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Clinical concepts for regenerative therapy in intrabony defects

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Periodontal-regenerative technologies are applied to improve the short- and long-term clinical outcomes of periodontally compromised teeth presenting with deep pockets and reduced periodontal support. The persistence of deep pockets following active periodontal therapy has been associated with an increased probability of tooth loss in patients attending supportive periodontal-care programs (81). Teeth with deep pockets associated with deep intrabony defects are considered a clinical challenge. Most authors have classified such teeth as having either a questionable or a hopeless prognosis. Key elements to support these opinions are the complex interplay of a reduced residual periodontal attachment, deep pocketing, functional demands and frequently the degree of residual tooth mobility (70, 72, 84, 85). It is therefore clear that the possibility of changing the prognosis of a tooth from 'questionable' or 'hopeless' into 'fair' or 'favorable' would greatly help clinicians and patients in the difficult job of maintaining teeth over time, and the possibility of gaining periodontal support would help patients improve their comfort and function.

The aims of periodontal regeneration are to obtain: (i) an increase in the periodontal attachment and bone of a severely compromised tooth; (ii) a decrease in pocket depth; and (iii) no, or a minimal, increase in gingival recession. Periodontal regeneration has been shown to be effective in the treatment of one-, two- and three-wall intrabony defects, or combinations thereof, from very deep to very shallow, and from very wide to very narrow (43, 44, 90, 91). However, the approaches used currently are technique sensitive and are burdened by a significant amount of clinical failures or incomplete success. At present, we know that most failures of regenerative therapy have an explanation in terms of negative patient factors, suboptimal use of surgical approaches and materials, and insufficient clinical skill and experience of the surgeon (26). Clinical success requires application of meticulous diagnostic and treatment strategies (26, 31). The aims of this narrative review were as follows: first, to review the current scientific literature highlighting the strengths and weaknesses of periodontal-regenerative approaches in intrabony defects; second, to discuss the patient, defect and surgery-associated factors that have an impact on clinical outcomes; and, third, to propose a step-by-step clinical approach in order to build up a scientifically sound strategy to optimize the clinical outcomes in different patients and in different defect anatomies.

Evidence for clinical efficacy and effectiveness

Questions of efficacy relate to the added benefit of a treatment modality under ideal experimental conditions (such as those of a highly controlled research-center environment). Effectiveness, on the other hand, relates to the benefit that can be achieved, in relation to morbidity and adverse events, in a regular clinical setting where the procedure is likely to be performed. Besides efficiency considerations, evidence for both efficacy and effectiveness need to be available in order to provide support for the adoption of treatment approaches in clinical practice.

The clinical efficacy of periodontal-regenerative procedures has been extensively evaluated in randomized controlled clinical trials that have compared regenerative procedures with a standard access flap approach. To limit sample size and study duration, these trials have utilized surrogate outcomes – clinical attachment-level changes, decrease in pocket depths, furcation closure or radiographic measurements – rather than changes in tooth survival. However, these surrogate outcomes are considered to be adequate

proxies of the true outcome represented by tooth survival: the persistence of deep pockets or furcation involvement are associated with a higher risk of periodontal breakdown and tooth extraction.

The majority of reported clinical trials have been small, single-center studies, with only a few being larger multicenter clinical trials. The evidence from these studies has recently been summarized in meta-analyses performed on data retrieved by systematic reviews of the published literature. In 2002, 2003 and 2008, the European Workshop on Periodontology (held by the European Federation of Periodontology) and the Workshop on Emerging Technologies in Periodontics (held by the American Academy of Periodontology) provided much of the systematic assessment of the evidence for currently available technologies. These include the use of barrier membranes (guided tissue regeneration), the use of bone-replacement graft materials, the use of biologically active regenerative materials and the use of combinations of the aforementioned materials/agents.

Evidence for clinical efficacy of barrier membranes in intrabony defects was assessed in the systematic reviews and meta-analyses performed by Murphy & Gunsolley (90) and Needleman et al. (91). The latter reported a significant added benefit of the use of barriers on top of open flap debridement alone in terms of clinical attachment level gain (16 studies) and probing pocket-depth reduction (11 studies). The results of large prospective multicenter studies in private practice settings (28, 134) conclusively support the additional benefit of membranes in reducing pocket depth and improving clinical attachment levels and bone levels in intrabony defects and thus their efficacy and effectiveness.

The efficacy of bone-replacement graft materials has been assessed in two systematic reviews performed by Trombelli et al. (141) and Reynolds et al. (106). As these two systematic reviews used significantly different criteria for study inclusion, the results did not show complete agreement. Trombelli et al. (141), who included only controlled studies that reported changes in clinical attachment level as the primary outcome, concluded that there was insufficient evidence to support the clinical use of bone-replacement graft materials in intrabony defects because there was significant heterogeneity among included studies, the size of the adjunctive effect was small and not statistically significant and there were differences that did not allow pooling of results obtained with different materials. In the other systematic review for intrabony defects, 27 controlled trials with 797 intrabony defects were included (106). The clinical efficacy of allografts in terms of bone fill and clinical attachment level gains was supported by a meta-analysis indicating that an additional bone fill of 1 mm and additional clinical attachment level gains of 0.4 mm were achieved compared with an access flap control (106). However, the total number of defects contributing to this meta-analysis was relatively small (136 for clinical attachment level gain and 154 for bone fill). Furthermore, no large-scale multicenter trial on allografts has ever been performed and hence the applicability of these results to clinical practice settings remains to be established.

The evidence of clinical efficacy of biologically active regenerative materials in intrabony defects has been summarized, through the years, in meta-analyses only for enamel matrix derivative (43, 44, 47, 141). The outcomes of eight studies, including 444 defects, have indicated that enamel matrix derivative provides significant additional benefits in terms of pocket-depth reduction, clinical attachment level gain and radiographic bone level. These data are in accordance with those of a large practice-based multicenter trial that demonstrated both efficacy and effectiveness of enamel matrix derivative in intrabony defects (135).

Combination therapy has been explored in two recent meta-analyses. Trombelli & Farina (142) evaluated the clinical effects of the use of bioactive agents when used in addition to open flap debridement, either alone or in association with grafts and/or barrier membranes. The authors concluded the following: there was evidence to support the use of amelogenins, either alone or in combination with grafts, to treat intra-osseous defects effectively and the additional use of a graft seemed to enhance the clinical outcome of amelogenins; the combined use of recombinant human platelet derived growth factor-BB and peptide P-15 with a graft biomaterial showed beneficial effects in intra-osseous defects; and contrasting results were reported for platelet-rich plasma and graft combinations.

Tu et al. (145) explored the additional treatment effect of barriers or bone grafts compared with amelogenins alone in 28 studies. Amelogenins plus bone grafts and amelogenins plus membranes attained 0.24 and 0.07 mm more probing pocket-depth reduction, respectively, compared with amelogenins alone, and amelogenins plus bone grafts and amelogenins plus membranes attained 0.46 and 0.15 mm, respectively, of clinical attachment level gain. When different types of bone grafts and barrier membranes were

treated separately, amelogenins with bovine bone grafts showed greater treatment effects. The authors concluded that there was little evidence to support the additional benefits of amelogenins in conjunction with other regenerative materials.

