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The Use of Single Flap Approach versus the Double Flap Approach in the Treatment of Intraosseous Periodontal Defects in Combination with Recombinant Human Platelet-derived Growth Factor-BB and Synthetic Beta-Tricalcium Phosphate: A Randomized Controlled Trial - Interim Report.

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The Use of Single Flap Approach versus the Double Flap Approach in the Treatment of Intraosseous Periodontal Defects in Combination with Recombinant Human Platelet-derived Growth Factor-BB and Synthetic Beta-Tricalcium Phosphate: a Randomized Controlled Trial – Interim Report.

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1.0 Introduction

The progression of the periodontal intrabony lesion, as manifested by damage to the connective tissue attachment and bone loss, can eventually lead to tooth loss in the absence of an intervention [1]. Decades of research in periodontal regeneration therapy have produced various materials and products as well as innovative surgical approaches resulting in clinically significant levels of regenerative success for patients.

One recent regenerative material showing promise is GEM-21 (rhPDGF-BB with β-TCP). *In vitro* studies, randomized clinical trials, and systematic reviews have confirmed its clinical and statistical benefit in treating intrabony defects [2-6]. Progress in flap management has moved towards smaller, less traumatic surgical approaches with the goal of maximizing the innate healing potential of the periodontium. The success of minimally invasive surgery has been confirmed in systematic reviews and meta-analyses, but to what extent one approach may have greater clinical benefit than another needs to be further investigated [7, 8].

To date, there is only one randomized clinical trial that evaluates minimally invasive surgery by directly comparing the single flap approach (SFA) to the double flap approach (DFA) [9]. On the contrary, these approaches have been tested extensively in the literature both alone or with other treatment adjuncts. These minimally invasive surgical approaches not only have the potential for greater clinical success as measured by traditional periodontal parameters, but also may have greater patient-centered outcomes in the form of less post-operative pain and analgesic consumption. What is not

known is whether a surgical approach like the SFA, which maintains space for clot maturation and enhances wound stability, will potentiate the regenerative efficacy of a treatment adjunct like rhPDGF-BB with β-TCP for a greater clinical benefit in comparison to another surgical approach like the DFA.

Therefore, the aim of this randomized, controlled, parallel-arm clinical trial was to compare the clinical, patient-centered, and radiographic outcomes of a regenerative strategy based on the use of rhPDGF-BB + β-TCP in deep intraosseous periodontal defects accessed with SFA versus DFA.

2.0 Background and Significance

2.1 Regenerative Periodontal Therapy

2.1.1 A History of Regenerative Periodontal Therapy

The progression of the periodontal lesion, as manifested by damage to the connective tissue attachment and bone loss, was considered irreversible until a series of studies proved that regenerative periodontal therapy was possible [10]. Early investigators observed in animal studies that new bone could grow in bone defects that were enclosed with an artificial device [11, 12]; whereas, Karring and Nyman showed in animal models specifically which cells contributed to the regeneration of the periodontium [13, 14]. They observed that a new periodontal ligament was only re-established on the root that had remnants of the periodontal ligament (PDL) preserved after scaling and root planing. The portion of the root without remnants of the PDL showed ankylosis and root resorption when in contact with bone and gingival connective

tissue. In another animal model, the authors induced periodontitis, scaled and root planed, and then submerged the roots for healing. They observed a formation of new connective tissue attachment on the previously diseased and root planed root area. It was concluded that new attachment is formed by coronal migration of cells originating from the periodontal ligament and not from the cells of the bone or gingival connective tissue [15]. In a human study, they used an occlusive barrier membrane with the purpose of excluding epithelium to allow PDL cells to populate the root and form new attachment. This study confirmed that use of this barrier membrane led to periodontal regeneration of a previously diseased root [16].

As these experiments were being conducted, Gore-Tex™ began testing the clinical application of its expanded polytetrafluoroethylene (ePTFE) occlusive membrane for treating periodontal defects. The clinical success of the ePTFE occlusive membrane was shown by histological evidence of periodontal regeneration in both intrabony defects and Class II and III furcation defects [17, 18], and by a randomized clinical trial using this membrane on Class II furcations showing 90% of the defects with complete resolution [19]. This occlusive barrier membrane with the purpose of excluding epithelium to allow PDL cells to populate the root and form new attachment seemed to explain the clinical success.

As the clinical success of Guided Tissue Regeneration (GTR) using e-PTFE membrane mounted, a group of researchers investigated the use of adding an osseous graft to the GTR protocol. Clinical studies displayed the added benefit of allografts for periodontal regeneration, in particular, a five year report showed a superior result of GTR with root conditioning and allograft in comparison to GTR alone [20-23].

Along with studies showing successful regenerative therapy, a group of reports focused on a notable level of GTR failure. An acknowledged reason for GTR failure was flap management resulting in membrane exposure and bacterial infection, but the level of failure was variable [24-29]. These reports fueled innovative research to develop new regenerative materials including resorbable membranes [30-32] and biologics, and surgical approaches such as papilla preservation flaps, minimally invasive surgery, and the single flap approach [33-35].

Decades of research in periodontal regeneration have produced various materials and products as well as innovative surgical approaches resulting in clinically significant levels of regenerative success for patients.

2.2 Wound Healing in Periodontal Regeneration

2.2.1 Biologic Factors for Periodontal Regeneration

2.2.1.1 Space Provision

In evaluating the proven success of guided tissue regeneration, the importance of space provision was tested by Wikesjö and coworkers. For years, the concept of tissue occlusion was used to explain GTR success, but in a study where a macroporous ePTFE membrane was compared to an occlusive ePTFE membrane it was shown that there was no difference in the level of regeneration between the groups [36]. Instead of excluding epithelium to allow PDL cells to populate the root and form new attachment, the importance of space provision became apparent. Polimeni et al. treated critical-sized defects with either a porous ePTFE membrane adapted according to the GTR protocol or no membrane at all [37]. There was a significantly greater level of regeneration when the

membrane was used proving that space provision enhanced regeneration. Moreover, Polimeni et al. further studied whether placing a graft with a membrane improved space provision and ultimately regeneration [38]. They showed significantly greater regeneration when the membrane and graft were used in conjunction. The authors explained that the biomaterial prevented a collapse of the membrane; thus, providing the space and support for the blood clot.

2.2.1.2 Blood clot stabilization

Other factors have been identified as important for promoting the innate regenerative potential of the periodontium. Early studies observed the importance of a stable root surface-adhering blood clot protected from mechanical and microbiological insults for regeneration [39-41]. Using the critical-size supraalveolar defect model in dogs, the group investigated what happened if the clot was prevented from adhering to the root by using heparin [42]. They found that heparin-treated roots had 50% connective tissue attachment and the saline-treated roots had 95% connective tissue attachment. In conclusion, manipulation of the clot's ability to adhere to the root prevented connective tissue attachment. To determine to what extent wound-rupturing forces may have disrupted re-attachment, Wikesjö et al. evaluated if placing a graft (polylactic acid) to support the clot could neutralize the effects of a heparin-treated root [43]. They found that placing a graft to stabilize the blood clot neutralized the effect of heparin on connective tissue attachment. The graft stabilized the wound against disruptive wound-rupturing forces acting on the gingival margin. These experiments show the importance of a stable blood clot adherent to the root for periodontal regeneration.

2.2.1.3 Wound infection

Unexposed primary intention healing has been shown to be an important factor for periodontal regeneration. An acknowledged reason for GTR failure was flap management resulting in membrane exposure and bacterial infection, but the level of failure was variable [24-29]. Trombelli et al. did a retrospective analysis to see the effect of wound dehiscence on GTR outcome [44]. Exposure versus no exposure was evaluated in 38 patients contributing 38 defects, and the average bone gain without exposure was 4.1 ± 2.3 versus 2.2 ± 2.3 with exposure and this was statistically significant. Wound dehiscence allows for bacterial penetration and provocation of the inflammatory response, which worsens the regenerative outcome.

There are biologic factors that contribute to creating an optimal environment for periodontal regeneration: space provision, stability of the root surface-adhering blood clot, and unexposed primary intention healing. By manipulating these factors, researchers can develop regenerative materials to potentiate the innate healing potential of the periodontium. Furthermore, developing a surgical approach that provides maximum support and space maintenance for the blood clot, reduces wound-rupturing forces, and ensures primary intention healing may enhance the regenerative outcome.

2.2.2 Innate Regenerative Potential of the Periodontium

The observation of the innate regenerative potential of the periodontium dates back to Björn et al. in 1957 in a dog model and then in humans [45, 46]. In a series of studies showing human histology, Bowers et al. showed periodontal regeneration in periodontally compromised teeth, which were treated with open flap debridement, with or

without demineralized bone matrix, and then submerged [47-50]. This highlighted the innate regenerative potential of the periodontium under optimal circumstances.

In a recent study in dogs, early periodontal wound healing events were observed using histological and immunohistochemical techniques. The dogs were sacrificed at 2, 5, 9, and 14 days, and at 4 and 8 weeks [51]. Using the critical-size supraalveolar defect model, pro-regeneration and pro-scar-forming domains were identified, which compete together to populate the wound space. The pro-regeneration cells and signals originated from the periodontal ligament and bone marrow, while the pro-scar-forming cells and signals originated from the gingival tissues. The net outcome of the competition between these two domains modulated by local and systemic factors determined to what extent the innate regenerative potential could occur. The pro-scar-forming domain was dominated by fibrous tissue and was separated from the regenerative domain by remnants of the clot. These observations indicated that most new tissue formation in the periodontium was complete by 14 days in the dog model; thus, making the assumption that in humans, regenerated periodontal tissues should be established within 4 weeks.

