REVIEW

Combination therapeutic strategies to improve the outcomes of reconstructive periodontal surgery

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Abstract

Substantial evidence from the literature indicates that following active periodontal therapy, persisting deep (≥ 6mm) residual periodontal pockets associated with bleeding on probing are risk factors for tooth loss. Therefore, from a clinician's point of view, the main goal of periodontal therapy is considered to be the presence of shallow pockets (≤ 5 mm) and absence of bleeding on probing, preferably with limited or no soft tissue recession. Deep periodontal pockets are frequently associated with intrabony defects which, in most cases, do not completely resolve following non-surgical or conventional surgical therapy. Even though periodontal infection can be successfully treated by means of nonsurgical therapy, intrabony defects often remain as a sequellae and are associated with deep localized residual pockets. Despite the fact that resective surgery can be successfully used to completely eliminate intrabony defects, these techniques are inevitably associated with substantial loss of hard and soft tissues leading to impaired esthetics, chewing comfort and increased hypersensitivity. Thus, the ideal goal of treating intrabony defects is the regeneration of the tooth`s supporting structures (i.e. formation of new root cementum with functionally orientated inserting periodontal ligament fibers connected to new alveolar bone), which should manifest clinically in shallow pockets, absence of bleeding on probing and no or minimal soft tissue recession. Over the past 20 years, many advancements including biologics, membranes and bone grafting materials have been made to predictably regenerate intrabony defects to additionally improve the clinical outcomes obtained with conventional periodontal surgery. Recent evidence indicates that the combination of different regenerative materials may additionally improve the clinical outcomes in defects with a complicated anatomy. The aim of the present review article was to summarize the potential additional effects of various combination procedures used in reconstructive periodontal surgery of intrabony defects as compared with those obtained with conventional flap procedures or monotherapies on periodontal pockets associated with intrabony lesions. The data appear to indicate that in defects with a complicated anatomy, certain combination approaches such as: bone grafting materials and biologics (i.e. enamel matrix proteins or recombinant platelet derived growth factor) or bone grafting material and membranes may not only result in periodontal regeneration but also in additional clinical improvements in terms of pocket depth reduction and clinical attachment gain compared to conventional periodontal surgery or the use of mono-therapies.

Keywords: Intrabony defects, periodontal regeneration, intrabony defects, enamel matrix derivative, EMD, growth factors, guided tissue regeneration

Introduction

Periodontal disease, which begins as superficial inflammation of the gingiva without attachment or bone loss (gingivitis) and later progresses to attachment loss with subsequent bone destruction (periodontitis), is one of the leading infectious diseases known to mankind. Results from a national survey conducted in the United States in 2010 analyzing the distribution of the disease found that over 47% of the adult population was affected [1]. Furthermore, 38.5% of the population had either moderate or severe forms of periodontitis, which are more difficult and cost-intensive to handle due to the advanced loss of the tooth's supporting apparatus and subsequently resulting niches that make infection control challenging [1]. Thus, it becomes vital for healthcare providers to diagnose the disease as early as possible and provide appropriate treatment. It has been extensively documented that periodontal infections can be successfully treated by non-surgical and surgical periodontal therapy associated with meticulous oral hygiene. Moreover, longitudinal studies have provided conclusive evidence on the effectiveness of periodontal therapy in the presence of a rigorous maintenance program demonstrating successful outcomes over 30 years [2]. On the other hand, substantial evidence indicates that persistence of residual periodontal pockets of

≥ 6 mm and presence of bleeding on probing following completion of active (non-surgical or surgical) periodontal therapy is associated with an increased risk of attachment and tooth loss [3,4].

Even though periodontal infection can be successfully treated by means of non-surgical therapy, intrabony defects often remain as a sequellae and are associated with deep localized residual pockets [5,6]. Moreover, periodontal pockets presenting with intrabony defects have been shown to deteriorate long-term tooth prognosis and, when left untreated, to increase tooth-mortality [7]. Despite the fact that various conventional surgical techniques have been successfully used to completely eliminate or reduce intrabony defects, such approaches are inevitably associated with significant gingival recession leading to impaired aesthetics and root hypersensitivity [8]. Therefore, the ideal goal of treating intrabony defects is the regeneration of the tooth's supporting structures (i.e. formation of root cementum with functionally orientated inserting periodontal ligament fibers connected to new alveolar bone), which should manifest clinically in shallow pockets (≤ 5mm), absence of bleeding on probing and no or minimal soft tissue recession [9]. Recent evidence indicates that the combination of different regenerative materials may additionally improve the clinical outcomes in defects with a complicated anatomy [9].

The aim of the present review article was to provide the biologic rationale and the clinical relevance in terms of pocket reduction/resolution of using various approaches in the regenerative therapy of intrabony defects.

Biologic Agents/Growth Factors

It has long been speculated that the use of biological agents/growth factors could accelerate wound healing and tissue regeneration [10]. A variety of novel research in the mid-1990s attempted to achieve these goals utilizing growth factor delivery. Due to their fluid consistency, growth factors are utilized in conjunction with various types of carriers or scaffolds such as bone grafts or collagens and therefore, these effects are discussed later in this manuscript [11].