Support for the clinical use of growth factors comes from two multicenter studies on recombinant human platelet-derived growth factor-BB and two on fibroblast growth factor-2. In one multicenter trial (92), 180 defects (comprising both intrabony and furcation defects) were treated with either one of two concentrations of human platelet-derived growth factor-BB (0.3 and 1.0 mg/ml) combined with the beta-tricalcium phosphate delivery device or with tricalcium phosphate alone. Clinical attachment level gains at 6 months failed to demonstrate a significant benefit of either concentration of platelet-derived growth factor compared with the bone replacement graft alone. However, with regard to radiographic assessments, the lower concentration of platelet-derived growth factor resulted in significantly higher percentages of radiographic bone fill of the defect (57% vs. 18%) and linear radiographic bone growth (2.6 vs. 0.9 mm). In the other multicenter trial on recombinant human platelet-derived growth factor-BB, Jayakumar et al. (71) studied 54 patients who were treated with human platelet-derived growth factor-BB1b combined with the beta-tricalcium phosphate delivery device or tricalcium phosphate alone. Clinical attachment level gain, bone growth and percentage bone fill at 6 months were significantly greater in the test group compared with the tricalcium phosphate control group.

A study on 74 patients (68) compared three different concentrations of fibroblast growth factor-2 vehicle with 3% hydroxypropylcellulose and with hydroxypropylcellulose alone. No difference was reported in terms of clinical attachment level gain between test and control groups. A significant difference in terms of bone gain was reported in favor of the 0.3% concentration of fibroblast growth factor-2 compared with hydroxypropylcellulose alone. No advantage, in terms of bone gain, was observed with the other two concentrations of fibroblast growth factor-2 (0.03% and 0.1%). A second randomized, double-blind, placebo-controlled clinical trial on 253 adult patients was performed, in which 0.2, 0.3 or 0.4% fibroblast growth factor-2 was compared with vehicle alone in two- or three-walled vertical bone defects (69). Each concentration of fibroblast growth factor-2 showed significant superiority over vehicle alone ($P < 0.01$) for the percentage bone fill 36 weeks after administration. No significant differences among groups were observed for clinical attachment gain. No clinical safety problems were reported in any of the four cited studies. Drawing conclusions from the four studies, it is apparent that both of the growth factors tested resulted in a measurable added benefit compared with controls in terms of bone gain, whereas three out of four did not induce a significant difference in terms of clinical attachment level gain. Both efficacy and effectiveness of human platelet-derived growth factor-BB1b and fibroblast growth factor-2 have to be further explored before clinical application.

A recent controlled study evaluated, clinically and histologically, wound healing/regeneration following surgical implantation of recombinant human growth/differentiation factor-5 adsorbed onto a particulate beta-tricalcium phosphate carrier into periodontal defects in 28 patients (128). Control defects were treated with open flap debridement alone. The authors reported greater probing pocket-depth reduction and clinical attachment level gain, and greater alveolar bone regeneration and periodontal regeneration at sites that received recombinant human growth/differentiation factor-5/beta-tricalcium phosphate compared with control sites. However, these differences were not statistically significant.

Block biopsies of the defect sites were collected 6 months postsurgery. Histologically, bone regeneration height was almost threefold greater for the recombinant human growth/differentiation factor-5/beta-tricalcium phosphate treatment compared with open flap debridement alone (2.19 ± 1.59 vs. 0.81 ± 1.02 mm, respectively; $P = 0.08$). Similarly, an increase of almost twofold was observed for periodontal ligament (2.16 ± 1.43 vs. 1.23 ± 1.07 mm, respectively; $P = 0.26$), cementum (2.16 ± 1.43 vs. 1.23 ± 1.07 mm, respectively; $P = 0.26$) and bone regeneration area (0.74 ± 0.69 vs. 0.32 ± 0.47 mm^2, respectively; $P = 0.14$). Root resorption/ankylosis was not observed. Future studies with larger sample sizes will have to be conducted to verify these findings.

Comparative studies between different regenerative approaches have been analyzed in a systematic review by Esposito et al. (44). The authors did not find any difference when comparing amelogenins with barriers, in terms of clinical attachment level gain and probing pocket-depth reduction, in the six studies examined. These data are supported by two large practice-based multicenter trials (110, 123). However, Sanz et al. (110) reported a significantly higher prevalence of complications in the barrier-treated group compared with the amelogenin-treated group.

Clinical periodontal regeneration

The data discussed above indicate that clinical improvements beyond those of flap surgery can be obtained by treating intrabony defects with regenerative therapies, but they also suggest a great variability in clinical outcomes among the different studies. In fact, regeneration is an advanced healing event that occurs when the systemic and local conditions are favorable and when therapy is properly applied. A significant 'center effect' was consistently observed in five randomized multicenter studies (28, 110, 134–136). The center variability, defined as the difference in clinical attachment level between the best and the worst centers, had a highly significant impact upon the outcomes, which was greater than the impact of the tested regenerative materials (Table 1).

The observed variability among centers may be a result of differences in the enrolled patients in terms of socio-economic background, form of periodontal disease, response to therapy, persistence of specific pathogens, differences in clinical experience, surgical skills and clinical organization of the clinicians. In addition, a series of prognostic factors associated with the clinical outcomes have been identified using multivariate approaches. The main sources of clinical variability are the patient, the defect and surgery-associated factors (26).

Patient and defect prognostic factors

Evidence suggests that the level of control of periodontitis is associated with clinical outcomes – the persistence of poor plaque control, high levels of bleeding on probing in the dentition (18, 19, 77, 82, 123, 131–133), as well as the persistence of high total bacterial loads or of specific microbial pathogens (or complexes of pathogens) – have all been associated, in a dose-dependent manner, with poor clinical outcomes (42, 62). The level of self-performed plaque control has a large 'dose-dependent' effect on the outcome of periodontal regeneration. Better clinical attachment level gains were observed in patients with optimal levels of plaque control than in patients with less ideal oral hygiene (18, 19, 131, 132).

A retrospective study showed that cigarette smokers displayed significantly impaired regenerative outcomes compared with nonsmokers (132). Cigarette smoking was associated with reduced gains in attachment level. The attachment gain in subjects smoking more than 10 cigarettes per day was 2.1 $\pm$ 1.2 mm compared with 5.2 $\pm$ 1.9 mm in nonsmokers. Thereafter, a series of investigations have confirmed that cigarette smoking displays a dose-dependent detrimental effect on clinical attachment level gains (19, 28, 42, 45, 127, 134, 140).

Defect morphology plays a major role in healing following periodontal-regenerative treatment of intrabony defects. This was demonstrated in studies showing that the depth of the intrabony component of the defect influenced the amount of clinical attachment and bone gained at 1 year: the deeper the defect, the greater was the amount of clinical improvement (42, 46, 123, 131, 133). However, in a multicenter controlled study, it was demonstrated that deep and shallow defects have the 'same potential' for regeneration (23). In other words, following the treatment of deep defects we would expect to achieve linear amounts of attachment gain that are larger than those obtained following the treatment of shallow defects, but both deep and shallow defects can express a regenerative potential up to the complete resolution of the intrabony component of the defect.