2.2.3 Phases of Periodontal Wound Healing

Wikesjö and coworkers studied the phases of periodontal wound healing [52]. The *immediate response* to injury is clot formation. The clot is protective and also a matrix for cell migration as red and white blood cells and platelets move to the site [53, 54]. The clot functions as a hemostatic plug through its matrix of fibrin and extracellular matrix glycoproteins, and plasma proteins begin absorbing and adhering to the root surface. In the *early stage of inflammation*, which is within hours of injury, neutrophils

and monocytes cleanse the wound by phagocytosis of bacteria. In the *late phase of inflammation*, which is within 3 days, macrophages debride the wound through phagocytosis, and they also release cytokines and growth factors targeting other cells involved in wound repair. There is also a proliferation and migration of fibroblasts, endothelial cells, and smooth muscle cells are seen. The next phase is the *formation of granulation tissue* occurring at one week. Granulation tissue begins maturing, and myofibroblasts express α-smooth muscle actin, which results in wound contraction. Endothelial cells vascularize the site, and a connective tissue attachment may be seen at the root surface. The last phase is *epithelialization*, which begins within hours of injury and completes by two weeks. Whether regeneration or repair occurs depends on the availability of the specific cell types and the signals necessary to recruit and stimulate these cells.

By understanding the intricacies of the innate healing potential of the periodontium, researchers can develop regenerative materials, which stimulate or amplify, specifically, the cells and signals contributing to regeneration. Moreover, the importance of the blood clot adhesion to the root surface for regeneration, emphasizes the need for a surgical approach developed to create a favorable environment for clot stabilization.

2.3 Flap Management

2.3.1 Progress in Flap Management

An acknowledged reason for GTR failure was flap management resulting in membrane exposure and bacterial infection, but the level of failure was variable [24-29]. In response, clinical innovators began developing new surgical approaches to preserve

soft tissue for primary intention healing with the goal of preventing wound dehiscence and infection of the regenerative material.

Takei et al. proposed in 1985 the Papilla Preservation Technique with the stated purpose of achieving primary closure after covering grafted bone defects [55]. The flap design began with sulcular incisions, and then was described as a series of semi-lunar incisions on the palatal or lingual that started and ended on the straight lingual of the involved teeth. This created a pedicle, which kept the interproximal tissue intact as it was passed under the contact point exposing the interproximal osseous defect. This was a double flap approach (DFA) in that both the facial and lingual flaps were elevated. In this case series, it was reported that primary intention healing always occurred, and interdental soft tissue craters did not develop.

Cortellini et al. further modified the double flap approach with the modified papilla preservation technique (MPPT) and the simplified papilla preservation flap (SPPF) [56, 57]. Cortellini described the purpose of the MPPT was to allow for primary closure, but also the coronal positioning of the interdental tissue. The clinical application required an interdental space of greater than 2mm, and the description of the flap design began with sulcular incisions around the teeth adjacent to the osseous defect. A horizontal incision was then placed on the buccal at the base of the papilla at the level equal to the most apical portion of the buccal gingival margin of the neighboring teeth. The interproximal tissue was pushed through the contact point and kept attached to the lingual flap. Vertical incisions were then placed to mobilize the buccal flap coronally for primary closure. Cortellini et al. tested GTR with the MPPT versus a conventional flap

in a randomized clinical trial where the clinical attachment level (CAL) gain using the MPPT was 5.3 ± 2.2mm versus 4.1 ± 1.9mm for the conventional flap [58].

The simplified papilla preservation flap (SPPF) had a similar application as the MPPT, but was instead developed for narrow interdental spaces of less than 2mm. Cortellini et al. described the flap design as first beginning with an incision that crossed the defect-associated papilla [57]. The incision started from the gingival margin at the buccal-line angle of the involved tooth, and extended to the mid-interdental portion of the papilla under the contact point of the adjacent tooth. Sulcular incisions were placed on the buccal and lingual of the teeth adjacent to the defect. The flaps were elevated with the interdental tissue intact and attached to both the buccal and lingual flaps. Vertical incisions and periosteal releasing incisions were placed to mobilized the buccal flap coronally for primary closure. This flap design was preliminarily tested in a case series of eighteen intrabony defects in combination with GTR [57]. The average CAL gain observed at one year was 4.9 ± 1.8 mm, and in all the cases it was possible to achieve primary closure of the flap over the membrane.

Harrel and Rees proposed minimally invasive surgery (MIS) with the aim to minimize wounds, limit flap reflection, and to gently handle the soft and hard tissues [34]. Minimally invasive surgery was described as a more precise surgical procedure made possible through the use of magnification and microsurgical instruments and materials. Harrel described MIS as consisting of an initial sulcular incision around the teeth adjacent to the defect, and then joining the sulcular incisions with a single horizontal incision placed 2 to 3 mm from the crest of the papilla [59]. Sharp dissection is used to elevate the buccal and lingual flaps, and the flap is kept limited to the teeth adjacent to

the defect. The case series reported that MIS had improved clinical parameters, a faster rate of healing, less postoperative pain, and improved soft tissue retention of height and contour. In a prospective study where 160 sites were treated with MIS and Emdogain, the mean CAL gain was 3.57 mm and the mean change in recession was 0.01 mm [60].

Cortellini et al. then published a similar concept, a minimally invasive surgical technique, and stressed that this approach maximized wound and blood clot stability and primary wound closure for blood clot protection while limiting patient morbidity [61]. This described a surgical approach that utilized the previously proposed flap designs, SPPF and MPPT, but instead, the surgery was done using high-power magnification, the mesial-distal extent of the flap was minimized, and no vertical incisions were placed. If possible, only the defect-associated papilla was involved, and only 1-2 mm of the defect-associated residual bone crest was exposed. In the two case series published, results showed significant improvements with CAL gains of 4.8 ± 1.9 mm and 88.7 ± 20.7% resolution of the defect, and there was a great reduction in morbidity [61, 62].

The movement towards more minimally invasive flap designs continued with the single flap approach (SFA), as first proposed by Trombelli et al. [35, 63],[64]. The SFA consists of the elevation of a limited mucoperiosteal flap to allow surgical access from either buccal or lingual aspect only leaving the interproximal supracrestal gingival tissues intact. The decision to access from buccal or lingual aspect depends on the position of the bone defect. Maintaining the interproximal supracrestal tissues intact helps facilitate flap repositioning and suturing by using the intact papilla to achieve passive primary closure. This limits the surgical trauma to the papilla's vascular supply allowing for faster wound healing, greater wound stabilization, and the intact papilla may minimize

recession [35, 63, 64]. Sulcular incisions follow the gingival margins of the teeth included in the surgical area and the mesio-distal extension of the flap is to be kept limited while ensuring access for defect debridement. An oblique or horizontal, butt-joint incision is 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. The interdental incision is placed at least 1 mm coronal to the underlying bone crest. The SFA has been subsequently tested in randomized clinical trials in comparison to the double flap approach as well as in conjunction with grafting materials.

In 2009, Cortellini et al. proposed a similar flap design to the SFA, which was named the modified minimally invasive surgical technique (M-MIST) [65] The surgical approach was described as beginning with either the SPPF or MPPT depending on the width of the interdental space. Then the incision continued intra-sulcular on the buccal to the two teeth adjacent to the intraosseous defect and was kept at minimum to allow exposure of the coronal edge of the buccal bone crest. Using a microsurgical blade, the interdental granulation tissue is separated from the supracrestal gingival tissues keeping the supracrestal gingival tissues attached to the root and continuous with the palatal tissue, which was not displaced. In a case series, the M-MIST was used in 15 patients resulting in a 4.5 ± 1.4 mm CAL gain after 1-year in defects 6 ± 1.5mm deep. Primary closure was attained and maintained in all sites, and gingival recession after 1-year increased 0.1 ± 0.3 mm [65].

Progress in flap management began with the goal of properly covering the regenerative materials to avoid exposure and bacterial infection. Years of research

showed a shift towards smaller, less traumatic surgical approaches with the intention of maximizing the innate healing potential of the periodontium by providing stability for the blood clot and wound. Therefore, primary wound closure achieves blood clot protection and patient comfort.

2.3.2 Biologic Advantage of the Single Flap Approach (SFA)

There are several biologic advantages in using a minimally invasive flap design like the single flap approach. By maintaining the interdental, supracrestal, gingival tissue intact, it facilitates flap repositioning and suturing by utilizing the undetached papilla as anchorage; thus, optimizing wound closure for primary intention healing [35, 63, 64, 66, 67]. By achieving primary wound closure, a faster wound healing process occurs particularly at the level of the incision line [66]. The wound stabilization and avoidance of secondary intention healing along with the preservation of an intact interdental papilla may minimize post-surgery recession [64, 65, 68]. Moreover, limiting the surgical trauma on the papilla diminishes the vascular impairment that occurs as a result of trauma, which may partly explain the favorable CAL gain and minimal recession seen with the SFA [69-73].

2.3.3 Systematic Reviews and Meta-analyses for Flap Designs

Tu et al. in a meta-analysis of randomized-controlled trials evaluated if there is a temporal trend in the reported treatment efficacy of periodontal regeneration [8]. By evaluating probing depth and CAL gain over time, a comparison between GTR and Enamel Matrix Derivatives (EMD) groups versus their Open Flap Debridement (OFD) controls showed improvement from 1998 to 2006, and the OFD controls had the same or

a slightly better rate of improvement. The authors attributed the improvement over time in both test and control groups to greater experience and improved surgical technique.