Role of grafting materials in Periodontal Regeneration

Substantial literature is available on the role of various types of bone grafting materials for supporting periodontal regeneration in intrabony defects and decreasing or eliminating residual pockets [12,13]. Originally grafts were developed to serve as a passive, structural supporting network with the main criteria being biocompatibility. Advancements in tissue engineering have allowed for a large array of bone grafting materials, each possessing various advantages and disadvantages. It is now estimated that the global market for bone grafting procedures has surpassed $2.5 billion with over 2.2 million procedures performed annually [14]. As such, the need for better biomaterials becomes vital due to the aging population and the number of bone grafting procedures for diseases such as osteoporosis, arthritis, tumors or trauma performed worldwide each year [15]. Bone grafting materials are typically classified under 3 main areas, that of osteoconduction, osteoinduction and osteogenesis [11]. However, it is important to point out that human histologic evidence has shown high variability among the various types of bone grafts in promoting regeneration of periodontal ligament, root cementum and bone [12]. While certain types of grafts such as autogenous bone, allografts or certain types of xenografts have been shown to support, at least to a certain extent, the healing process, synthetic grafts such as hydroxyapatite, beta tricalcium phosphate, polymers or bioactive glasses appear to have limited to no biologic effects when used in periodontal pockets associated with intrabony defects [12]. The data from human histological studies are in line with the findings from randomized controlled clinical studies evaluating the additional effect of various type of grafting materials in conjunction with open flap debridement (OFD) over the use of OFD alone and have demonstrated a high degree of heterogeneity (varying from 0.04 mm obtained with coralline calcium carbonate, to 0.41 mm with allografts, 0.60 mm with bioactive glass and 0.98 mm with hydroxyapatite) [16]. Therefore at present, it is difficult to draw any conclusion on the biological and clinical benefit of using grafting

materials alone to improve the results obtained with OFD in intrabony defects [16]. Therefore, it has to be understood that the main rationale for using grafting materials for reconstructive surgery is not necessarily to promote periodontal regeneration "per se", but rather to provide a carrier for biologic molecules/growth factors, to prevent flap collapse, and to enhance wound stability.

Role of barrier membranes in Periodontal Regeneration

Guided tissue regeneration (GTR) is one of the more thoroughly documented techniques in reconstructive periodontal surgery. By means of either non-bioresorbable or bioresorbable barriers, the epithelial and gingival connective tissue cells are excluded from the wound area, thus enabling periodontal ligament and bone cells to selectively repopulate the root surfaces and the bony defects [17]. Furthermore, the GTR barrier will stabilize the blood clot and support the regeneration process [18]. Evidence from systematic reviews indicates that GTR promotes periodontal regeneration in humans [12] and has a greater effect on probing measures of periodontal treatment than open flap debridement, including improved attachment gain, reduced pocket depth, less increase in gingival recession and more gain in hard tissue probing at re-entry surgery. The additional use of a barrier membrane has yielded about 1.21 mm higher probing depth reduction than the use of flap surgery alone [19].

Enamel Matrix proteins with bone grafting materials

The most widely utilized biologic agent for regeneration of intrabony defects is via the use of enamel matrix proteins (EMPs). Substantial evidence is available demonstrating that EMPs affects cells earlier in their differentiation process [20] and has various roles on the multi-lineage differentiation of periodontal ligament cells in vitro [21,22]. Since enamel matrix proteins are known to contribute to root development during tooth formation, the biological effects of EMPs on PDL, bone, cementum and gingival connective tissue cells have been shown to promote regeneration of intrabony defects [22].

An extensive systematic review on the topic showed that EMPs possesses a significant influence on cell behavior of many cell types by mediating cell attachment, spreading, proliferation and survival as well as expression of transcription factors, growth factors, cytokines, extracellular matrix constituents and other molecules involved in the regulation of bone remodeling [23]. EMPs further possesses the ability to reduce tissue inflammation and improve soft tissue wound healing [24] The use of EMPs in conjunction with OFD has been shown to promote periodontal regeneration of intrabony defects in humans [12] while clinically EMPs treated sites displayed statistically significant higher CAL gains (mean difference 1.1 mm, 95% CI 0.61 to 1.55) and PPD reductions (0.9 mm, 95% CI 0.44 to 1.31) when compared to OFD alone or placebo [25,26,27,28,29,30,31,33,34].

To date, a large number of clinical studies have evaluated the combination of EMPs with a bone grafting material when compared to either bone grafting material alone or EMPs alone (Table 1). Large variability exists and while the reasons are difficult to explain, they may at least in part be due to the defect anatomy and the utilized grafting material. Two separate studies have investigated the effects of EMPs + autogenous bone [35,36]. In a parallel study of 28 intra-osseous lesions, EMPs did not offer a statistically significant advantage when compared to EMPs alone for mean PD and CAL, however EMPs significantly improved healing in pockets greater than 6mm, and significantly increased recession coverages [35]. Yilmaz et al. also studied this combination and found that the combination approach led to statistically superior results in all areas measured including CAL gains and PD reductions [36].