Another important morphological characteristic of the defect is the width of the intrabony component, measured as the angle that the bony wall of the defect

Table 1. Outcomes of regression analyses in studies performed to explain variability in terms of clinical attachment gain at 1 year

References	No. of patients	Treatment	Treatment effect	Center effect
Tonetti et al. (134)	143	Bioresorbable barriers vs. flap	0.6 mm	2.4 mm
Cortellini et al. (28)	113	Bioresorbable barriers vs. flap	1.0 mm	2.1 mm
Tonetti et al. (135)	166	Amelogenins vs. flap	0.5 mm	2.6 mm
Sanz et al. (110)	67	Amelogenins vs. bioresorbable barriers	0.8	2.6
Tonetti et al. (136)	120	Bioresorbable barriers + filler vs. flap	0.8	2.8

Treatment effect = added clinical benefit on top of control treatment; Center effect = clinical outcomes of the best center vs. the worst center.

forms with the long axis of the tooth (129). Wider defects have been associated with reduced amounts of clinical attachment level and bone gain at 1 year (46, 131, 133). In a study on 242 intrabony defects treated with membranes, Cortellini & Tonetti (24) demonstrated that defects with a radiographic angle of $\leq 25°$ gained consistently more attachment (1.6 mm on average) than did defects of $\geq 37°$. A follow-up study addressed the significance of the baseline radiographic angle of the intrabony defect following the use of enamel matrix derivative (144). The analysis revealed a negative association between the radiographic angle of the defect and the clinical attachment level gains observed at 1 year. Another secondary analysis of a multicenter study on a combination of bone replacement graft with a barrier membrane, in contrast, has indicated a reduced effect of the radiographic angle on clinical attachment gain at 1 year (74). Similarly, an earlier secondary analysis of a controlled clinical trial using titanium-reinforced expanded-polytetrafluoroethylene membranes (133) indicated that the negative impact of an unfavorable defect morphology may be reduced with the use of supported membranes. In particular, the width of the defects was not correlated with the clinical outcomes.

It has also been shown that the number of residual bony walls is correlated with the outcomes of various regenerative approaches (48, 112). This point, as related to regenerative therapy, was addressed in a few investigations with nonresorbable barriers. In one study, clinical attachment level gain was reportedly related to the number of bony walls (120), whereas, in another study with the same barriers, gains in attachment were not related to the defect configuration in terms of one-, two- and three-wall subcomponents (131). Although the number of walls did not result in a significant difference when titanium barriers (133) or combination therapy (136, 137) were used, when bioresorbable barriers (45, 123) and amelogenins were used, significant differences were reported (123, 135). In particular, a secondary analysis of a multicenter trial showed that, in intrabony defects, the added benefit of amelogenins was greater in three-walled defects than in one-walled defects (135). These data have questioned the suitability of the gel formulation of amelogenins for the treatment of defects with a nonsupporting anatomy (i.e. wide defects with missing bony walls). However, more recently, two studies demonstrated a reduced impact of the number of residual bony walls and of defect width on the outcomes obtained with amelogenins under a minimally invasive surgical technique (34, 35). This clearly differs from the evidence, discussed above, of a strong impact of the defect anatomy in terms of residual bony walls and defect width on the clinical outcomes when amelogenins were used under conventional large, and intrinsically less stable, papilla preservation flaps (135, 136).

The endodontic status of the tooth has been suggested as a potentially relevant factor in periodontal therapy. Cortellini & Tonetti (27), in a clinical study on 208 consecutive patients who had one intrabony defect each, demonstrated that when performed properly, root canal treatment does not negatively affect the healing response and the long-term stability of results of deep intrabony defects treated with barrier membranes.

Tooth mobility has long been considered an important factor for periodontal regeneration (111). A multivariate analysis of a multicenter controlled clinical trial demonstrated that increased tooth mobility is negatively and dose-dependently associated with the clinical outcomes of regeneration (28). Although significant, the size of the effect was small within the range of physiologic mobility. The study concluded that Miller grade III mobility (88) negatively affects periodontal regeneration. Another recent secondary analysis of three previously reported trials assessed the regenerative outcomes of mobile teeth (139). This report indicated that teeth with baseline mobility amounting to < 1 mm horizontally could be successfully treated by periodontal regeneration.

Based on these results, it can be concluded that deep and narrow intrabony defects at either vital or endodontically succesfully treated teeth are those in which the most significant and predictable outcomes can be achieved by guided tissue-regeneration treatment. The number of walls and the width of the defect are influential when nonsupportive biomaterials are used. The influence of defect anatomy appears to be reduced, to some extent, when a more stable design of the flap is applied. Severe, uncontrolled tooth mobility (Miller grade II or higher) (88) may impair the regenerative outcomes. Significant clinical improvements can be expected only in patients with optimal plaque control and with reduced levels of periodontal contamination, and in nonsmokers.

Surgery-associated factors

Basic and clinical research indicate that the absolute requirements for successful regeneration include the presence of space for the formation of the blood clot at the interface between the flap and the root surface (18, 19, 55, 67, 121, 133, 151), the stability of the blood clot to maintain continuity with the root surface,

avoiding formation of a long junctional epithelium (55, 64, 75, 150) and the soft-tissue protection of the treated area to avoid bacterial contamination (39, 40, 94, 95, 110, 119). Development of periodontal-regenerative medicine in the last 25 years has followed two distinctive, yet totally intertwined, paths. The interest of researchers has so far focused on regenerative materials or products on the one hand and on novel surgical approaches on the other.

Materials for regenerative surgery

In the area of materials and products, three different regenerative concepts have been explored – barrier membranes, grafts and wound-healing modifiers – plus many combinations of those. Historically, barrier membranes have been used to mechanically select the cells able to repopulate the blood clot (104). In addition, barrier membranes also possess the capacity to provide space and to increase blood-clot stability (104). In the first attempts of guided tissue regeneration, a bacterial filter produced from cellulose acetate (Millipore®) was used as an occlusive membrane (49, 79, 97). Although this type of membrane served its purpose, it was not ideal for clinical application. Later studies utilized nonresorbable membranes of expanded-polytetrafluoroethylene that were specially designed for periodontal regeneration (Gore-Tex Periodontal Material®). This type of membrane must be removed in a second operation. Membranes of expanded-polytetrafluoroethylene have been used successfully in animal experiments and in several clinical studies (90, 91).

Natural or synthetic bioabsorbable barrier materials for guided tissue regeneration were introduced in order to avoid a second surgery for membrane removal. Barrier materials of collagen from different species and from different anatomic sites have been tested in animals and in humans (3, 4, 11, 100, 103, 130, 149). Barrier materials of polylactic acid or copolymers of polylactic acid and polyglycolic acid have been evaluated in animal and human studies and are commonly used (9, 12, 22, 28, 50, 52, 66, 73, 80, 114, 125, 134).

The biologic principles supporting the use of autologous and heterologous grafts include osteoconductivity and osteo-inductivity, and also their capacity for space provision and blood-clot stabilization (109, 142). Bone-replacement grafts comprise a heterogeneous group of materials of human, animal or synthetic origin. Some consist of bone or exoskeletal mineral, whereas others contain mainly bone matrix. Few materials present evidence of periodontal regeneration. A randomized controlled clinical trial provided histological support that the healing outcome following application of demineralized freeze-dried bone allograft in intrabony defects had a regenerative component in the apical to middle portion of the depth of the defect (6–8).