In a more recent systematic review, Graziani et al. analyzed the clinical performance of access flap surgery in the treatment of the intrabony defect [7]. Conservative surgeries were included in the meta-analysis, and they were defined as surgical procedures aimed at gaining access to the root surface with no active removal of bone and most often no resection of soft tissues. Flap designs were categorized from least to most conservative approaches. The least conservative group consisted of open flap debridement and modified widman flap, the papilla preservation flap, the MPPT and SPPF, and finally the M-MIST. The authors concluded that clinical performance may vary according to the type of flap used with the most conservative approach having the greatest CAL gain, probing depth reduction, and a decrease in recession.

2.3.4 Minimally Invasive Surgery and Patient-Centered Outcomes

Studies have reported on the clinically significant outcomes of minimally invasive surgical approaches. Furthermore, some of these studies have published patient-centered outcomes, which suggest a less traumatic patient experience when more minimally invasive approaches are used. Several studies have reported the postoperative pain at one week, the pain intensity as measured by a visual analogic scale (VAS), and the number of analgesics consumed [33, 61, 74-76]. Cortellini et al. 2001 used SPPF/MPPT + bioresorbable barriers, Tonetti et al. 2004 used SPPF/MPPT + Emdogain; Cortellini et al. 2007 used MIST + Emdogain; and Cortellini et al. 2011 used M-MIST + Emdogain. The outcomes for subjects with postoperative pain at 1 week in the aforementioned order

were 46%, 50%, 30% and 0%. The pain intensity measurements were 28.1±2.5, 28±20, 19±10, and "not measured." Lastly, the numbers of analgesics consumed were 4.1±2.5, 4.3±4.5, 1.1±2, and 0.3±0.6. This comparison of studies suggests a less traumatic surgical experience from the perspective of the patient, but no study has directly compared the double flap approach to the single flap approach.

2.3.5 Double Flap Approach (DFA) and Single Flap Approach (SFA) with and without Grafting for Intrabony Defects: Clinical Studies

There are several clinical studies evaluating the use of the DFA and SFA with and without grafting. The studies vary in the quality of evidence in that some are retrospective and others are prospective, randomized controlled clinical trials, but they are consistently showing remarkable clinical outcomes using these flap designs.

There are a group of studies where the DFA was used in the form of MIS (minimally invasive surgery) or MIST (minimally invasive surgical technique), and all results can be seen in Table 1. In the DFA flap design, the entire papilla is elevated [33]. The outcomes from these seven studies show clinically significant levels of CAL gain and minimal changes in recession when MIS and MIST were used. In another group of studies, the Single Flap Approach (SFA) was used with treatment adjuncts, and in this surgical approach only one side of the interdental defect was accessed keeping the interdental papilla mostly intact. The results can be seen in Table 2. The four SFA studies consistently show excellent CAL gains with minimal amounts of recession, which are comparable and often superior to the outcomes seen in the literature for the DFA.

There are only two studies in the literature that include the DFA and SFA in the same clinical trial. The first study is a prospective study by Cosyn et al. where 95 patients were treated with either MIST or M-MIST and grafted with a collagen-enriched bovine-derived xenograft [77]. After 1-year of healing, the CAL gain was 3.1 and the change in recession was 0.5 mm. This study demonstrated that using MIS and collagen-enriched bovine-derived xenograft had favorable clinical outcomes in CAL gain and change in recession, and it also identified risk factors for failure. In the second study, Trombelli et al. conducted a Randomized Controlled Trial (RCT) to compare the SFA to the DFA in treating 28 patients with 28 intrabony defects without treatment adjuncts [9]. After six months of healing, the CAL gains for the SFA versus the DFA were 4.5 ± 1.1 and 3.4 ± 1.4 mm, respectively, and this was a statistically significant greater CAL gain for the SFA. For the change in recession, the SFA had 0.7 ± 0.8 mm and the DFA had 0.5 ± 1.1 mm, and there was no statistically significant difference.

The literature reports excellent clinical results using minimally invasive surgeries either as a double flap approach or a single flap approach. Moreover, the volume of evidence concludes that using these surgical approaches with treatment adjuncts also results in remarkable clinical outcomes, while it also suggests in some studies that the outcomes may be similar to the control groups where treatment adjuncts were not used. To date, there is only one randomized clinical trial directly comparing the SFA to the DFA. It is well established that currently available regenerative grafts and biologics may lead to a true regeneration of the periodontal tissues lost to periodontitis [78-81]. What is not known is whether a surgical approach like the SFA, which maintains space for clot maturation and enhances wound stability, will potentiate the regenerative efficacy of a

regenerative material for a greater clinical benefit in comparison to another approach like the DFA.

TABLE 1. Clinical studies where the double flap approach was used in conjunction with grafting and/or biologics

MIS/MIST	Type of study	Interventions	# of patients	# of Defects	CAL gain	Δ Recession
Harrel et al 1999 [82]	Retrospective	MIS+DFDBA	87	194	4.87±0.27	Not reported
Harrel et al 2005 [60]	Case cohort	MIS+EMD+DFDBA	16	160	3.57±1.75	0.01
Cortellini et al 2007 [61]	Case cohort	MIST+EMD	13	13	4.8±1.9	0.1±0.9
Cortellini et al 2007 [62]	Case cohort	MIST+EMD	40	40	4.9±1.7	0.4±0.7
Cortellini et al 2008 [83]	Case cohort	MIST+EMD	20	44	4.4±1.4	0.2±0.6
Ribeiro et al 2011 [84]	RCT	MIST MIST+EMD	15 14	15 14	2.82±1.19* 3.02±1.94*	0.54±0.58* 0.46±0.87*
Ribeiro et a 2011 [85]	RCT	MIST MINST	14 13	14 13	2.85±1.19* 2.56±1.12*	0.48±0.51* 0.45±0.46*

EMD, enamel matrix derivative; MIS, minimally invasive surgery; MIST, minimally invasive surgical technique; MINST, minimally invasive nonsurgical technique; DFDBA, Decalcified Freeze Dried Bone Allograft
*No statistical difference

TABLE 2. Clinical studies where the single flap approach was used in conjunction with grafting and/or biologic

SFA/M-MIST	Type of study	Interventions	# of patients	# of Defects	CAL gain	Δ Recession
Cortellini et al 2009 [65]	Case cohort	M-MIST+EMD	15	15	4.5±1.4	0.07±0.3
Cortellini et al 2011 [74]	RCT	M-MIST	15	15	4.1±1.4*	0.3±0.6*
		M-MIST+EMD	15	15	4.1±1.2*	0.3±0.5*
		M-MIST+EMD+BioOss	15	15	3.7±1.3*	0.3±0.7*
Trombelli et al 2010 [68]	RCT	SFA	12	12	4.4±1.5*	0.8±0.8*
		SFA+HA+GTR	12	12	4.7±2.5*	0.4±1.4*
Mishra et al. 2013 [86]	RCT	M-MIST	12	12	2.64±0.67*	0.55±0.52*
		M-MIST+rhPDGF-BB	12	12	3.00±0.89*	0.82±0.60*

EMD, enamel matrix derivative; M-MIST, modified minimally invasive surgical technique; BioOss®; SFA, single flap approach; HA, hydroxyapatite, GTR, guided tissue regeneration; rhPDGF-BB, recombinant human platelet derived growth factor BB
*No statistical difference

2.4 GEM-21 (rhPDGF-BB and β-Tricalcium Phosphate)

GEM-21 has been released as a promising therapeutic for periodontal regeneration of intrabony defects. The active component is recombinant human platelet-derived growth factor-BB and the carrier is β-Tricalcium Phosphate.

2.4.1 An Introduction to Polypeptide Growth Factors and PDGF-BB

Polypeptide growth factors, such as platelet derived growth factor (PDGF), are hormone-like substances that bind cell membranes receptors, trigger intracellular signaling pathways, and activate genes causing an alteration in cell activity [87, 88]. The major source of PDGF in the body is the α-granules of the platelets. PDGF is generally not detectable in the blood because it is rapidly cleared by PDGF-binding protein making its presence highly localized [89]. This important mechanism keeps distal tissues from inadvertently being exposed to the growth factor. PDGF is expressed as different isomers: AA, AB, BB, CC, DD. There are also two receptors, α and β, that bind to PDGF. Periodontal tissues specifically express β-receptors, and β-receptors have the highest affinity for the BB isomer of PDGF[90]. Consequentially, GEM-21 uses rhPDGF-BB isomer.

2.4.2 Mechanism of Action of rhPDGF-BB on Cells (*in vitro* studies)

Growth factors have been developed to enhance the regenerative potential of the periodontium. In particular, rhPDGF-BB has been combined with bone substitutes in vitro to show promising effects on cells of the periodontium. For example, in two studies human allografts were enriched with rhPDGF-BB to show positive effects on osteoblasts and periodontal ligament cells [91, 92]. In addition, Vavouraki et al. showed bovine bone

grafts with PDGF-BB stimulated PDL cells, and Bateman et al. showed two alloplastic bone matrices with PDGF-BB positively effected osteoblastic proliferation [93, 94]. There are several studies that have identified the mechanisms of action that rhPDGF-BB exerts on the cells of the periodontium.

Platelet-derived growth factor has been shown to have various effects on periodontal ligament cells (PDL cells). Nishimura et al. showed that PDGF induced a migratory response in a dose-dependent manner on PDL cells showing that PDGF had a chemotactic effect on PDL cells [95]. Two studies tested the effect of PDGF on PDL cell mitogenesis showing that PDGF had a mitogenic effect [96, 97]. Two studies investigated the effect of PDGF on matrix synthesis by PDL cells and their mitogenesis [98] [97]. PDGF was strongly mitogenic, and it also increased the production of matrix synthesis seen by an increase in proteoglycans measured (versican, biglycan, decorin). Lastly, Zaman et al. studied the effect of PDGF on the presence and attachment of human PDL cells to EDTA-demineralized dentin [99]. They showed that PDGF increased PDL cell attachment to the root, which is a prerequisite for periodontal ligament formation. These *in vitro* studies show that PDGF has a profound effect on PDL cell chemotaxis, mitogenesis, matrix synthesis, and PDL cell attachment to the root surface.