The combination of allografts (either DFDBA or FDBA) with EMPs has been investigated in 5 clinical studies. In a split mouth study of 40 patients, Gurinsky et al found that EMPs + DFDBA offered no statistical difference in mean PD or CAL levels after a 6 month healing period. However, in the same study it was found statistically significant improvements in bone fill, crestal resorption

and percentage of sites gaining greater than 50% and 90% bone fill when compared to EMPs alone (P < 0.001) [37]. Hoidal et al. validated this finding in a parallel study with 32 patients and found that the combination approach did not lead to any significant changes after a 6 month healing period when compared to the control group receiving DFDBA alone [38]. Contrary to these results, Aspriello et al. found in a parallel study with 56 intra-osseous defects that the combination of DFDBA with EMPs led to significant improvements in mean CAL and PD reduction after 6 months of healing [39]. Recently, Ogihara and Tarnow found that the combination of either DFDBA or FDBA in combination with EMPs led to significant improvements in PD and CAL changes when compared to EMPs alone with no differences observed between either DFDBA + EMPs or FDBA + EMPs [40].

Table 1. Human clinical studies utilizing a combination approach including bone grafting material + EMPs

Author & Year	Study Desing & Patient Number	Clinical Defects	Healing Period	Treatment Groups	Mean PD change (mm)	P value	Mean CAL Change (mm)	P value
Lekovic, 2000	Split mouth with 21 patients	42 intrabony defects > 6 mm	6 months	EMPs	1.91	<0,001	1.71	<0,001
				EMPs + DBBM	3.43		3.31	
Velasquez-Plata, 2002	Split mouth 16 patients	32 intrabony defects ≥ 5 mm	6-8 months	EMPs	3.8	n.s.	2.9	n.s.
				EMPs + DBBM	4.0		3.4	
Sculean, 2002	Parallel 24 patients	24 intrabony defects	12 months	DBBM	6.5	n.s.	4.9	n.s.
				DBBM+ EMPs	5.7		4.7	
Scheyer, 2002	Split mouth with 17 patients	34 intrabony defects ≥ 5 mm	6 months	DBBM	3.9	n.s.	3.7	n.s.
				DBBM + EMPs	4.2		3.8	
Sculean, 2002	Parallel with 28 patients	28 intrabony defects ≥ 6 mm	12 months	BG	4.22	n.s.	3.07	n.s.
				EMPs + BG	4.15		3.22	
Zucchelli, 2003	Parallel with 16 patients	16 intrabony defects > 5 mm	12 months	EMPs	5.8	n.s.	4.9	<0,01
				EMPs + DBBM	6.2		5.8	
Gurinsky, 2004	Split mouth with 40 patients	67 intrabony defects ≥ 3 mm	6 months	EMPs	4.0	n.s.	3.2	n.s.
				EMPs + DFDBA	3.6		3.0	
Sculean, 2005	Parallel with 30 patients	30 intrabony defects ≥ 6 mm	12 months	EMPs	4.5	n.s.	3.9	n.s.
				EMPs + BG	4.2		3.2	
Kuru, 2006	Parallel with 23 patients	23 intrabony defects ≥ 6 mm	8 months	EMPs	5.03	<0,05	4.06	<0,05
				EMPs + BG	5.73		4.17	

Bokan, 2006	Parallel with 56 patients	56 intrabony defects ≥ 3 mm	12 months	EMPs	3.9	n.s.	3.7	n.s.
				EMPs + β-TCP	4.1		4.0	
Guida, 2007	Parallel : 27 patients	28 intra-osseous lesions	12 months	EMPs	5.6	n.s.	4.6	n.s.
				EMPs + AB	5.1		4.9	
Jepsen, 2008	Parallel with 73 patients	73 intrabony defects ≥ 4 mm	6 months	EMPs	2.55	n.s.	1.83	n.s.
				EMPs + BCP	1.93		1.31	
Hoidal, 2008	Parallel with 32 patients	41 intrabony defects ≥ 3 mm	6 months	DFDBA	2.45	n.s.	1.63	n.s.
				DFDBA + EMPs	2.56		1.47	
Yilmaz, 2010	Parallel : 40 patients	40 2,3 wall intrabony defects	12 months	EMPs	4.6	<0,001	3.4	<0,001
				EMPs + AB	5.6		4.2	
Aspriello, 2011	Parallel with 56 patients	56 intra-osseous defects	12 months	DFDBA	3.75	<0,05	3.5	<0,05
				DFDBA + EMPs	5.0		4.0	
Meyle, 2011	Parallel with 73 patients	73 intrabony defect ≥ 4 mm	12 months	EMPs	2.9	n.s.	1.9	n.s.
				EMPs + BC	2.8		1.7	
Jaiswal, 2013	Parallel with 30 patients	30 class II furcation defects	12 months	DFDBA + GTR	0.81	<0,05	1.5	<0,05
				DFDBA + GTR + EMPs	1.74		2.12	
De Leonardis, 2013	Parallel with 34 patients	34 intrabony defects ≥ 3 mm	12 months	EMPs	3.51	<0,001	2.73	<0,001
				EMPs + HA/β-TCP	4.00		3.47	
Ogihara, 2014	Parallel: 69 patients	69 intrabony defects > 6 mm	12-36 months	EMPs	1.91	<0,001	3.04	<0,001
				EMPs + DFDBA	3.7		3.52	
				EMPs + FDBA	3.26		4.14	

The combination of xenografts (DBBM) with EMPs has been investigated in 5 clinical studies. It was first demonstrated by Lekovic et al. in a split mouth study with 21 patients having intrabony defects > 6 mm that the combination of EMPs with DBBM led to statistically improved mean PD and CAL change after a 6 month healing period when compared to EMPs alone [41]. Since then, 1 other study showed significant advantages for the combination approach whereas 3 others demonstrated no statistically significant advantage for the combination approach [42,43,44,45]. Zucchelli et al. found that EMPs + DBBM showed statistically significantly greater CAL gains, radiographic bone level gains and less gingival recessions following a combination therapy with EMPs + DBBM [45].