The adoption of biologic products/compounds is based on their ability to induce or accelerate the processes of matrix formation and cell differentiation (5). These products enforce the healing process but lack the mechanical properties to help in the provision of space and blood-clot stabilization. Accordingly, some of these products are loaded onto solid, bioresorbable carriers to provide some mechanical properties (98, 142). Currently, two preparations consisting of growth and/or differentiation factors are available for use in periodontal regeneration: enamel matrix derivative in a gel form; and platelet-derived growth factor mixed in a beta-tricalcium phosphate bone-replacement graft.

Significant pre-clinical evidence supports the positive effect of recombinant human platelet-derived growth factor-BB associated with recombinant human insulin-like growth factor-1 on periodontal wound healing and regeneration (65). Support for the clinical use of growth factors comes from two multicenter studies on recombinant human-derived growth factor (71, 92) and two on fibroblast growth factor-2 (68, 69). Drawing conclusions from the four studies, it is apparent that both the tested growth factors resulted in a measurable added benefit compared with controls in terms of bone gain, but in three of the four studies a significant difference in terms of clinical attachment level gain was not achieved.

The benefit of the use of amelogenin (enamel matrix derivative) gel in the treatment of intrabony defects is supported by human histologic evidence, case report studies, meta-analyses of randomized controlled clinical trials and large multicenter trials (43, 44, 47, 59–61, 63, 86, 115, 122, 123, 135). Clinically, the rate of wound healing following application of amelogenins seems to be enhanced. A study investigating soft-tissue density in the surgical site using underexposed radiographs (137) reported that the rate of increase in soft-tissue density following the application of amelogenins may be faster than in the access flap control. Such modulation has been interpreted as the outcome of the local release of growth and differentiation factors by the cells involved in the local wound-healing process (5).

The biologic principles supporting combination therapy relate to the possibility of obtaining an additive effect from one regenerative principle when used in combination with another one, such as

osteoconductivity and osteo-inductivity, the capacity for space provision and blood-clot stabilization, and the ability to induce or accelerate the processes of matrix formation and cell differentiation that are inherent in barriers, grafts and bioactive substances. Various modalities of combination therapy based on the use of barrier membranes plus grafting materials have been proposed. Pre-clinical (i.e. animal) studies presenting histologic support for periodontal regeneration using the combination of barrier membranes and grafting materials have been recently reviewed (118). The 10 papers completely fulfilling the inclusion criteria demonstrated superior histologic healing following use of the combination of barrier membranes and grafting materials than following open flap debridement. Histologically superior healing following use of the combination of barrier membranes and grafting materials compared with use of barrier membranes alone or grafting materials alone were only obtained in noncontained two-wall intrabony and supra-alveolar defects. The cited analysis indicates that the combination of barrier membranes and grafting materials may result in histologic evidence of periodontal regeneration, predominantly bone repair.

From a clinical standpoint, Schallhorn & McClain (113) reported on improved clinical results in intrabony defects and degree II furcations, following a combination therapy that included barrier membranes plus demineralized freeze-dried bone allograft and citric acid root conditioning. In three controlled clinical trials, the treatment of a total of 45 pairs of intrabony defects with demineralized freeze-dried bone allograft grafting and guided tissue regeneration were compared with guided tissue regeneration alone. The differences between the two treatments did not reach statistical significance, thus indicating no added effect of combining demineralized freeze-dried bone allograft with barrier materials in the treatment of intrabony defects. Guillemin et al. (53) compared the effect of demineralized freeze-dried bone allograft alone with a combination of barrier materials plus demineralized freeze-dried bone allograft in 15 pairs of intrabony defects. Both treatments resulted in significant amounts of clinical attachment level gains and bone fill at 6 months, but no difference was found between the treatments. The same outcomes were reported by Trejo et al. (138) in a randomized clinical trial that compared polylactic acid barriers plus demineralized freeze-dried bone allograft with polylactic acid barriers alone.

Promising clinical results with a clinical attachment level gain ranging from 1.0 to 5.5 mm were obtained in human case reports, in which the combination of barrier membranes and Bio-Oss® was used for the treatment of intrabony periodontal defects (76, 87, 99). The combined Bio-Oss® and guided tissue-regeneration treatment resulted in greater pocket-depth reduction, clinical attachment level gain and defect fill compared with the implantation of Bio-Oss® alone in a case series (11) and flap surgery alone in a split-mouth study (10). Three randomized controlled clinical studies (116, 126, 136) confirmed that clinical improvements in defects treated with barrier membranes in combination with Bio-Oss® grafting were significantly better than those obtained with flap surgery alone. In a controlled study (101), similar clinical improvements were obtained when Bio-Oss® combined with guided tissue regeneration was compared with biomodification of the root surface with enamel matrix protein.

Combination therapy, including the use of amelogenins plus barrier membranes and/or grafting materials, has been tested. A systematic review (142) concluded that there is evidence to support the use of amelogenins, either alone or in combination with grafts, to treat intra-osseous defects effectively. The additional use of a graft seems to enhance the clinical outcome of amelogenins; the combined use of human platelet-derived growth factor-BB and P-15 with a graft biomaterial has shown beneficial effects in intra-osseous defects; contrasting results were reported for platelet-rich plasma and graft combinations. The systematic review of Tu et al. (145) concluded that there was little evidence to support the additional benefits of amelogenins in conjunction with other regenerative materials when compared with amelogenins alone. When different types of bone grafts and barrier membranes were treated separately, amelogenins with bovine bone grafts showed greater treatment effects.

The surgical approach

Application of all the aforementioned regenerative strategies, including combinations, requires stable protection by soft tissues to avoid bacterial contamination. Membrane exposure with consequent bacterial contamination during healing represented the major complication of periodontal-regenerative procedures previously, with prevalence in the range of 50–100% (1, 2, 14–16, 39, 40, 45, 82, 89, 119, 140). Cortellini et al. (19, 20) reported that the prevalence of membrane exposure could be greatly reduced with the use of access flaps, specifically designed to preserve the interdental tissues (i.e. the modified papilla preservation technique). Many studies have shown that exposed membranes are contaminated with

bacteria (39, 40, 77, 94, 95) and contamination of exposed nonbioabsorbable as well as bioabsorbable membranes is associated with lower probing attachment-level gains in intrabony defects.

Another important issue associated with clinical results is the coverage of the regenerated tissue after removal of a nonbioabsorbable membrane. Many authors have reported that the frequent occurrence of a gingival dehiscence over barrier membranes is likely to result in insufficient protection of the interdental regenerated tissue (1, 2, 15, 16, 119, 131). Exposure of the regenerated tissue to the oral environment enhances the risks of mechanical and infectious insults that may, in turn, prevent complete maturation of the regenerated tissue into a new connective tissue attachment. In fact, incomplete coverage of the regenerated tissue was associated with reduced attachment and bone gain at 1 year (131). The positioning of a saddle-shaped free gingival graft over the regenerated interdental tissue was suggested to offer better coverage and protection than a dehiscent gingival flap (18).

In general, the development of new procedures was aimed at complete preservation of the marginal tissue in order to achieve and maintain primary closure on top of the applied regenerative material/substance during the critical stages of healing. Specifically, flap designs attempted to achieve passive primary closure of the flap combined with optimal wound stability.