There are *in vitro* studies that also show an effect of PDGF on the other cells of the periodontium: osteoblasts and cementoblasts. In a study by Bateman et al., two alloplastic bone matrices enriched with PDGF-BB had a greater mitogenic effect on osteoblasts [93]. In another study, Park et al. showed that PDGF increased osteoblast mitogenesis and chemotaxis [100]. In regards to the effect of PDGF on cementoblasts, studies have investigated the effect of PDGF on cementoblasts *in vitro* and *ex vivo* [101,

102]. When PDGF was used, the results showed an increased mitogenic activity, and a level of biomineralization with greater incorporated neovascularization. The results of this series of studies showed that PDGF increases mitogenesis and chemotaxis of osteoblasts, and that it increases mitogenesis in cementoblasts while increasing neovascularized biomineralization.

2.4.3 The Use of β-Tricalcium Phosphate (β-TCP)

In vitro studies have elucidated the various effects that PDGF exerts on the cells of the periodontium by specifically showing the mechanisms through which it potentiates periodontal regeneration. Choosing the proper carrier for the growth factor depended on the performance of the bone substitute in experimental studies.

In two experiments by Stahl and Froum, the healing of intrabony defects grafted with β-TCP was evaluated with human histology after eight and eighteen months. At eight months, the β-TCP was shown to be a non-irritating filler, there was no inflammatory infiltrate, but the material did provide a scaffold for ingrowth of fibroblasts and showed encapsulation by connective tissue [103]. By eighteen months, particles remaining were surrounded by connective tissue without induction of inflammation, and it was almost fully resorbed [104].

In another study by Bateman et al., β-TCP enriched with PDGF was evaluated *in vitro* and *in vivo* in a mouse model, and the release of PDGF by the graft was measured [93]. The study showed after ten days, 45% had been released showing a slow, incremental release. Besides these studies, several animal and human studies have demonstrated the biocompatibility of the β-TCP with no reports of adverse reactions

[105]. β-TCP has been shown to be not only biocompatible, but also an effective carrier for PDGF. Also, after 18 months it has been shown to resorb almost completely without the presence of inflammation. The product GEM-21 was developed using β-TCP and rhPDGF-BB for the treatment of human intrabony defects.

2.4.4 Animal and Human Studies using rhPDGF-BB and β-TCP

PDGF has been topically applied to periodontally diseased root surfaces in beagle dogs, and substantial amounts of new bone, cementum, and periodontal ligament were present after two weeks [105-107]. These results were confirmed in three other studies using beagle dogs and also nonhuman primates [108-110]. The promising results from these animal studies lead to experimental case series in humans where rhPDGF-BB was used with bone allograft to treat Class II furcations and intrabony defects, and the clinical, radiographic, and histologic results were excellent [111-114].

These results prompted the organization of the multicenter randomized controlled trial where the safety and effectiveness of rhPDGF-BB and β-TCP was tested on intrabony defects in humans[4]. The results after six months of healing showed that β-TCP/0.3mg/ml of rhPDGF-BB had the greatest clinical benefit. In comparison to the β-TCP/Buffer control, β-TCP/0.3mg/ml of rhPDGF-BB had a statistically significant difference for linear bone gain and percent bone fill, but no difference for CAL gain. Moreover, there were no adverse events reported, and the authors concluded that the regenerative material was safe and showed a clinical benefit at six months.

In another study, McGuire et al. showed human histology for the use of rhPDGF-BB and β-TCP (GEM-21) [3]. All the sites treated with rhPDGF-BB and β-TCP (GEM-

21) had evidence of regeneration defined by reformation of cementum, supporting alveolar bone, and a periodontal ligament with inserting connective tissue fibers. The authors concluded regeneration of the periodontium was possible with rhPDGF-BB and β-TCP (GEM-21).

A more recent multi-center randomized clinical trial evaluated the safety and efficacy of a formulation containing rhPDGF-BB with β-TCP versus β-TCP alone in patients with intrabony defects [2]. All three measurements were statistically significant in favor of the rhPDGF-BB with β-TCP test group. The authors concluded that the treatment was safe because there were no adverse events reported, and that there was a clinically and statistically significant greater benefit using rhPDGF-BB with β-TCP.

2.4.5 Systematic Reviews: rhPDGF-BB and β-TCP

Two systematic reviews evaluated the clinical benefit of using rhPDGF-BB with β-TCP for treating human intrabony defects. Trombelli et al. found rhPDGF-BB in combination with a graft material showed beneficial effects in intrabony and furcation defects [6]. When rhPDGF-BB was used with allogenic bone grafts, substantial CAL gain and probing depth reduction were seen in case reports. One randomized clinical trial showed the efficacy of rhPDGF-BB with β-TCP for linear bone growth and percent defect fill. It was concluded that further studies are needed to determine to what extent rhPDGF-BB with graft may be effective for periodontal reconstructive procedures.

A more recent systematic review and meta-analysis evaluated the use of rhPDGF-BB with β-TCP for treating human intrabony defects [5]. A review of the literature resulted in two randomized clinical trials that qualified for a meta-analysis where

rhPDGF-BB with β-TCP (test) versus β-TCP alone (control) was used. For CAL gain at 3 months, the weighted mean difference in favor of the test group was 0.55 with a 95% CI [0.13, 0.96]. For CAL gain at 6 months, the weighted mean difference in favor of the test group was 0.62 with a 95% CI [0.23, 1.00]. For linear bone growth, the weighted mean difference in favor of the test group was 1.44 with a 95% CI [1.08, 1.80]. For percent bone fill, the weighted mean difference in favor of the test group was 24.87 with a 95% CI [15.40, 34.34]. The authors concluded within the limits of this systematic review and meta-analysis it appears that rhPDGF-BB with β-TCP has a greater clinical benefit than β-TCP alone, but more studies are needed to confirm this result.

In vitro studies have elucidated the various effects that PDGF exerts on the cells of the periodontium by specifically showing the mechanisms through which it potentiates periodontal regeneration. The clinical efficacy of rhPDGF-BB with β-TCP in treating human intrabony defects is promising in the small amount of studies published to date. More randomized clinical trials are needed to confirm these results.

2.5 Concluding Remarks on the Literature

Decades of research in periodontal regeneration have produced various materials and products as well as innovative surgical approaches resulting in clinically significant levels of regenerative success for patients. The success of regenerative therapy has been extensively reported and results have been shown to be stable over time in longitudinal clinical trials. Along with research into regenerative materials, a significant volume of research has investigated the innate regenerative potential of the periodontium. Progress in flap management has moved towards smaller, less traumatic surgical approaches with

the goal of maximizing the innate healing potential of the periodontium. The success of minimally invasive surgery has been confirmed in systematic reviews and meta-analyses, but to what extent one approach may have greater clinical benefit than another needs to be further investigated. To date, there is only one randomized clinical trial directly comparing the single flap approach (SFA) to the double flap approach (DFA), whereas, these approaches have been tested extensively in the literature with other treatment adjuncts. One recent regenerative material showing promise is GEM-21 (rhPDGF-BB with β-TCP). *In vitro* studies have elucidated the mechanisms through which it potentiates periodontal regeneration by acting on the cells of the periodontium, and randomized clinical trials and systematic reviews have confirmed its clinical and statistical benefit in treating intrabony defects. What is not known is whether a surgical approach like the SFA, which maintains space for clot maturation and enhances wound stability, will potentiate the regenerative efficacy of a treatment adjunct like GEM-21 for a greater clinical benefit in comparison to another surgical approach like the DFA. Moreover, will there be a more favorable patient-centered outcome with the more minimally invasive SFA?

3. Aim, Hypothesis, and Objectives

3.1 Aim

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.

3.2 Hypothesis

The treatment of intraosseous periodontal defects with single flap approach + rhPDGF-BB/β-TCP will improve clinical, radiographic, and patient-centered outcomes when compared with the use of a double flap approach + rhPDGF-BB/β-TCP.

3.3 Objectives

3.3.1 Primary Objectives

To evaluate the clinical outcome (CAL, PD, Recession (REC)) after treatment of intraosseous periodontal defects with SFA+ rhPDGF-BB/β-TCP versus DFA+ rhPDGF-BB/β-TCP.

To evaluate patient perception of pain and discomfort between SFA+ rhPDGF-BB/β-TCP versus DFA+ rhPDGF-BB/β-TCP.

3.3.2 Secondary Objectives

To evaluate radiographic bone changes after treatment of intraosseous periodontal defects with SFA+ rhPDGF-BB/β-TCP versus DFA rhPDGF-BB/β-TCP.

To evaluate the quality of wound closure after using SFA+ rhPDGF-BB/β-TCP versus DFA+ rhPDGF-BB/β-TCP.

4. Study Design and Procedures

4.1 Study Design

The study was a randomized, controlled parallel-arm trial. Patients were recruited at the Division of Periodontology, School of Dental Medicine, University of Connecticut (Farmington, CT). Each patient contributed one intraosseous periodontal defect to the trial. All intraosseous defects were grafted with rhPDGF-BB (0.3 mg/ml) + β-TCP. The clinical procedures differed between groups for the soft tissue management (SFA or DFA) only. Flow chart and visit schema are reported in Figure 1. For Visit 6 the clinical parameters included clinical attachment level (CAL), probing depth (PD), recession (REC), bleeding index (BI), plaque index (PI), and bleeding sites (BS). For Visit 11, the clinical parameters included CAL, PD, REC, and BS.