The use of EMPs in combination with synthetic materials including Bioglass, (BG), β-Tricalcium phosphate (β-TCP), biphasic calcium phosphate (BCP), and hydroxyappatite (HA) has led to variable results for the regeneration of human intrabony defects. In 2 parallel studies evaluating the combination of EMPs with BG, no significant change in mean PD or CAL were observed when compared to either BG alone or EMPs alone following a 12 month healing period [46,47]. In contrast,

Kuru et al. found after an 8 month healing period that a statistically significant increase in PD reduction and CAL was observed when compared to EMPs alone [48]. The combination of EMPs with β-TCP was investigated by Bokan et al. who showed that no statistical difference in mean PD change and CAL was observed after a 12 month healing period [49]. Similar results were also found for the combination of EMPs with BCP following a 6 and 12-month healing period [50,51]. A study combining HA/□-TCP with EMPs found that EMPs statistically improved the mean PD and CAL change for intrabony defects greater or equal to 3mm [52]. Moreover, the combination of EMPs and various types of grafting materials has provided evidence for periodontal regeneration in animal and humans, thus justifying its biologic rationale in reconstructive periodontal surgery [53,54,55-57].

In a recent systematic review including meta-analysis, Matarasso et al. found that the combination of EMPs + grafting material resulted in additional clinical improvements in terms of CAL gain and PD reduction compared with those obtained with EMPs alone [58]. Mean CAL gain amounted to 3.76 ± 1.07 mm (median 3.63 95% CI: 3.51-3.75) following treatment with a combination of EMPs and bone graft and to 3.32±1.04 mm (median 3.40; 95% CI 3.28; 3.52) following treatment with EMPs alone. Mean PD reduction measured 4.22±1.20 mm (median 4.10; 95%CI3.96-4.24) at sites treated with EMPs and bone graft and yielded 4.12±1.07 mm (median 4.00; 95% CI 3.88-4.12) at sites treated with EMPs alone. A clinical case is provided demonstrating periodontal regeneration following application of DBBM + EMPs.

On the other hand, a large variability amongst the studies was found which was partly explained by the fact that reconstructive surgery was performed in defects with different types of morphology (i.e. contained 2 and 3 walled defects and non-contained 1 and -2 walled ones), using different types of grafts and surgical techniques. Interestingly, the potential influence of the used graft or the surgical procedure (i.e. the used flap design) on the clinical outcomes is unclear. Furthermore, no

differences in terms of tooth survival rate were found between the combination approach and EMPs (i.e. both approaches yielding 100% tooth survival) and none of them reported on the outcomes in terms of residual pockets ≥ 5 mm. Furthermore, no data on the cost-effectiveness of using the combination approach is available.

Combination of PDGF with bone grafting materials

The second most utilized biological growth factor for intrabony defect regeneration has been Platelet Derived Growth Factor (PDGF). Initially it was used as a concentrated form isolate from platelets by isolating autologous blood followed by centrifugation allowing super-physiological concentrations [59,60]. Following rigorous preclinical testing, recombinant human PDGF (rhPDGF) was granted FDA approval as the first such growth factor of its kind built from recombinant proteins [61,62]. Its main action is derived following injury by promoting rapid cell migration, proliferation and angiogenesis to defect sites [63]. Three different isoforms PDGF-AA, PDGF-AB and PDGF-BB are available [64].

The use of rhPDGF with a bone grafting carrier system has been investigated in comparison to bone grafting material alone in 4 different human randomized clinical studies. In the first study investigating the use of rhPDGF-BB in combination with a synthetic beta-TCP for the healing of advanced periodontal osseous defects, 11 clinical centers enrolled 180 subjects with 4mm or greater intrabony defects. It was found that PDGF at a concentration of 0.3 mg/ml led to significantly improved CAL gain and percentage of bone fill at 3 months following surgery when compared to beta-TCP alone; however, this difference was no longer significant at 6 months [65]. Nevertheless, on a long-term basis (up to 36 months), a consistent improvement in CAL gain was observed compared to the baseline. Moreover, the regenerative technique yielded significantly higher clinical improvements in terms of bone fill compared to the control (i.e. synthetic beta-TCP).

In a second double blinded, prospective, parallel, randomized multi-center clinical trial of 54 patients, it was found that rhPDGF-BB+β-TCP also performed better than β-TCP alone [66]. Thakare and Deo also found that following 12 months of healing, a concentration of 0.3 mg/ml was able to significantly improve CAL and PD reductions when compared to the bone grafting material utilized alone [67]. Furthermore, it was also concluded in a split mouth study comparing the regenerative potential of intrabony defects that rhPDGF-BB+β-TCP led to significantly higher CAL and PD reduction when compared to of β-TCP alone [68]. Despite a small number of clinical trials with appropriate controls, there have been numerous other case reports and retrospective studies that have demonstrated that PDGF-BB in combination with a bone grafting material is capable of periodontal regeneration of intrabony defects and therefore, it could be suggested that it remains a viable treatment option[69,70-72,73,74-76,77,78].