Papilla preservation flaps. The modified papilla preservation technique was developed in order to achieve and maintain primary closure of the flap and to increase the ability to create space for regeneration in the interdental area, (20). This approach is based on the elevation of ample full-thickness buccal and lingual flaps, followed by a buccal periosteal incision to increase buccal flap mobility. Vertical releasing incisions are traced when needed. Flaps are generally coronally positioned on top of barriers and/or grafts or combinations and are sutured with a double-layer suturing technique to provide stable interdental closure. The double-layer suturing approach is mandatory. The deep internal crossed mattress suture is aimed at coronally advancing the buccal flap and the second suture is aimed at sealing the papilla in the absence of tension. The application of this technique reduced wound failure and subsequent bacterial contamination to about 30% of the treated sites (Fig. 1A–J). The modified papilla preservation technique allowed stable primary closure of the flap in the interdental space in 70% of the sites, providing protection of the regenerative materials and the underlying regenerating tissues from the oral environment. In a randomized controlled clinical study on 45 patients (19), significantly greater amounts of attachment gain were obtained with the modified papilla preservation technique and titanium-reinforced barriers (5.3 ± 2.2 mm) in comparison with either conventional guided tissue regeneration (4.1 ± 1.9 mm) or flap surgery (2.5 ± 0.8 mm). The sites treated with the modified papilla preservation technique also developed less gingival recession compared with control therapies. This controlled clinical study demonstrated that a papilla preservation flap can result in improved clinical outcomes compared with regeneration performed using conventional flap approaches without interdental soft-tissue preservation.

A meta-analysis (90) showed a trend of increased clinical outcomes in studies using flap designs and suturing techniques considered conducive to the achievement and maintenance of primary closure of the flap. The modified papilla preservation technique can be successfully applied in sites in which the interdental space width is at least 2 mm at the most coronal portion of the papilla and in conjunction with a variety of regenerative materials, including barriers, biologically active materials such as amelogenins (135) or growth factors and bone replacement grafts (31, 136).

When interdental sites are narrower, a different papilla preservation procedure has been proposed, the simplified papilla preservation flap (25). In the cited study, 100% of the narrow interdental papilla could be closed on top of bioresorbable barriers, and 67% maintained primary closure over time, resulting in gains of clinical attachment level of 4.9 ± 1.8 mm (Fig. 2A–I). This approach has been successfully applied in several multicenter randomized clinical trials designed to test the generalizability of the added benefits of using barrier membranes in deep intrabony defects (28, 134) and in conjunction with a variety of regenerative materials, including biologically active materials such as amelogenins (135) and bone-replacement grafts (31, 136).

Following the path of surgical refinements, the potential of soft-tissue manipulation to obtain stable protection of the regeneration site has been further explored, applying a microsurgical approach in the regenerative therapy of deep intrabony defects. Cortellini & Tonetti (29, 31) and Wachtel et al. (148) tested the use of operative microscopes and microsurgical instruments to increase visual acuity and surgical accuracy in the application of papilla preservation flaps in periodontal regeneration (Fig. 3A–G). Microsurgery reduced wound failure to a

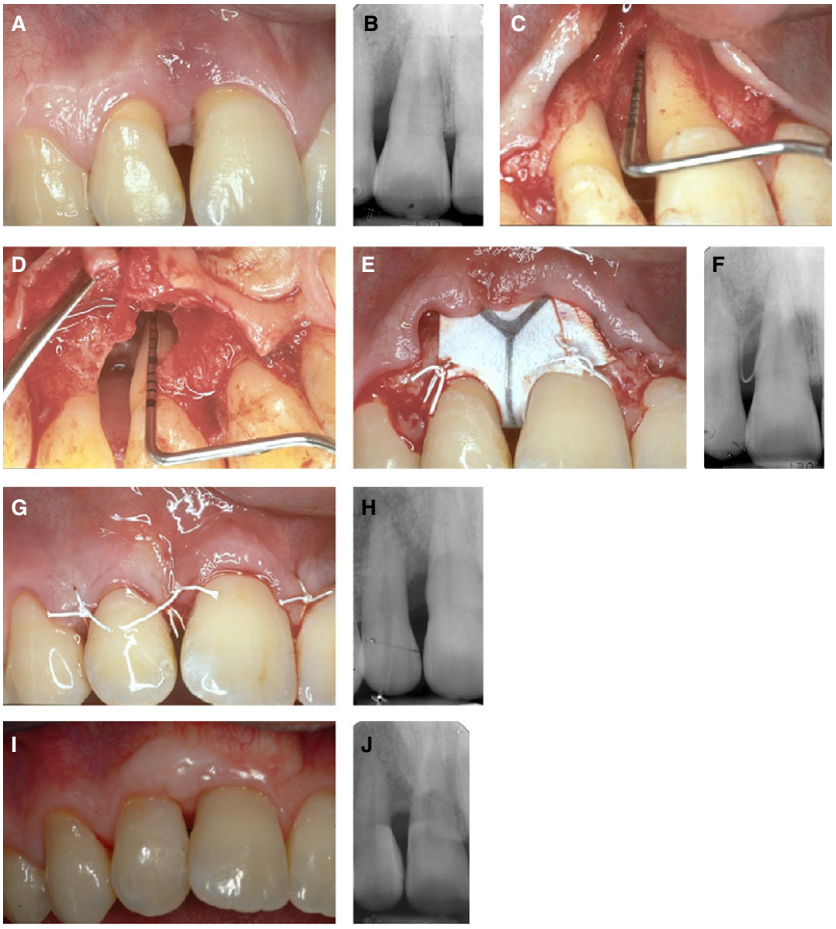

Fig. 1. (A) Preoperative image of a right central incisor presenting with a pocket depth of 16 and 2 mm of gingival recession. (B) Baseline radiograph showing an intrabony defect reaching the apex of the tooth. (C) A large buccal flap has been raised to obtain proper access to the severe defect. After debridement, a two-wall, wide, 14-mm-deep intrabony defect associated with a buccal bone dehiscence is evident on the central incisor. The lateral incisor shows loss of bone on the buccal and mesial root surfaces. (D) The severe bone defect also involves the lingual root surface. The lingual flap is extended to the neighboring teeth. (E) A titanium-reinforced expanded-polytetrafluoroethylene barrier has been positioned. (F) Postoperative radiograph showing the 'space' provided by the titanium-reinforced barrier. (G) The modified papilla preservation large flap has been coronally advanced and sutured with a double-layer suturing technique. (H) One-year radiograph showing the consistent bone fill of the defect. (I) Clinical image after 15 years. An interdental free gingival graft was positioned 2 years after regeneration, partly to solve the baseline gingival recession. (J) Radiograph after 15 years, showing complete mineralization of the defect area.

mere 6% of the treated sites when applying the modified papilla preservation technique and the simplified papilla preservation flap (29, 31). In a patient cohort study on 26 patients with 26 intrabony defects treated with papilla preservation techniques, primary closure on the barrier was obtained in 100% of the defects and maintained over time in 92.3% of the sites (29). Treatment resulted in large amounts of clinical attachment level gains (5.4 $\pm$ 1.2 mm) and minimal gingival recession (0.4 $\pm$ 0.7 mm).