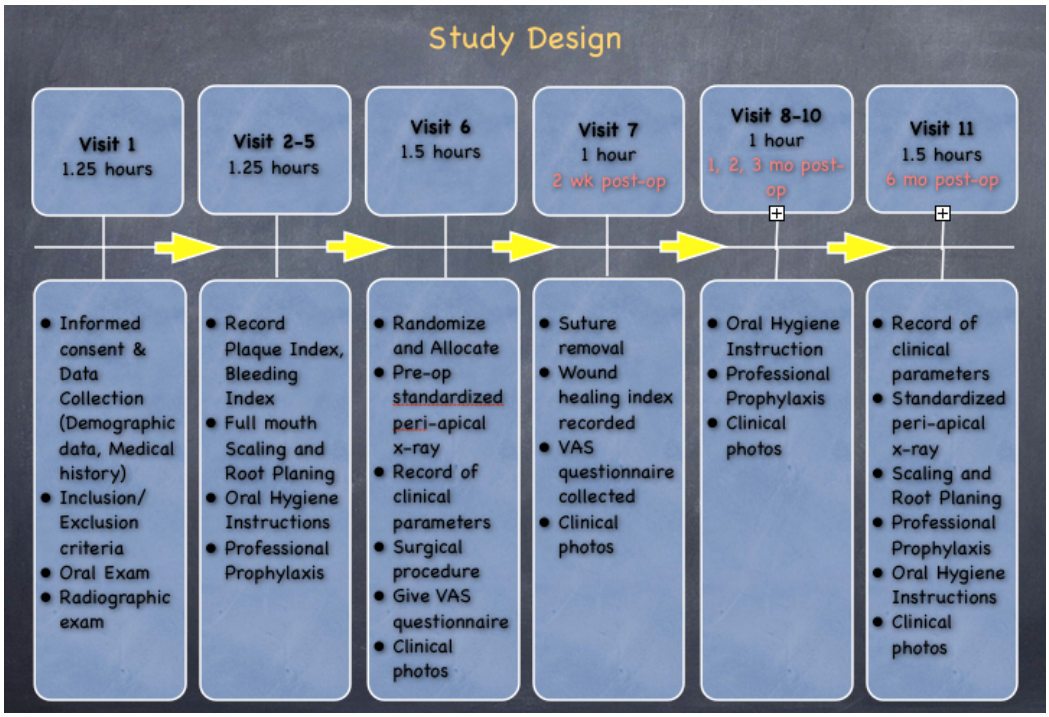

Figure 1: Flow chart and visit schema.

4.2 Screening Procedures

The subjects for the investigation were recruited among patients diagnosed with chronic or aggressive periodontitis in the post-graduate periodontology clinic at University of Connecticut Health Center. An initial evaluation was conducted to determine whether a patient met the study inclusion criteria. This evaluation included medical history, clinical examination, and radiographic examination (from existing full mouth x-ray or orthopantomograms).

Inclusion and exclusion criteria are summarized in Table 3.

Table 3: Inclusion and exclusion criteria.

Inclusion Criteria	Exclusion Criteria
• Males and females at least ≥ 18 years of age • Provision of informed consent • Diagnosis of chronic or aggressive periodontitis • Presence of at least one deep (probing depth ≥ 6mm, radiographic depth of ≥ 3mm) interproximal intraosseous periodontal defect; limited to no extension of the defect on the lingual/palatal side as assessed by	*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

bone sounding • Full Mouth Plaque Score [115] and Full Mouth Bleeding Score < 20% at the time of the surgical procedure • Third molars will be excluded	*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 • Active carious lesion

4.3 Pre-surgical Procedures

Each patient had a full-mouth session of scaling and root planing (SRP) using mechanical and hand instrumentation, and they 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. Up to four sessions of SRP were considered to achieve this goal. Patients were excluded from the trial if after this period the plaque index and bleeding index were higher than 20%.

4.4 Allocation and Allocation Concealment

Every patient was given a subject identification number. An independent investigator, not involved with patient treatment, generated the allocation list. Computer software was used to randomize the subject identification numbers into one of the two groups. This information was concealed in sealed envelopes, which were opened before the surgical treatment. Neither the surgeon, nor the patient was aware of the group assignment until the day of surgery.

In addition, a calibrated examiner masked with respect to treatment allocation performed the clinical measurements.

4.5 Surgical Procedures

The same experienced operator 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 the defect morphology and extension, the probing bone level, and the horizontal component of bone loss. Defects with an

extension past the line angles determined by bone sounding were treated with the DFA but excluded from the study.

4.5.1 Single Flap Approach Group

In the SFA group, the surgical access was performed by the elevation of either a buccal or lingual mucoperiosteal flap according to previously detailed principles [35, 63, 64]. Briefly, a buccal or lingual 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 or lingual incision in the interdental area. However, the interdental incision was placed at least 1 mm coronal to the underlying bone crest. This provided an adequate amount of supracrestal soft tissue connected to the undetached papilla to ensure subsequent flap adaptation and suturing, and it permitted proper surgical access to the defect. For each defect, a microsurgical periosteal elevator was used to raise a flap only on the buccal or lingual side, leaving the other portion of the interdental supracrestal soft tissues undetached. Root and defect debridement were performed using Minifive™(Hu-Friedy) curettes and ultrasonic instruments. After surgical debridement, defects were grafted with rhPDGF-BB + β-TCP (GEM 21S®; BioMimetic Therapeutics, Franklin, TN). β-TCP was mixed with rhPDGF-BB (0.3 mg/ml) and allowed to sit for □ 10 minutes to permit binding of the rhPDGF-BB protein to the β-TCP before being placed into the defect. For wound closure, a horizontal

internal mattress suture (Monosoft 6.0 Covidien Mansfield MA, USA) was placed between the flap and the base of the attached papilla to ensure repositioning of flap. A second internal mattress suture (vertical or horizontal) was placed between the most coronal portion of the flap and the most coronal portion of the papilla as needed. See Figure 2 for an illustration of the technique.

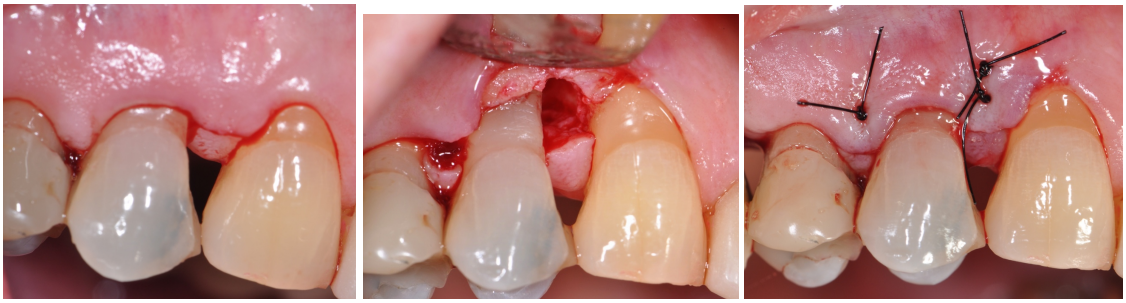

Figure 2: Surgical steps for the Single Flap Approach.

4.5.2 Double Flap Approach Group

In the DFA group an envelope flap without vertical releasing incisions was performed at both buccal and lingual aspects. Sulcular incisions were placed following the gingival margin of the teeth included in the surgical area. The defect-associated interdental tissue was approached with surgical techniques for the preservation of the interdental papilla, namely the simplified papilla preservation flap (SPPF) [57] or the modified papilla preservation technique (MPPT) [56]. The selection of the flap design depended on the operator's evaluation [116]. The mesio-distal extension of the flaps was minimized while maintaining an adequate surgical access for a proper root and defect debridement. Partial-thickness dissection was limited to the apical portion of the buccal flap to obtain the desired flap mobilization and ensure tension-free suturing. Root and

defect debridement were performed using hand and ultrasonic instruments. After surgical debridement, defects were grafted with rhPDGF-BB + β-TCP (GEM 21S®; BioMimetic Therapeutics, Franklin, TN). The material was prepared as previously described. When MPPT was applied, the flaps were sutured using horizontal and vertical internal mattress (or interrupted) sutures [56]. A horizontal internal mattress suture was placed between the base of the lingual flap and the buccal flap coronal to the mucogingival junction in order to coronally displace the buccal flap. Then, a vertical internal mattress suture (or an interrupted suture) was placed between the most coronal portion of the lingual flap, which included the interdental papilla, and the most coronal portion of the buccal flap. See Figure 4 for an illustration of the MPPT. When the SPPF was applied, primary closure was achieved as previously described [57]. First, a horizontal internal mattress suture was placed in the defect-associated interdental space, from the base of the gingiva (close to the mucogingival junction) at the mid-buccal aspect of the tooth not involved by the defect to a corresponding location at the base of the lingual flap. This suture rested on top of the highest peak of the interdental bone crest and was anchored to the lingual flap. This "offset" suture allowed for tension-free coronal positioning of the buccal flap by rubbing against the root surface and lying on top of the residual bone crest. The interdental tissue above the defect was then closed with one or two interrupted sutures or an internal vertical mattress suture (as in MPPT), depending on the width of the interdental space and the thickness of the interdental tissue [57]. See Figure 3 for an illustration of the SPPF. In all cases a non-resorbable monofilament suture (Monosoft 6.0 Covidien Mansfield MA, USA) was used.