Furthermore, studies have also confirmed the ability for rhPDGF + bone grafting material to demonstrate histologically periodontal regeneration in humans [73,79]. However, despite the fact that the use of rhPDGF + bone grafting is based on a biologic rationale and has provided evidence for periodontal regeneration in humans, no data are available on the potential beneficial effect of this approach over the outcomes obtained with OFD alone (i.e. without any grafting material). Moreover, the effect of this treatment on minimizing the number of sites < 6 mm has not yet been reported and requires further studies.

Combination of PRP with bone grafting materials and membranes

Platelet Rich Plasma (PRP) is an autologous concentration of growth factors derived from typical platelets following centrifugation to reach super-natural concentrations [80,81]. It was first introduced in the 1970s as a fibrin glue and has since exploded in popularity for a variety of dental treatments and procedures. Since 1990, the understanding of concepts involved in tissue repair have demonstrated the ability for several key factors found in blood that improve the speed and quality of wound healing. PRP has since been utilised by many oral surgeons for extensive dental procedures [82,83,60]. Its main drawback includes the use of anticoagulants which has been shown to limit the natural healing process despite containing a number of growth factors implicated in tissue repair [80,81]. As PRP is a blood concentrate, its main use has been in combination with a bone grafting material.

The combined use PRP and several bone grafts for the treatment of intrabony human defects has been documented in several randomized controlled clinical trials [84,85, 86, 87, 88, 89-91, 92, 93-95, 96, 97, 98, 99] (Table 2) with contradictory conclusions. Five of the studies have investigated the additional effect of PRP in conjunction with bovine porous bone mineral (BPBM) and only two have reported positive results. Also, four studies have used β-TCP in combination with PRP and just one has claimed adjunctive beneficial effect of PRP. Furthermore, another study failed to show a positive result when PRP was added to bioglass (BG). On the other hand, five studies have claimed an additional effect of PRP when it combined with autograft (AUG), DFDBA or hydroxyapatite (HA).

In summary, despite a number of the available studies showing a tendency to indicate that the use of PRP may improve the CAL in the treatment of intrabony human defects, there is no solid evidence regarding the potential of PRP application. Additionally, although more than half of these studies reported a positive additional effect of PRP on the treatment of the intrabony defects, this effect was minimal to modest in the most of them. Nevertheless, the use of PRP was demonstrated by all selected RCTs to be entirely safe, without causing complications or adverse events and postoperative healing was uneventful in all RCTs. Despite its common use, to date there are no human histological studies documenting the use of PRP for periodontal regeneration and animal histological evidence is also quite limited.

Regarding the combined use of PRP with GTR, only few randomized controlled clinical trials are available [100,101,102-104,105] (Table 2). The majority of the studies (five out

of six) used the combination GTR plus bone graft as control vs. PRP plus GTR plus bone graft as test group. In summary, when platelet concentrates are used in conjunction to GTR, no adjunctive effect can be detected after 6 to 12 months. Among the six human studies using GTR, none accomplished to report any significant positive adjunctive effect of PRP. A possible explanation is that the proven efficacy of GTR in regenerative periodontal procedures could mask the potential effect of the platelet concentrate. Unfortunately, the vast majority of the selected RCTs provide no scientific data on whether the adjunctive use of PRP was associated with lower incidence of exposure of non-resorbable and resorbable barrier membranes, improved aesthetics, higher progression of soft and hard tissue healing or improved clinical handling of the combinations of PRP with various regenerative materials [106]. Furthermore, to date, no clinical investigation has evaluated this combination approach with human histology, thus it remains to be determined what type of regeneration/reparation is occurring following periodontal regenerative therapy.

In addition, a few studies have compared the effects of PRP + bone grafting material + barrier membrane [100,103,104,99]. Yassibag-Berkman et al. compared three groups on intrabony defect regeneration including, 1) graft alone (beta-TCP), 2) graft + PRP, and 3) graft + PRP + collagen membrane. No statistically significant difference could be observed between the groups [99]. Dori et al. investigated PRP in a study containing twenty-four patients with advanced chronic periodontal disease and displaying one intrabony defect whom were randomly treated with a combination of either PRP + DBBM + GTR or DBBM + GTR [103]. No difference in any of the investigated parameters including plaque index (PI), gingival index (GI), bleeding on probing (BOP), PD, GR and CAL was observed [103]. Furthermore, Dori et al. also investigated the same combination approach with beta-TCP [20]. Once again no significant difference could be observed for any of the measured parameters [104]. Camargo et al. compared twenty-three paired intrabony defects treated with DBBM + GTR + PRP or DBBM + GTR [100]. No significant differences could be observed [100]. Therefore, the additive effect of combining 3 therapies including barrier membrane + bone grafting material + PRP did not lead to significant clinical improvements in any of the above mentioned studies.