Minimally invasive surgical technique

In the last decade, a growing interest for more patient-friendly and patient-oriented surgery has urged clinical investigators to focus their interest in the development of less-invasive approaches. Harrel & Rees (56) proposed the minimally invasive surgical technique with the aim to produce minimal wounds, minimal flap reflection and gentle handling of the soft and hard tissues (57, 58). In order to provide even greater wound stability and protection, and to limit patient morbidity further, Cortellini & Tonetti proposed a papilla preservation flap in the context of a minimally invasive, high-power, magnification-assisted surgical technique (32). The minimally invasive approach is particularly suited for treatment in conjunction with biologically active agents, such as amelogenins or growth factors, which are eventually associated with grafting materials. In the minimally

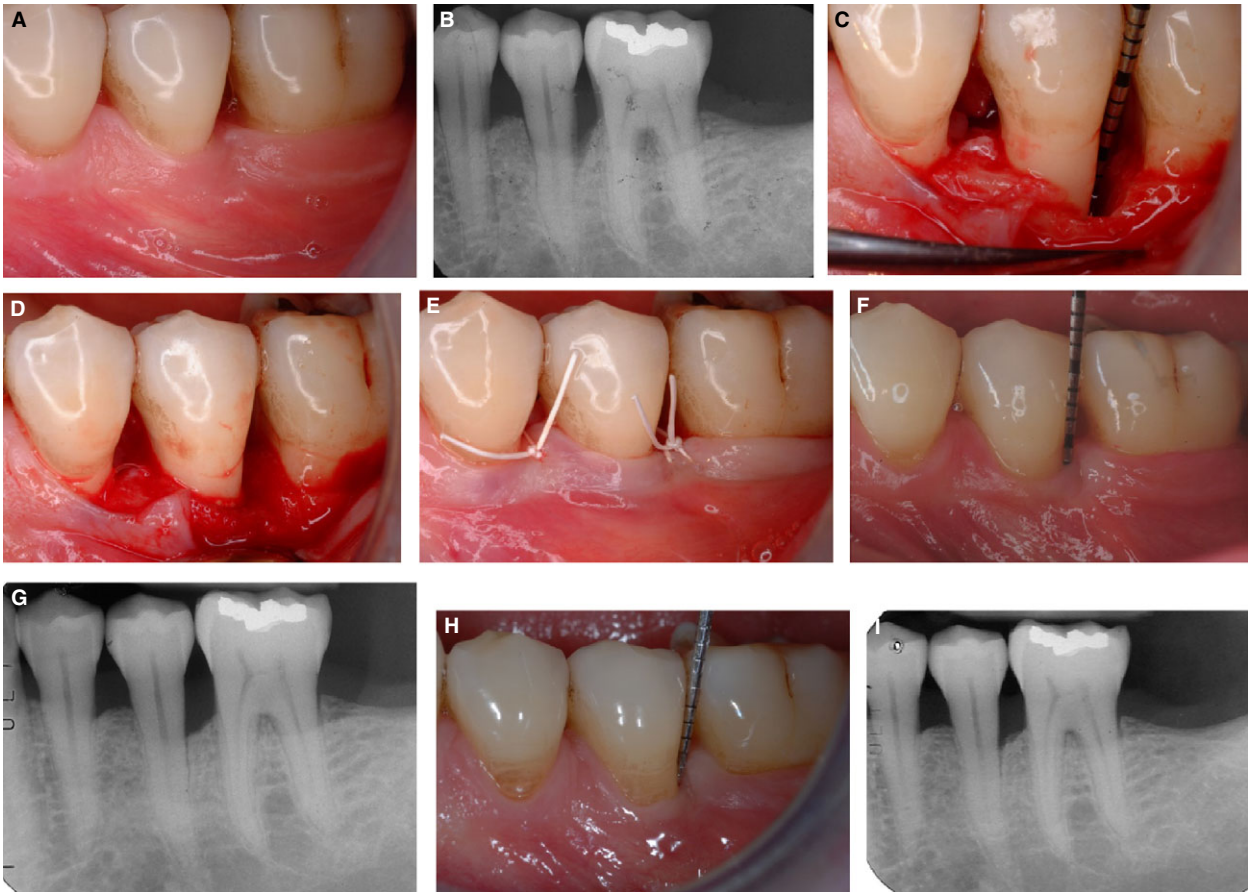

Fig. 2. (A) Preoperative image of a second lower-left premolar presenting with a distal pocket of 6 mm. (B) Distal narrow intrabony defect reaching the mid-third of the root. (C) A large buccal flap, extending to the neighboring teeth, uncovers a two- to three-wall 5-mm intrabony defect. (D) A bioresorbable barrier was positioned on the defect area. (E) The simplified papilla preservation large flap has been coronally positioned and sutured to cover the barrier completely. (F) At 1 year, a 3-mm sulcus is associated with minimal gingival recession with respect to baseline. (G) The 1-year radiograph showing almost complete resolution of the intrabony component of the defect. (H) Clinical stability after 10 years. (I) Radiographic evidence of stability of the regenerated bone after 10 years.

invasive surgical technique approach, the defect-associated interdental papilla is accessed either with the simplified papilla preservation flap (25) in narrow interdental spaces (Fig. 4A–I) or the modified papilla preservation technique (20) in large interdental spaces (Fig. 5A–G). After elevation of the interdental tissues, the buccal and the lingual incisions are minimally extended mesial-distally and the full-thickness flaps are minimally elevated in order to expose just the coronal edges of the residual bony walls. Periosteal incisions are never performed. Vertical releasing incisions are placed in very few instances. The suturing approach is based on the use of a single internal modified mattress suture. Additional sutures can be applied to further increase primary closure, when needed. All surgical procedures are performed with the aid of an operating microscope or magnifying loops at 4–16× magnifications (29, 31). Microsurgical instruments are utilized, whenever needed, as a complement to the normal set of periodontal instruments. The minimally invasive surgical technique associated with the application of amelogenins has undergone preliminary testing in two case series with a total of 53 deep intrabony defects (32, 33). The 1-year results have shown clinically significant improvements (clinical attachment level gains of 4.8 ± 1.9 mm and 88.7 ± 20.7% clinical resolution of the defect) accompanied by greatly reduced patient morbidity. The same approach was successfully applied to multiple intrabony defects in 20 patients (34). The 44 treated defects gained, on average, 4.4 ± 1.4 mm of clinical attachment. Of the defects, 73% showed clinical attachment level improvements of ≥ 4 mm. This corresponded to an 83 ± 20% resolution of the defect (15 defects were completely filled). Residual probing pocket depth was 2.5 ± 0.6 mm. A minimal increase of 0.2 ± 0.6 mm in gingival recession between baseline and 1 year was recorded.

