defects received surgical debridement according to either SFA or DFA and no regenerative devices were used in addition to surgical access (Trombelli et al. 2012). At 6 months, SFA resulted in 1-mm greater CAL gain and PD reductions compared to access

with DFA, largely exceeding CAL gain values reported for conventional access flap (Graziani et al. 2012). In the light of these findings, the SFA seems to be at least as effective as traditional papilla preservation techniques based on double flaps, when evaluated either as stand-alone protocol or in combination with regenerative devices (such as BAs). The tendency of SFA to show good clinical results is probably favored by the better quality of early wound healing at the incision margin (see the following paragraphs for details). In particular, the elevation of a single flap with the preservation of the interproximal supracrestal gingival tissues may limit the surgical trauma on the vascular supply of the interdental papilla. It can be speculated that a faster recovery of the vascular stability of the flap within the papillary area (Retzepi et al. 2007), determines a higher stability of the blood clot in the interproximal area and therefore a more favorable healing.

When treating an intraosseous defect with BA, does the SFA improve postoperative morbidity of the intervention compared to traditional double flap approaches?

Simplified surgical procedures aims at maximizing the reconstructive outcomes while reducing patient post-operatory morbidity. To date, no infective complications or adverse reactions (edema or hematoma) were ever reported after regenerative treatments of periodontal intraosseous defects with SFA with or without BAs. Furthermore, no treatment failure related the use of various reconstructive technologies, including graft materials, membranes or BAs was described (Trombelli et al. 2009, 2010, Farina et al. 2013, 2014, 2015, Rizzi at al. 2013, Simonelli et al. 2013). Only one study investigated the patient's self-perceived pain and analgesic consumption after SFA+ rhPDGF-BB + β-TCP and DFA+ rhPDGF-BB + β-TCP (Schincaglia et al. 2015). Significantly lower pain levels were self-reported during the first postoperative days by patients treated with SFA, compared to patients undergoing DFA. The mean number of analgesics consumed during the first 2 postoperative weeks was 2.73 in the SFA group and 8.69 in the DFA group, with a significantly greater dose of analgesics being used in the DFA group compared to the SFA group (3.2 versus 1.1, respectively) at day +1. Similar results were reported in a case series (Cortellini e Tonetti 2009) in which a minimally invasive flap design (M-MIST) –similar to SFA- was used in conjunction with EMD. In this study, only 3 patients reported very limited discomfort in the first 2 days of the first post-operative week, and none of the 15 treated patients reported significant postoperative pain at week 1. In another study from the same group (Cortellini e Tonetti 2011), none of the patients experienced postoperative pain

at week 1. Average VAS scores (on a 100-mm scale) for postoperative discomfort ranged from 10.7 to 12.3. The mean number of analgesics was below one, with a maximum of 3 analgesics used during the postoperative period (Cortellini e Tonetti 2011). In the light of these findings, we can speculate that the minimal invasiveness and limited surgical trauma provided by the SFA (or similar surgical techniques) may influence the postoperative course of the intervention, limiting pain and consumption of analgesics.

What is the quality of early post-operative healing at the incision margin following regenerative treatment of periodontal intraosseous defects performed with the SFA with/without reconstructive devices (including bioactive agents)?

The significance of primary intention healing as a determinant of periodontal wound healing following regenerative procedures has been universally recognized (Wikesjö et al. 1992, Polimeni et al. 2006). In particular, the first postoperative weeks seem to be critical for the maintenance of wound stability (Wikesjö et al. 1992, Werfully et al. 2002, Hiatt et al. 1968). Wound dehiscence may compromise wound stability, which in turn would jeopardize the cascade of biologic events leading to periodontal regeneration (Wikesjö et al. 1990, Linghorne et al. 1950, Yumetet al. 1985). Furthermore, when flap surgery is used in association with regenerative technologies, the postoperative loss of primary closure may lead to partial or complete exfoliation of the implanted graft, contamination of the membrane surface, or premature clearance of the BAs. In addition, primary intention healing seems to influence the clinical outcomes of the regenerative procedure. The results of a recent study suggest an impact of the different early healing patterns on the 6-month clinical outcomes of the procedure, with a trend towards better clinical outcomes (greater CAL gain, less buccal REC increase) when defects showed optimal wound closure compared to incomplete wound closure (Farina et al. 2013).