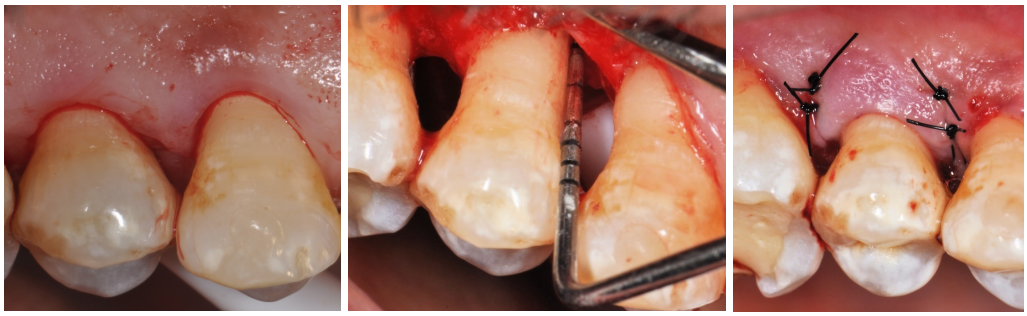

Figure 3: Surgical steps for the Double Flap Approach, specifically the Simplified Papilla Preservation Flap (SPPF).

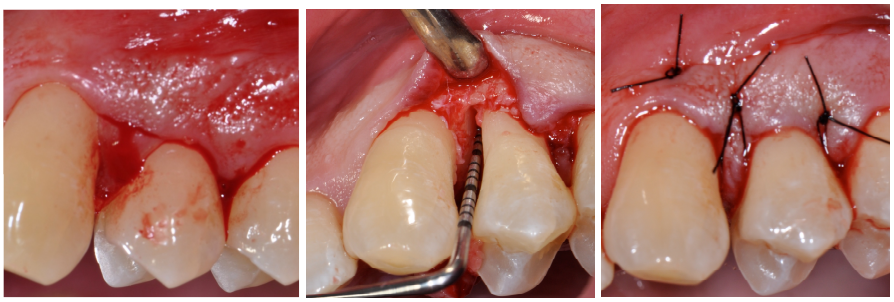

Figure 4: Surgical steps for the Double Flap Approach, specifically the Modified Papilla Preservation Technique (MPPT).

4.6 Post-surgery Procedures

At the end of each session, patients were prescribed a rescue analgesic (Ibuprofen 600 mg) to control post-treatment pain and discomfort. 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/2 wks) was used to support local plaque control. 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 months post-surgery.

4.7 Sample Size and Power Analysis

The sample size was calculated assuming a standard deviation of CAL change of 1.5 mm. A minimum sample size of 24 patients (12/group) was needed to detect a difference in CAL change of 1.5 mm between groups, using a parametric test with a 0.05 two-sided significance level at a statistical power of 87%.

5. Data Collection

5.1 Training and Calibration

Before the study initiation, a calibration session was performed to evaluate (i) the intra-examiner agreement in the assessment of clinical recordings and (ii) the intra- and inter-examiner agreement in the assessment of radiographic measurements.

5.2 Clinical Records

Calibrated masked examiners performed all clinical recordings. The following parameters were recorded on the treated tooth and the two adjacent teeth if applicable. This was done immediately pre-surgery and at the completion of study at 6 months using a manual pressure sensitive probe (UNC 15, Hu-Friedy, Chicago, IL, USA) with 1-mm increments and using approximately 0.3-N force. Measurements were rounded off to the nearest mm.

- Probing depth (PD), measured from the gingival margin to the bottom of the pocket;
- 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;
- Local bleeding score (BS): recorded as positive when bleeding on probing was present at the surgical site.

PD, CAL and REC were recorded at six aspects per tooth: mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, and disto-lingual.

5.3 Full Mouth Bleeding Score

Four sites per tooth were evaluated for bleeding on probing. The total number of positive sites was divided by the total number of sites and presented as a percentage. A score of ≤ 20% was needed to proceed to the surgical phase.

5.4 Full Mouth Plaque Index

Four sites per tooth were evaluated for the presence or absence of dental plaque. The number of positive sites was divided by the total number of sites and presented as percentage. A score of ≤ 20% was needed to proceed to the surgical phase.

5.5 Intra-surgical Measurements

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. In addition, the configuration of the defect with respect to the number of bony walls was also recorded.

5.6 Early Wound-healing Index

At suture removal, 2 weeks post-surgery, any complications were recorded. Wound closure was evaluated using the early wound-healing index [117]:

1 - complete flap closure – no fibrin line in the inter-proximal area;

2 - complete flap closure – fine fibrin line in the inter-proximal area;

3 - complete flap closure – fibrin clot in the inter-proximal area;

4 - incomplete flap closure – partial necrosis of the inter-proximal tissue;

5 - incomplete flap closure – complete necrosis of the interproximal tissue.

5.7 Radiographic Measurements

Standardized radiographs were obtained immediately before surgery as well as at 6 months after surgery using a bite plane indexed with composite material. The clinical examiner assessed the quality of the films prior to the release of the patient to ensure diagnostic quality. Radiographs were sent to a blinded operator. The films were digitized, and linear radiographic measurements were analyzed for bone changes. The following linear measurements were taken on the pre-surgery and 6-month digitized periapical radiographic images:

- Distance (in mm) between the CEJ and the most apical extension of the defect (i.e., where the periodontal ligament space is considered having a normal width);
- Distance (in mm) between the CEJ and the bone crest of the adjacent tooth;
- Distance (in mm) between the CEJ and the root apex.

For each digitized radiograph, linear bone growth (LBG) was calculated as CEJ to base of the defect at baseline minus CEJ to base of the defect at 6 months. Also, percent bone fill was calculated by dividing LBG by the depth of the original bone defect.

All radiographs to date have been sent to the blinded examiner who will complete the measurements once all the patients have finished the study. These measurements will not be available for the purpose of the present thesis.

5.8 Patient Perception of Pain

A visual analog scale (VAS, 100 mm) was used to assess the subject's self-perceived pain (VAS). Assessments were performed immediately after surgery, at 8 a.m., 1 p.m. and 8 p.m. on the 1st, 2nd and 3rd postoperative day, and at 8 p.m. on the 4th, 5th and 6th, 7th and 14th postoperative day.

For the use of self-administered anti-inflammatory drugs/analgesics, the patients were asked to record any postoperative consumption (timing, dosage) of the rescue analgesic.

5.9 Method of Data Analysis

Statistical software (SPSS v.18; Chicago, IL) was used for data analysis. The patient was regarded as the statistical unit. The aspect of the tooth topographically related to the intraosseous defect presenting the largest CAL value pre-surgery was used for comparisons and statistical analysis of outcome variables. Data were expressed as mean ± standard deviation (SD).

The Kolmogorov-Smirnov's test was used to assess the normal distribution of each parameter. Intra-group comparisons were performed with the Paired t test and the Wilcoxon signed-rank test for parametric and non-parametric variables, respectively. Inter-group comparisons were performed with the Independent t test and the Mann Whitney rank-sum test for parametric and non-parametric variables, respectively. For nominal and ordinal data the Chi-square test and Mann-Whitney rank-sum test were used, respectively. The level of significance was set at 5% for all statistical tests.

6. Results

6.1 Study Population

The patient flow and allocation during the study are reported in Figure 5.

A total of 29 patients were treated. All patient healing was uneventful. One patient was excluded from the statistical analysis because of an endodontic complication detected at visit 11. As per intent to treat, the data relative to this participant until Visit 11 were included in the analysis. In the present interim report we are presenting data relative to 22 patients completed. Six patients are still in the recall stage. For the 22 patients who completed the study, the characteristics of the study population are summarized in Table 4. There was no significant difference between the groups for age (P=0.5 Independent T-Test), smokers (P=1.0 Chi-squared Test), full mouth bleeding score (FMBS) (P=0.2 Independent T-Test), and full mouth plaque score (FMPS) (P=0.4 Independent T-Test). Defect characteristics were summarized in Table 5. There was no significant difference between the groups for depth of the intraosseous component of the defect (IBD)

(*P*=0.053 Independent T-Test). None of the patients in the SFA group were excluded from the study because of an insufficient surgical access or an extension of the defect morphology preventing an adequate root and defect instrumentation. All 22 patients who completed the study fully complied with the recall program. All defects were reevaluated at 6-months post-surgically.