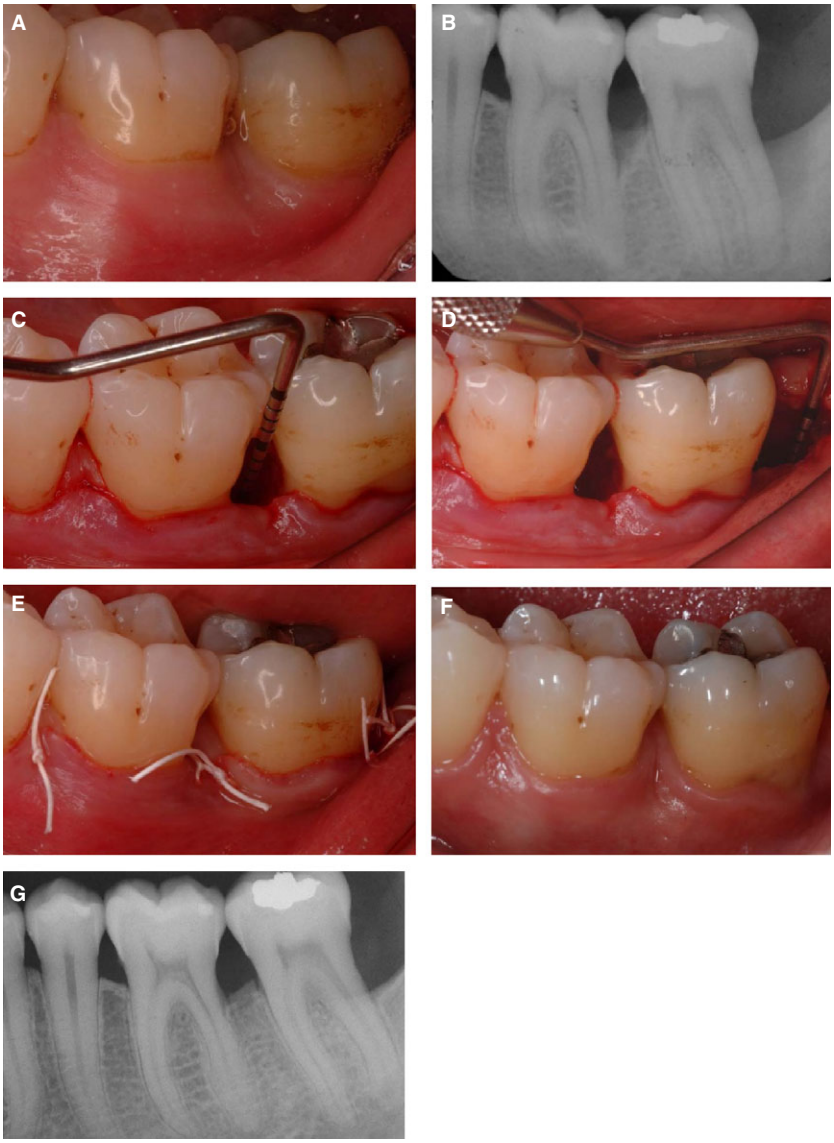

Fig. 3. (A) Baseline clinical image of the first and second lower-left molars. The two teeth present deep distal pockets. (B) Baseline radiograph showing the deep intrabony components of the defects. (C) A 6-mm three-wall defect distal to the first molar, accessed using a modified papilla preservation technique. (D) A 9-mm three-wall intrabony defect distal to the second molar, accessed with a crestal incision. (E) The flap is sutured to cover the treated area completely, after application of amelogenins. (F) Clinical image of the area after 10 years. (G) Radiograph showing the complete resolution of the defect after 10 years.

A recent controlled clinical study on 30 patients compared the minimally invasive surgical technique plus amelogenins with the minimally invasive surgical technique alone (107). The authors reported significant probing pocket-depth reductions, clinical attachment level gains and radiographic bone gain at 3 and 6 months in both groups. No differences were detected between therapies at any time. They concluded that the use of amelogenins did not provide superior benefits on the outcome of the minimally invasive surgical approach for the treatment of intrabony defects.

An enhancement of this technique, the modified minimally invasive surgical technique (36), has been recently tested. The modified minimally invasive surgical technique was designed specifically to improve flap stability and to provide self-ability to maintain space for regeneration. The surgical approach consists of a tiny interdental access in which only a buccal triangular flap is elevated, while the papilla is left in place, connected to the root of the crest-associated tooth with its supracrestal fibres (Fig. 6A–I). Access to the defect is gained through the tiny buccal triangular flap: from the buccal 'window' the soft tissue filling the defect (i.e. the so-called granulation tissue) is sharply dissected from the papillary supracrestal connective tissue and from the bony walls with a microblade and removed with a minicurette. Then, the root surface is carefully debrided with hand and mechanical instruments. The supracrestal fibres of the defect-associated papilla and the palatal tissues are left untouched. The minimal wound and the minimal flap elevation allows for preservation of most of the vessels providing blood supply to the interdental tissues, with obvious advantages for the healing process of the interdental wound. This

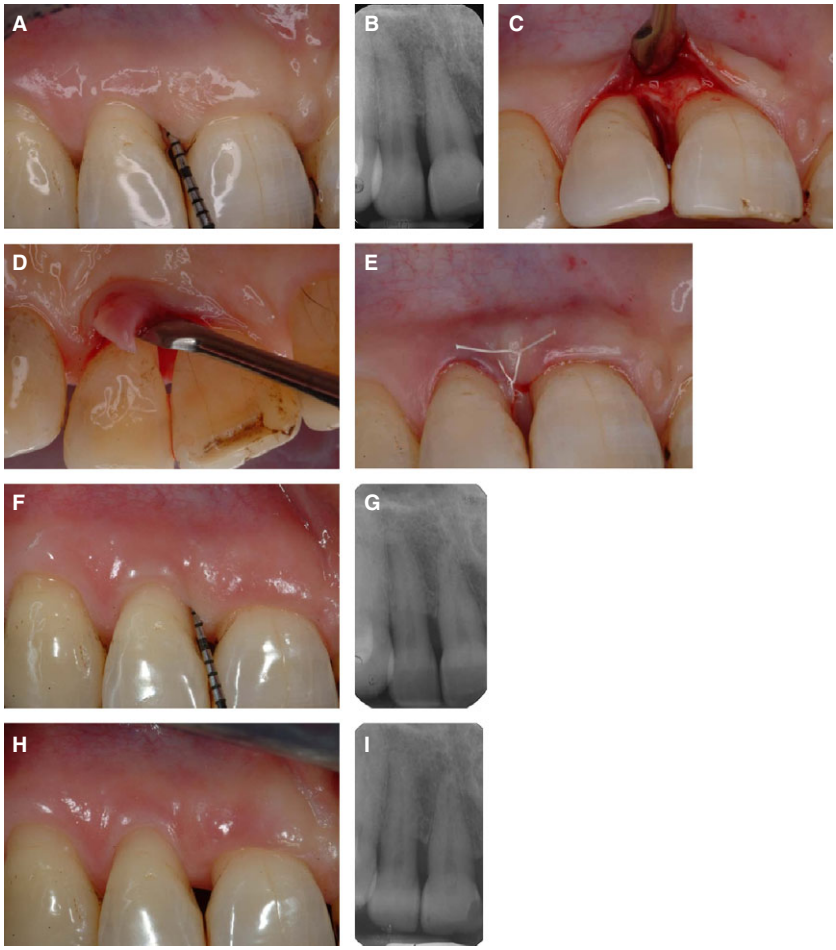

Fig. 4. (A) Upper left lateral incisor presenting with a 7-mm pocket. (B) The radiograph shows a narrow mesial intrabony defect. (C) The defect was accessed using a minimally invasive surgical technique involving only the defect-associated papilla. (D) The defect-associated papilla, incised according to the simplified papilla preservation flap oblique interdental incision, was minimally elevated to the palatal side. (E) A single internal modified mattress suture seals the area, after treatment with amelogenins. (F) The 1-year image shows stability of the gingival margin associated with a 3-mm normal sulcus. (G) One-year radiograph showing the resolution of the intrabony component of the defect. (H) Clinical stability of the treated area after 5 years. (I) Radiographic stability of the treated area after 5 years.