Table 2. Human clinical studies comparing bone grafting material in combination with either PRP

Author & Year	Study Desing, patient number	Healing Period	Treatment Groups	Mean PD change (mm)	P value	Mean CAL Change (mm)	P value
Hanna et al. 2004	RCT/split-mouth, 13 patients	6 months	BPBM	2.5 ± 1.0	0.033	2.3 ± 1.2	0.026
			BPBM+PRP	3.5 ± 1.2		3.2 ± 1.0	
Okuda et al. 2005	RCT/parallel, 70 patients	12 months	HA	3.7 ± 2.0	<0.05	2.0 ± 1.2	<0.001
			HA+PRP	4.7 ± 1.6		3.4 ± 1.7	
Ouyang & Qiao 2006	RCT/mixted, 10 patients	12 months	BPBM	3.5 ± 0.4	<0.01	2.9 ± 0.8	<0.01
			BPBM+PRP	4.8 ± 1.0		4.5 ± 1.1	
Yassibag-Berkman et al. 2007	RCT/mixted, 25 patients	12 months	b-TCP	4.1	n.s.	2.4	n.s.
			bTCP+PRP	3.6		2.1	
Demir et al. 2007	RCT/parallel, 29 patients	9 months	BG	3.3 ± 0.5	n.s.	2.9 ± 0.4	n.s.
			BG+PRP	3.6 ± 0.5		3.1 ± 0.5	
Döri et al. 2008	RCT/parallel, 26 patients	12 months	BPBM/EMPs	5.9 ± 1.3	n.s.	5.0±0.9	n.s.
			BPBM/EMPs+PRP	5.8 ± 1.8		4.8±1.3	
Piemontese et al. 2008	RCT/parallel, 60 patients	12 months	DFDBA	3.5 ± 1.9	<0.05	2.4 ± 2.2	<0.001
			DFDBA+PRP	4.6 ± 1.3		3.6 ± 1.8	
Döri et al. 2009	RCT/parallel, 30 patients	12 months	BPBM	5.3±1.7	n.s.	4.7±1.6	n.s.
			BPBM+PRP	5.2±1.6		4.6±1.7	

Harnack et al. 2009	RCT/split-mouth, 22 patients	6 months	b-TCP	_0.4_	n.s.	_0.3_	n.s.
			bTCP+PRP	_0.8_		_0.1_	
Parimala & Mehta 2010	RCT/split-mouth, 14 patients	9 months	BPBM	6.2±1.4	n.s.	4.1±1.1	n.s.
			BPBM+PRP	6.6±1.4		4.7±0.8	
Kaushick et al. 2011	RCT/split-mouth, 10 patients	6 months	HA/b-TCP	3.3±0.8	0.03	2.9±0.7	0.002
			HA/b-TCP+PRP	4.3±0.9		4.4±0.8	
Saini et al. 2011	RCT/split-mouth, 20 patients	3 months	b-TCP	2.2±0.2	0.036	1.1±0.2	0.042
			bTCP+PRP	2.8±0.3		1.8±0.3	
Ozdemir & Okte 2012	RCT/split-mouth, 14 patients	6 months	b-TCP	3.0 ±1.3	n.s.	2.0 ±1.3	n.s.
			bTCP+PRP	3.0 ±1.3		2.0 ±2.0	
Hassan et al. 2012	RCT/split-mouth, 12 patients	12 months	AUG	4.4	<0.01	2.9	<0.01
			AUG+PRP	5.0		3.8	
Gupta 2014	RCT/split-mouth, 10 patients	12 months	HA	1.9	<0.05	1.2	<0.05
			HA+PRP	3.4		3.1	
Agarwal & Gupta 2014	RCT/split-mouth, 24 patients	12 months	DFDBA	2.7 ± 0.4	<0.05	2.4 ± 0.6	<0.05
			DFDBA+PRP	3.3 ± 0.5		3.2 ± 0.5	

Taken together, the additional use of PRP to various types of grafting materials used in conjunction with EMPs or membranes, did not reveal statistically or clinically significant benefits in terms of CAL gain and PPD reduction. Moreover, the additional efforts and costs related to the use of PRP (i.e. sampling blood, use of various centrifuges and anticoagulants) along with patient centred outcomes have has not yet been evaluated. Since at present no data on the effect of various combination modalities including PRP on reducing periodontal pockets are available and as such, its clinical benefit appears to be more than questionable.

Combination of biologic agents/growth factors and membranes

The use of barrier membranes has been demonstrated to provide additional space-maintenance for the regrowth of periodontal tissues [107]. The rationale behind a combination strategy is based on the fact that while the barrier membrane is able to create additional space for the repopulation of periodontal tissues, the additional use of a growth factor is then capable of speeding their regeneration by either providing faster cell repopulation or influencing their differentiation towards specialized tissues [107]. While less studies with appropriate controls have investigated this combination, it does possess theoretical advantages when compared to the use of membranes alone or growth factors alone. Below the use of EMPs are discussed in combination with a barrier membrane for their possible improvement of periodontal regeneration of intrabony defects.