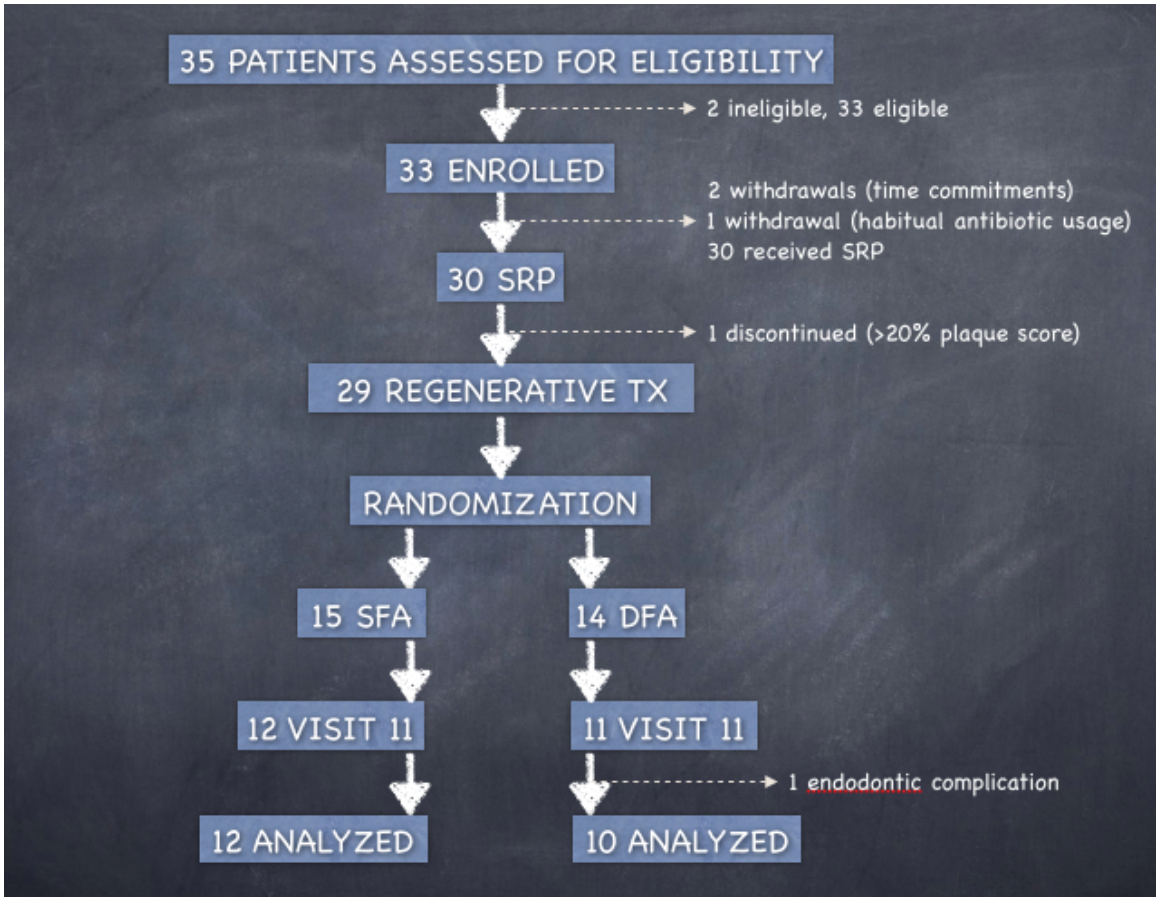

Figure 5: Patient flow and allocation of the study

Table 4: Study Population

Characteristic	SFA (n = 12)	DFA (n = 11)	P
Gender (M/F)	7/5	6/5	
Age (mean ± SD)	55.1 ± 13.8	48.0 ± 13.8	.5
Smoker (yes/no)	2/10	0/10	1.0
FMBS (mean ± SD)	8.0 ± 6.1	5.8 ± 5.9	.2
FMPS (mean ± SD)	8.8 ± 6.0	6.4 ± 6.5	.4

FMBS = Full Mouth Bleeding Score; FMPS = Full Mouth Plaque Score

Table 5: Defect characteristics (mean ± SD)

Characteristic	SFA (n = 12)	DFA (n = 11)	*P*
Dental Arch (n)			
Maxillary	6	5	
Mandibular	6	6	
Tooth type (n)			
Incisor	3	2	
Canine	0	2	
Premolar	5	3	
Molar	4	4	
IBD (mm)	7.1 ± 2.08	5.8 ± 1.69	.053

IBD = intraosseous component of the defect

6.2 Clinical Measurements

Baseline and 6-months values of the clinical parameters are reported in Table 6. Figures 6-8 compare SFA and DFA from baseline to 6-months. CAL significantly

decreased from baseline to 6-months with the SFA (P=0.002 Wilcoxon Signed Ranks Test) and the DFA (P=0.005 Wilcoxon Signed Ranks Test). There was no significant difference between the groups (P=0.5 Independent T-Test).

PD significantly decreased from baseline to 6-months with the SFA (P=0.002 Wilcoxon Signed Ranks Test) and the DFA (P=0.002 Wilcoxon Signed Ranks Test). The change from baseline to 6-months for the SFA and DFA were 4.1 ± 1.8 and 3.9 ± 1.0, respectively. There was no significant difference between the groups (P=0.9 Mann-Whitney Test).

There was no significant increase in REC from baseline to 6-months for the SFA (P=1.0 Wilcoxon Signed Ranks Test) or DFA (P=0.3 Wilcoxon Signed Ranks Test). The change from baseline to 6-months for the SFA and DFA were 0.0 ± .8 and -.3 ± 1.4, respectively. There was no significant difference between the groups (P=0.9 Mann-Whitney Test).

There was no significant difference for Bleeding Sites (BS) between baseline and 6-months for the SFA (P=0.08 Chi-squared Test) and DFA (P=1.0 Chi-squared Test) groups.

The Early Healing Index (EHI) summarized by type was reported for SFA and DFA in Table 7. There was no significant difference between the groups (P=0.9 Mann-Whitney Test).

6.3 Patient-Centered Outcomes

Average self-perceived pain VAS score and the total number of analgesic doses consumed are reported for SFA and DFA in Table 8. There was a significant difference between the groups for average VAS score with the SFA groups reporting a lower level of pain (P=0.01 Mann-Whitney Test). There was no significant difference between the groups for the total number of analgesic doses (P=0.09 Mann-Whitney Test).

Table 6: CAL (mean ± SD), PD (mean ± SD), REC (mean ± SD), BS (frequency).

Parameter	Baseline (mm)	6 Months (mm)	*P*	Change (mm) Baseline – 6 Months
CAL				
SFA	9.6 ± 2.6	5.2 ± 2.9	.002	4.3 ± 2.3
DFA	8.3 ± 1.5	4.9 ± 1.7	.005	3.7 ± 1.4
P				.5
PD				
SFA	8.4 ± 1.9	4.1 ± 1.3	.002	4.1 ± 1.8
DFA	7.9 ± 1.5	4.0 ± 1.5	.002	3.9 ± 1.0
P				.9
REC				
SFA	1.2 ± 1.4	1.3 ± 1.7	1.0	0.0 ± .8
DFA	.4 ± .8	.9 ± 1.6	.3	-.3 ± 1.4*
P				.8
BS (positive/negative)				
SFA	6/6	6/6	.08	
DFA	8/3	2/8	1.0	
P				

* Negative value for Recession represents an increase in recession.

Table 7: Early Healing Index

Early Healing Index (Type)	SFA (n = 12)	DFA (n = 11)	*P*
1	9	7	
2	1	2	
3	0	1	
4	2	0	
5	0	1	
Median	1.0	1.0	.9

Table 8: Patient-centered outcomes.

Parameter	SFA	DFA	*P*
VAS Score (mean ± SD)	2.1 ± 3.0	14.0 ± 17.8	.01
Total # of Analgesic Doses (Mean ± SD)	3.2 ± 5.9	9.6 ± 13.2	.09

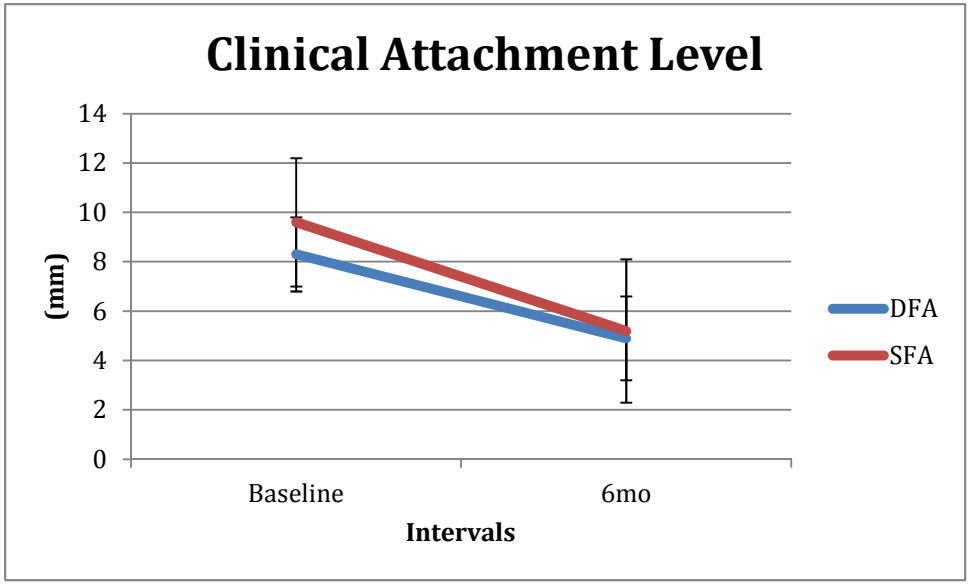

Figure 6: CAL from baseline to 6-months for SFA and DFA.

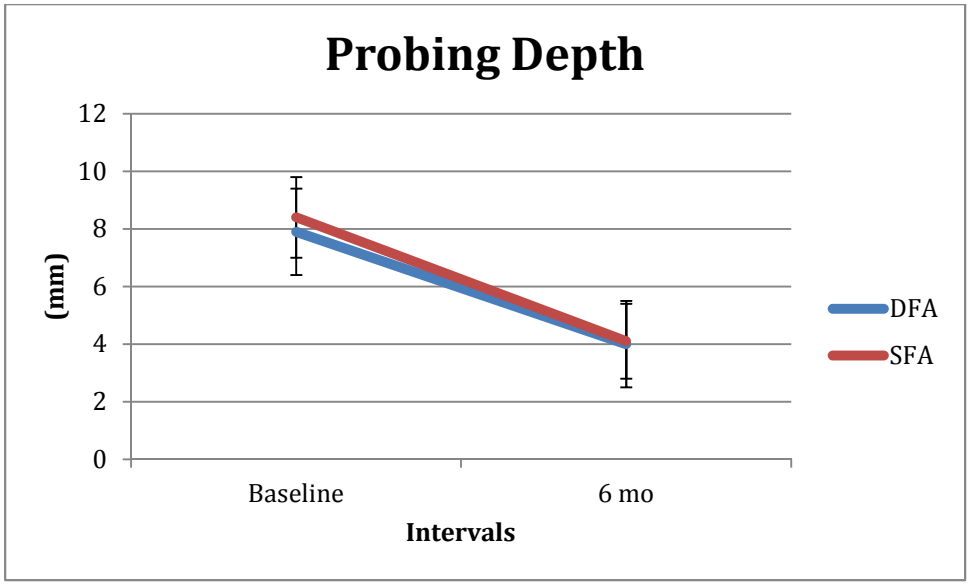

Figure 7: PD from baseline to 6-months for SFA and DFA.