Data from recent studies on the early postoperative healing following SFA either alone or in combination with BAs indicate that the use of this simplified surgical technique may result in high proportion of sites showing complete flap closure during the first postoperative weeks (Farina et al. 2013, Schincaglia et al. 2015). In particular, a retrospective analysis of defects treated with SFA with and without BAs consistently showed that the 84% of defects presented a complete closure of the incision wounds at 2 weeks post-surgery, as assessed by an EHI score of 1 to 3. In particular, 54% of the treated defects showed optimal conditions (i.e. EHI=1) of wound closure. More recently, a

randomized clinical trial compared the quality of early wound healing after regenerative treatment of deep intraosseous defects with SFA + rhPDGF-BB + β-TCP vs DFA + rhPDGF-BB + β-TCP (Schincaglia et al. 2015). The results clearly showed that SFA might optimize the quality of early wound healing of defects compared to a DFA. At 2 weeks, 12 sites in the SFA group and 6 sites in the DFA group showed complete flap closure (i.e., EHI = 1, 2 or 3). The frequency of sites showing optimal wound healing (i.e., EHI = 1) was 8 and 3 in the SFA and DFA group, respectively. It can be speculate that the enhanced early wound healing may have an influence on the improved clinical outcomes in SFA group compared to DFA group. Overall, these results confirm that a simplified surgical access, such as SFA (Trombelli et al. 2007), may be associated with a high prevalence of sites maintaining wound closure at 2 weeks following surgery especially when associated to BAs. This condition may positively influence the final clinical outcomes.

Undoubtedly, the quality of early wound healing after regenerative treatment of intraosseous defects may be influenced by some surgical factors, in particular by the reconstructive devices placed inside the defect. In this respect, a previous study compared the early post-operative healing of sites approached with SFA alone vs. sites treated with SFA in combination with graft biomaterial + resorbable membrane (GTR) (Trombelli et al. 2010). The results clearly showed that sites treated with SFA+GTR presented a worst post-operative healing compared to the ones treated with SFA alone. It can be speculated that the presence of a membrane may result in a transient impairment of the revascularization process of the gingival flap during the early phase of healing (Vergara et al. 1997). In this respect, a relationship between reduced blood perfusion in a mucoperiosteal flap covering a membrane and the incidence of wound dehiscence has been reported (Zanetta-Barbosa et al. 1993). Differently, when BAs -used alone or in combination with scaffold biomaterials- were associated to the SFA in the regenerative treatment of intraosseous defects, no differences were observed when compared to the SFA alone. Unfortunately, this consideration is only supported by a clinical feeling and not from scientific data. In this respect, further investigations are needed.

Which factors (either related to the patient or the treated site) may influence the quality of early wound healing following regenerative treatment of periodontal intraosseous defects with BAs in combination with the SFA?

The influence of site-specific factors and patient-related factors on the post-operatory wound healing following regenerative treatment of intraosseous periodontal defects was analyzed in a recent retrospective study (Farina et al. 2013). Among site-specific factors, higher risk for sub-optimal wound closure was associated with a narrower base of the interdental papilla and the presence of either interdental contact point or interdental soft tissue crater. These data can be explained by the vascular anatomy of gingival tissues. In particular, at the buccal aspect, blood vessels in the gingival tissues are oriented mainly in an apico-coronal direction (Mörmann et al. 1979). A horizontal incision performed in the gingiva results in a transient reduction of blood perfusion to the gingival tissues coronal to the incision margin (Mörmann et al. 1979, Retzepi et al. 2007). When considering the characteristics of the vascular system of the interdental tissues, which consists of a mixed pattern of anastomosing capillaries and loops (Kohl & Zander 1961), it is therefore reasonable to admit that the dimensions as well as the morphological characteristics of the soft tissues occupying the interdental space may affect the re-establishment of the normal blood perfusion after gingival incision. Differently, when considering the influence of patient-related factors on the post-operatory wound healing following regenerative treatment of intraosseous periodontal, no association between EHI and age, gender, or smoking status was evident (Farina et al. 2013). In contrast with our finding, a delayed and impaired healing process following gingival biopsies was observed in older compared with younger patients (Holm-Pedersen et al. 1971). The effect of age on the early healing of gingival incision wounds and its clinical relevance on the reconstructive outcomes need to be further investigated since there is wide consensus that wound healing is negatively affected by the aging process (Ashcroft et al. 2002, Gosain & DiPietro 2004).

What is the rationale for combining BAs and graft materials when approaching an intraosseous defect with the SFA?