Combination EMPs + Membrane

An animal study was first investigated following surgically created intrabony defects in monkeys. Treatment groups included 1) OFD + GTR, 2) OFD + EMPs, 3) OFD + EMPs + GR or 4) OFD alone (control) [108]. The results from this histological investigation demonstrated that although OFD + EMPs + GTR may enhance new attachment and bone, the results were not superior to those found in OFD + EMPs alone or OFD + GTR alone [108].

In the first human study analyzing this combination, a series of 56 patients were either assigned to 1) OFD, 2) EMPs alone, 3) GTR alone or 4) EMPs + GTR [31]. Mean PD change and CAL were investigated. No significant difference between treatment sites receiving EMPs + GTR, EMPs alone or GTR alone could be observed [31]. Furthermore, a second parallel study and a split mouth study found no preferential regeneration of periodontal tissues for the combination of EMPs + GTR when compared to EMPs alone, or GTR alone (Table 3) [62,108]. Furthermore, no human histological study has investigated this combination.

Table 3. Results from clinical short-term (6 - 12 months) and long-term (3 - 10 years) studies. (adapted and amended from Murphy & Gunsolley 2003 (short-term studies) and Figueiro et al. 2014 (long-term studies))

	No of Studies	no of teeth	CAL gain range	tooth survival	follow up time
Short-term					
GTR	9	126	2.0 ± 0.4 - 5.9 ± 1.2	100%	6 - 12 m
GTR+BG/BS	9	134	2.0 ± 1.4 - 5.4 ± 1.7	100%	6 - 12 m
Long-term					
GTR	8	137	2.1 ± 1.1 - 3.0 ± 2.0	81.8% - 100%	3 - 10 y
GTR+BG/BS	4	56	2.3 ± 2.1 - 4.1 ± 1.6	90.9% - 100%	6 – 10 y

Taken together with the additional histological evidence from animals and humans, it can be concluded that the combination of EMPs with a GTR barrier membrane provides little to no additional support for the regeneration of intrabony defects.

Combination of grafting materials with membranes

A critical factor for periodontal regeneration is provision of adequate space for new tissue formation during healing [111,112]. Since the majority of currently available membranes consist of supple materials, there is an inherent risk for membrane collapse in cases of non-supportive defect anatomy, i.e., the membrane collapses/falls (partially or totally) into the defect and/or towards the root surface, thus physically reducing the space available for new tissue invasion. Indeed, reduced amounts of periodontal regeneration, and of new bone formation in particular, due to membrane collapse was noticed in several preclinical in vivo studies on GTR [113,114,115,116]. Haney et al. [115] observed a statistically significant correlation between the space provided by the membrane and the amount of regenerated alveolar bone using a supra-alveolar defect model in dogs. Similarly, in the clinic, Cortellini et al. reported that application of self-supporting (reinforced with titanium strips) e-PTFE membranes, which could be positioned more coronally than ordinary e-PTFE membranes, yielded statistically significant more CAL gain in intrabony defects [117]. In a subsequent publication, a retrospective analysis of the results of this study showed that the most significant factor associated with the amount of regenerated tissue was the amount of space available under the membranes, and not the type of membrane [118]. Thus, membranes have often been combined with the use of particulated bone grafts and substitutes, with the aim to support the membrane and assure space provision, and thereby enhance the histological and clinical outcome of GTR treatment.

Almost all available bone graft and substitute materials have been combined with GTR membranes for the treatment of periodontal intrabony defects [119,120].

Murphy & Gunssoley, in a systematic review, evaluated all randomized control studies, cohort studies, and case-control studies on GTR (both non-resorbable and resorbable barriers) in combination with bone grafts/substitutes [121]. The review identified 7 studies providing direct comparisons between intrabony defects treated with membranes in combination with bone grafts/substitutes and defects treated with membranes only. Significant clinical improvements, i.e. CAL gains (average range 2.0-5.4 mm) and PD reduction (average range 2.3-5.8 mm), were observed in sites treated with the combination approach (Table 4).

Table 4. Histomorphometric results of animal and human histological studies (Adapted from Ivanovic et al. 2014 (animal data) and Sculean et al. 2015 (human data)

	No of Studies	No of Teeth	Defect depth (mm)	Osseouswal ls	CF (%)	NC (%)	NB (%)	JE (%)
Animal								
GTR	18	201	4.9 ± 0.9	1-3	43 ± 29	66 ± 25	58 ± 25	25 ± 17
GTR+BG/BS	14	95	5.2 ± 1.1	1-3	28 ± 20	64 ± 21	58 ± 6	15 ± 13
Human								
GTR	8	20	5.1 ± 2.5	1-3	3.1 ± 2.1	2.6 ± 1.6	1.7 ± 1.3	-
GTR+BG/BS	8	39	6.22 ± 2.04	1-3	0.47 ± 0.63	2.32 ± 1.94	2.41 ±1.61	2.53 ± 1.14

However, the meta-analysis performed in that study failed to reveal any statistically significant or clinically meaningful differences between the two treatment modalities in any of the evaluated parameters; differences were in a range of magnitude 0.01-0.7 mm. On the same conclusion led the results of another very recent systematic review [122] which identified only a few studies providing direct comparisons between membranes with and without bone grafts/substitutes, that were published at a later time-point then those included in the former review [121]. For instance, Stavropoulos et al observed an average CAL gain and residual PD of 2.5 mm and 4.9 mm, respectively, in sites treated with a PLA/PGA membrane in combination with DBBM [123]. The corresponding values in sites treated only with membranes were 2.9 mm and 4.9 mm, respectively. Similarly, no significant difference was observed between the combination approach and the monotherapy, in terms of radiographic bone formation (2.8 mm and 3.1 mm, respectively). In this context, it has also to be remembered that DBBM is a radiopaque material, and thus radiographic bone fill in DBBM treated sites does not necessary imply bone and/or periodontal regeneration. On the other hand, human histologic evidence indicates that the combination of DBBM and membranes may not only result in substantial clinical and radiographic improvements, but may also promote periodontal regeneration in human intrabony defects [12].