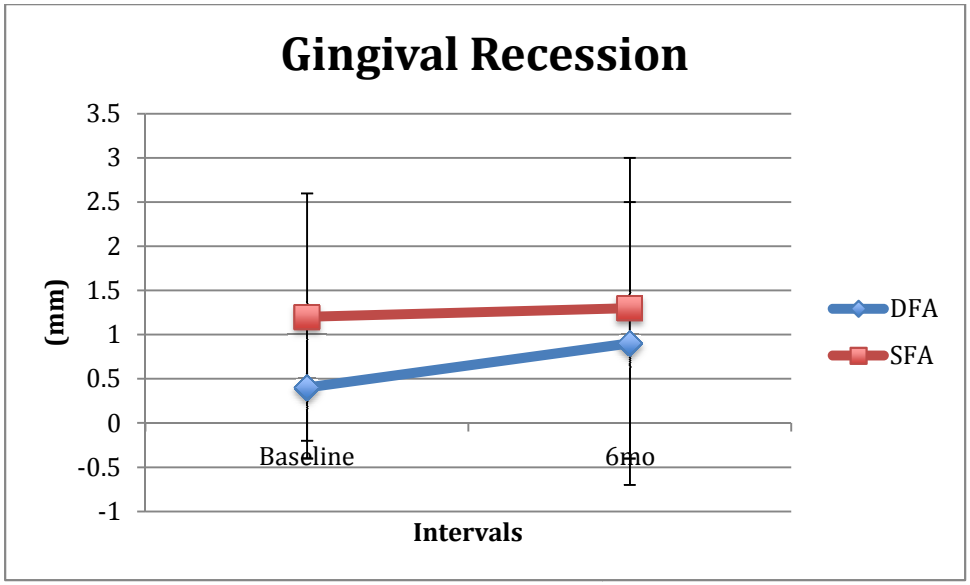

Figure 8: REC from baseline to 6-months for SFA and DFA.

7. Discussion

The present study compares clinical and patient-centered outcomes of intraosseous periodontal defects accessed with either the SFA or DFA and grafted with rhPDGF-BB + β-TCP. For this interim report we present data from 12 patients treated with the SFA, and 10 patients treated with the DFA. None of the patients were excluded due to inadequate access and debridement. All the defects received a graft, and primary wound closure was achieved.

The present study was based on the assumption that a minimally invasive approach like the SFA, which maintains space for clot maturation and enhances wound stability, would potentiate the regenerative outcome of a treatment adjunct like GEM-21 for a greater clinical benefit in comparison to another surgical approach like the DFA.

The analysis showed the SFA and DFA combined with rhPDGF-BB + β-TCP resulted in significant CAL gains and PD reductions at 6 months post-surgery compared to baseline, and they were similarly effective when compared for the change between baseline and 6-months. These results are consistent with a previously published RCT using SFA vs. DFA without the use of any treatment adjuncts [9]. In a recent systematic review, Graziani et al. showed the greatest CAL gain when the SFA and the DFA were used in comparison to less conservative surgical approaches [7]. Moreover, the additions of treatment adjuncts like Demineralized Freezed Dried Bone Allograft (DFDBA) and EMD with the DFA [60-62, 82-84] as well as the use of EMD, BioOss, and HA/GTR with the SFA have resulted in significant CAL gains, which is consistent with the results of the present report [65, 68, 74]. It is well established that these conservative surgical

approaches when used alone or with treatment adjuncts have resulted in significantly improved clinical outcomes. The regenerative material, GEM-21, has been shown *in vitro* to have various effects on PDL cells, osteoblasts, and cementoblasts such as enhancing mitogenesis and chemotaxis [93, 95-97, 100-102]. Furthermore, a recent systematic review and meta-analysis evaluated the use of rhPDGF-BB with β-TCP for treating human intrabony defects, and they showed significant CAL gain, linear bone growth and percent bone fill [7]. Both the present study and Trombelli et al. 2012 have exceeded the reported means for CAL gain reported by Graziani et al. 2012. It can be speculated that the level of CAL gain achieved with these approaches may be due to smaller, less traumatic surgery that maximizes the innate healing potential of the periodontium by providing stability for the blood clot and wound, and also by achieving primary wound closure for blood clot protection.

The minimally invasive approaches used in this study may have minimized the surgical trauma during the manipulation of the soft tissues resulting in no significant increase in REC from baseline to 6-months post-surgery for both the DFA and SFA. While intergroup comparisons showed no significant difference, there was a trend for a smaller increase in REC for the DFA compared to the SFA. It should be considered that no difference between groups might be due to the small sample size of this interim report. The SFA maintains the interdental, supracrestal, gingival tissue intact, and it facilitates flap repositioning and suturing by utilizing the undetached papilla as anchorage; thus, optimizing wound closure for primary intention healing. Moreover, limiting the surgical trauma on the papilla diminishes the vascular impairment that occurs as a result of trauma, which may partly explain a trend for minimal recession seen with the SFA. This

speculation was supported by the results of Retzepi et al. that showed the conservative simplified papilla-preservation flap (SPPF) having a faster restoration of the normal perfusion of the gingival tissues compared to sites accessed with the less conservative modified Widman flap design [73]. The present study resulted in less post-surgical recession than the aforementioned study where SFA and DFA were used without treatment adjuncts [9]. This may suggest that by using rhPDGF-BB + β-TCP for space provision it provided support for the interdental, supracrestal tissue preventing tissue collapse, which otherwise would result in increased REC due to post-surgery tissue remodeling. Trombelli et al. when comparing the SFA versus the SFA with GTR/Hydroxyapatite graft also observed this trend [68]. The grafted group had slightly less post-surgical REC although it was not significantly different.

There was no significant difference between the SFA and DFA in the quality of early wound healing as both groups reported a median of Type 1 using the Early Wound Healing Index [117]. Type 1 is the best quality healing, and it is described as complete flap closure with no fibrin line in the inter-proximal area. This can be partly due to the surgical design ensuring primary closure at the time of surgery for both approaches. The SFA maintains the interdental, supracrestal, gingival tissue intact, and it facilitates flap repositioning and suturing by utilizing the undetached papilla as anchorage. The DFA mobilizes the flaps on both sides of the defect allowing for passive coronal displacement of the flaps for primary closure. Minimal levels of dehiscence and better quality healing using the DFA (SPPF and MPPT) and the SFA have been reported in other studies [61, 62, 64, 65]. In two studies, the SFA/M-MIST was compared to the SFA/M-MIST with treatment adjuncts, and the authors reported more incidences of poorer quality healing or

dehiscence in the grafted groups [68, 74]. This is contrary to the outcome observed in the present study, which may indicate that the minimally invasive surgical approaches used enhanced the regenerative potential GEM-21 allowing rhPDGF-BB to potentiate the speed and quality of healing. Retzepi et al. showed a faster restoration of the normal perfusion of the gingival tissues when a more minimally invasive surgical approach is used, and vascularity is one determinant of the speed and quality of healing [73]. In addition, studies have shown that rhPDGF-BB upregulates angiogenesis by necessitating recruitment and differentiation of pericytes [118-120]. These are smooth muscle-like cells that regulate the morphology and function of vessels.

Regarding the patient-centered outcomes, the SFA group reported a significantly lower level of pain than the DFA group as assessed by a VAS scale delineating self-perceived pain. While there was no significant difference between groups for the number of analgesic doses consumed, there was a trend for less consumption in the SFA group. It should be considered that no difference between groups might be due to the small sample size of this interim report. A review of the literature indicates several studies have reported the patient's pain intensity as measured by a visual analogic scale (VAS), and the number of analgesics consumed when either a DFA or SFA were used [33, 61, 74-76]. A comparison of the studies shows a lower pain score and less consumption of analgesics when the SFA/M-MIST was used. This was confirmed through a direct comparison between the SFA and DFA in the present RCT, and this suggests that the elevation of only a buccal or lingual flap rather than both is a less traumatic surgical experience from the perspective of the patient.

In the present study, only intraosseous defects limited to the interdental space and not extending past the line angles were selected. This inclusion criterion created the proper clinical conditions for the application of the SFA. As a result, the conclusions drawn from this study are only applicable in clinical situations reflecting the selection criteria suitable for the SFA. Any lack of significance found in this interim report should be taken with caution, and these results need to be confirmed once all the data are collected for all subjects.

8. Conclusion

The results of the study indicate that the surgical debridement of deep intraosseous periodontal defects using the SFA and DFA and then grafting with rhPDGF-BB + β-TCP resulted in significant CAL gains, PD reductions, and no significant increase in REC. Utilizing both of these treatment approaches resulted in a superior quality of healing at 2-weeks post-surgery. Within the limitations of the study, it appears that the SFA with rhPDGF-BB + β-TCP results in excellent clinical outcomes while minimizing post-surgery morbidity from the perspective of the patient.

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