When considering the pertinent literature on the use of BAs (EMD) alone or in combination with graft materials in defects treated with conventional double flap papilla preservation techniques (DFA), the combined regenerative approach demonstrated better clinical outcomes compared with EMD alone (De Leonardis et al. 2013). The combination

of BAs and graft materials in the regenerative treatment of deep intraosseous periodontal defects accessed with SFA has been widely validated (Farina et al. 2014, 2015, Schincaglia et al. 2015). In fact, when considering the 6-month clinical outcomes of a regenerative treatment based on SFA+EMD+DBBM or SFA+rhPDGF-BB+β-TCP, statistically significant CAL gain and PD reduction were observed (see above). The rational for combining BAs and graft materials was recently described (Farina et al. 2014, 2015). In this context, the 2014 study by Farina et al. described the regenerative treatment of 24 deep periodontal intraosseous defects with the SFA combined to EMD with or without DBBM, according to the surgeon's discretion. Both treatments resulted in significant CAL gain and PD reduction. In the selection of DBBM as adjunctive scaffold to EMD, the defect morphology played a role of paramount importance. In fact, the results showed that the composition of defects treated with EMD and EMD+DBBM was markedly different: the EMD-treated defects showed a dominant 3-wall component while EMD+DBBM defects showed a predominant 1-wall component. In addition, defects in EMD+DBBM group showed a tendency to have a wider radiographic angle and mesio-distal space. When interpreting these results, it appears that deep intraosseous defects with an unfavorable morphology (i.e., mainly non self-contained due to a dominant 1-wall component, ample defect angle and width) treated with EMD+DBBM may respond similarly to defects with a more favorable morphology (i.e., mainly self-contained due to a dominant 3-wall component, narrow defect angle and width) treated with EMD only. Interestingly, when mainly 3-wall defects with a narrow defect angle were accessed with a simplified surgical procedure similar to SFA and treated with EMD with or without EMD+DBBM, no adjunctive benefit of DBBM over EMD was observed (Cortellini et al. 2011). These observations suggest that the adjunctive use of a graft in a regenerative strategy based on a simplified surgical access (i.e. SFA) and including the application of EMD may be indicated in defects with an unfavorable morphology, i.e. defect configuration/features that may affect the endogenous regenerative potential. A second condition that may justify the combination of BAs with graft materials was reported in a more recent study (Farina et al. 2015) that evaluated the aesthetic impact, in terms of gingival recession (REC) increase, after regenerative treatment of deep periodontal defects. The results showed that the change in REC at the interproximal level was significantly predicted by pre-surgery interproximal PD and treatment modality. In particular, defects treated with SFA in combination with a BA plus a graft material (i.e. EMD+DBBM or rhPDGF-BB+β-TCP) were less prone to REC increase compared to defects treated with open flap debridement with or without EMD. This finding was consistent with previous studies showing that the

combined use of EMD and a graft may significantly temper postoperative recession increase compared to EMD alone in the treatment of deep intraosseous defects (Zucchelli et al. 2003, Guida et al. 2007, Matarasso et al.2015). Overall, findings from the above mentioned studies seem to indicate that the treatment of intraosseous defects with the SFA in combination with bioactive agents and graft materials is indicated for deep intraosseous defects with unfavourable (non self-containing) morphology located at esthetically-sensitive areas.

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CHAPTER 9

Conclusive remarks

The general purpose of the studies included in this Ph.D. activity was to evaluate the effectiveness of bioactive agents (BAs) alone or in combination with graft materials in the regenerative treatment of periodontal intraosseous defects accessed with a simplified surgical procedure (the *Single Flap Approach*, SFA; Trombelli et al. 2007, 2009).

On the basis of the produced evidence, the following conclusions can be drawn:

1. The treatment of periodontal intraosseous defects with the SFA in combination with BAs (with or without graft materials) may result in substantial clinical attachment gain and probing depth reduction as well as limited postoperative increase in gingival recession (chapters 2-5, 7).

2. When treating an intraosseous defect with BA, the SFA results in better quality of early wound healing, better clinical outcomes, and lower pain and postoperative consumption of analgesics compared to a double flap approach based on papilla preservation techniques (chapter 4).

3. The SFA alone or in combination with BAs may result in a highly predictable (>80%) complete flap closure and substantial prevalence of sites with optimal healing at 2 weeks following surgery. Our findings also indicate that local, site-specific characteristics (i.e. narrower base of the interdental papilla) may influence the early postoperative healing of the papillary incision (chapter 2).

4. The adjunctive use of a graft material to a BA at defects approached with the SFA seems indicated at defects (i) with unfavorable osseous morphology (i.e., predominantly one- or two-walled) (chapter 3), and (ii) located at esthetically-sensitive areas (chapter 5).