Several randomized controlled clinical studies and systematic reviews have evaluated the effects of combining DBBM and collagen membranes on the healing of intrabony defects. The available data indicate that in deep intrabony defects, this combination may lead to statistically and clinically higher improvements in terms of CAL gain and PD reduction compared to OFD alone (Sculean et al. 2003, 2005, Tonetti et al. 2004, Stoecklin-Wasmer et al. 2013). In a systematic review including meta-analysis Stoecklin-Wasmer et al. 2013 have shown that the combination of bone grafts and GTR with collagen membranes resulted in significantly higher CAL gain (i.e. weighted mean difference of 1.71 mm (95% CI, 1.26 to 2.15)) when compared with OFD. Despite the fact that the available studies did not clearly report the number of residual pockets ≥ 6 mm, the combination approach has led to statistically and clinically significantly higher PPD reduction compared to OFD alone (i.e. weighted mean difference of 1.44 mm (95% CI, 1.04 to 1.85)). Therefore, these data suggest that this combination approach is a valuable modality to promote periodontal regeneration and pocket closure at deep intrabony defects.

In context, in an earlier systematic review looking specifically on pre-clinical in vivo studies on combinations of barrier membranes and grafting materials, Sculean et al. concluded that clear additional benefits of combination treatments over the use of membranes alone were detected only in defects sites with non-supportive anatomy [124]. Specifically, larger amount of periodontal regeneration were observed only in non-contained two-wall intrabony or supra-alveolar defects, i.e. in sites

with a larger risk of membrane collapse [124]. These preclinical findings are in line with clinical data indicating that a combination of GTR and bone grafts may only bear certain advantages over the use of GTR alone in defects with a non-self-containing configuration (for example in one wall defects), whereas in 2 and 3-wall defects the advantages may be limited to non- existent (Stoecklin-Wasmer et al. 2013). This aspect needs to be carefully considered during the decision making process in treating intrabony defects in order to avoid overtreatment and limit the possibilities of complications such as infections that may occur once several biomaterials are used simultaneously.

One relevant aspect, in this discussion, is the long-term outcome of treatment. According to the results of a recent systematic review of controlled clinical studies [125] that identified 11 publications reporting on treatment outcomes after an observation period of 3 to 10 years post-operatively, the clinical improvements achieved at short-term after regenerative treatment with the use of membranes in combination with bone grafts/substitutes can in general be preserved on the long-term. In particular, the average CAL gain reported for sites treated with a membrane in combination with a bone graft/substitute was 2.3 – 4.1 mm (Table 4). However, this range of values was not much different from what it was observed for sites treated with only a membrane (2.1 – 3.0 mm). Most of the studies included in the review reported that only a small number of sites showed some CAL loss (mostly < 1 mm on average) between the short- and long-term evaluation time-point. Importantly, in one of the studies included in the review, specific analysis failed to show any association of DBB grafting (in combination with a resorbable membrane) with sites showing CAL loss within a 6-year period after treatment [126]. The results of Stavropoulos et al. [126] suggest, thus, that mere presence of bone graft substitute particles in the regenerated and/or repaired periodontal tissues may have no consequence per se on the stability of the improved clinical conditions as long as optimal plaque control is maintained.

Overall, treatment of periodontal intrabony defects with membranes in combination of bone grafts/substitutes may result, at least in part, in periodontal regeneration while clinically, the results are accompanied by significant clinical improvements evidenced by CAL gain and pocket closure [12]. These improvements are in general preserved on the long-term for the majority of cases, provided the patient performs adequate oral hygiene and receives supportive periodontal therapy. Nevertheless, it appears that this combination may bear clinical relevance in non-contained defects or defects with a complicated anatomy.

Conclusions and future perspectives

Although numerous attempts and combination approaches have been made to enhance periodontal regeneration, it remains an optimistic future goal as complete periodontal regeneration at present is not predictably achievable.

Despite this, numerous combination approaches have been utilized to improve intrabony defect regeneration and decrease residual pockets around teeth. The available literature suggests that the combination of biologic agents/growth factors/GTR with bone grafting materials may possess a biologically sound rationale which also bears clinical relevance evidenced in superior clinical outcomes compared to treatment with OFD alone. However, it also appears that combination or biologic agents/growth factors or bone grafting materials and GTR may only bear certain clinical relevance in defects with a more complicated (i.e. so called non-self-containing defects) while in two or three wall defects no clear advantage is present. Furthermore, it might be speculated that the use of GTR barriers may be replaced by the use of biologic agents/growth factors when used in combination with bone grafting materials.

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