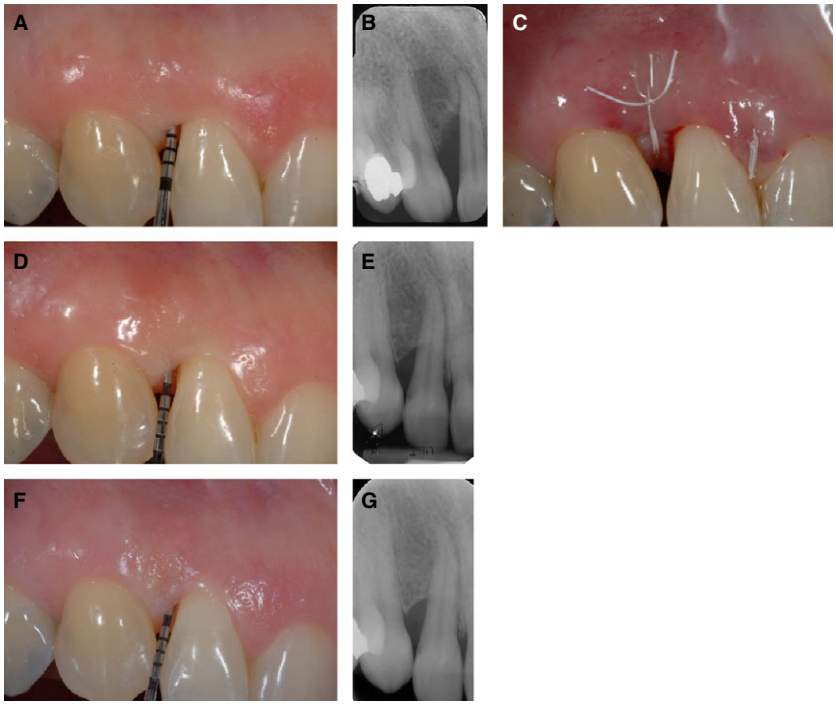

Fig. 5. (A) An 11-mm-deep pocket distal to the upper right lateral incisor. (B) A deep and wide intrabony defect is evident in the baseline radiograph. (C) A single interdental suture is positioned to seal the defect area after application of amelogenins. The area was accessed using a modified papilla preservation technique-like horizontal interdental incision. The buccal flap was extended to the mesial side of the lateral incisor to gain better access to the defect. (D) The 1-year probing shows a clinical attachment gain of 7 mm. (E) The 1-year radiograph showing the resolution of about 70% of the intrabony component of the defect. (F) Six-year clinical stability of the outcomes. (G) Clinical stability of the regenerated bone after 6 years.

surgical approach, with its particular design, ensures self-support to the interdental soft tissues through the 'hanging' papilla, thereby enhancing space provision. The flap is extremely stable because most of the soft tissues around the bony defect are not incised or elevated, thereby enhancing blood-clot stability. Minimal flap trauma, integrity of the blood supply and absolute passivity in the suturing technique ensures primary closure of the interdental wound in the majority of cases, thereby preventing bacterial contamination. The suturing approach is based on the use of a single internal modified mattress suture. Additional sutures can be applied, when needed, to ensure primary closure. However, the reduced buccal access means that this approach is not applicable to very deep defects that involve the lingual side of a tooth in which the diseased root surface cannot be reached easily for instrumentation from the small buccal window (36).

Recently, a three-armed randomized controlled clinical trial was designed to compare the clinical efficacy of the modified minimally invasive surgical technique alone with the modified minimally invasive surgical technique plus amelogenins (enamel matrix derivative) and with the modified minimally invasive surgical technique plus amelogenins plus bone mineral-derived xenograph, in the treatment of isolated, interdental intrabony defects (37). The study was performed on 45 deep, isolated, intrabony defects accessed using the modified minimally invasive surgical technique and randomly assigned to three experimental groups: 15 to the modified minimally invasive surgical technique alone; 15 to the modified minimally invasive surgical technique + enamel matrix derivative; and 15 to the modified minimally invasive surgical technique + enamel matrix derivative + bone mineral-derived xenograph. Differences between baseline and 1 year were statistically significant in the three groups in terms of probing pocket-depth reduction ($P < 0.0001$, Student's t-test) and clinical attachment level gain ($P < 0.0001$). Comparisons among the three groups showed no statistically significant

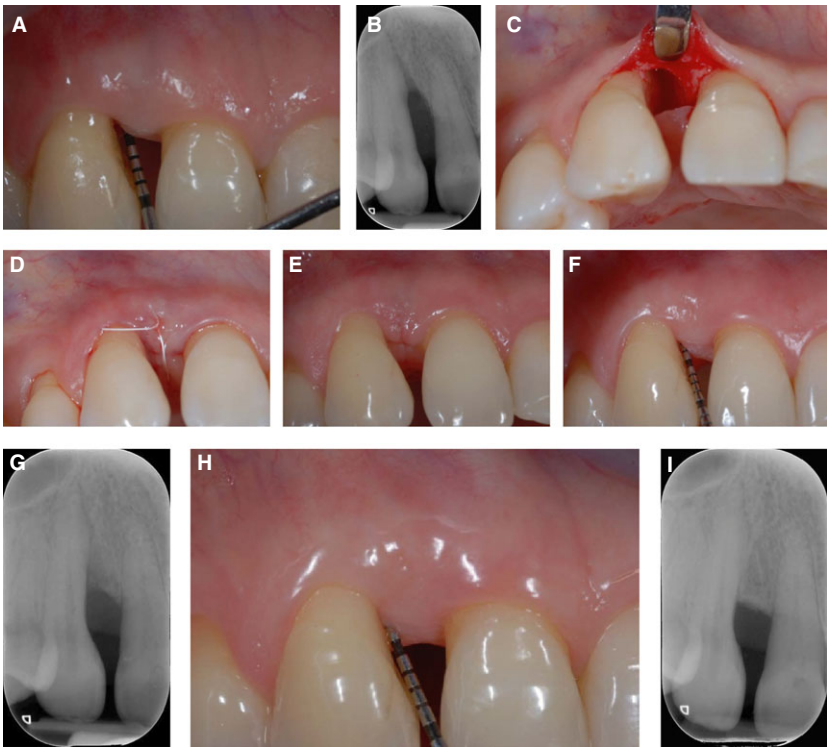

Fig. 6. (A) A 10-mm pocket mesial to the upper right cuspid. (B) The baseline radiograph shows a deep intrabony defect reaching the mid-third of the root. (C) The area was accessed using the modified minimally invasive surgical procedure. The buccal flap is minimally elevated to the midbuccal contour of the cuspid and the lateral incisor. The defect-associated interdental papilla has been left untouched and the lingual flap was not elevated. The intrabony defect and the exposed root surface were instrumented through the small buccal surgical 'window'. (D) Single modified internal mattress suture positioned to close the area. No regenerative material was placed in the defect, leaving the natural blood clot alone to fill the intrabony component. (E) Integrity of the primary closure of the wound after 1 week. (F) Light probing after 6 months showed penetration of the probe to a depth of 3 mm. (G) The defect was mineralized almost completely after 6 months. (H) The 1-year clinical image shows a 3-mm normal sulcus, associated with 7 mm of clinical attachment gain and no increase in gingival recession. (I) The 1-year radiograph shows complete resolution of the intrabony component of the defect.

difference in any of the measured clinical outcomes. In particular, clinical attachment level gains were 4.1 ± 1.4 mm in the modified minimally invasive surgical technique control group, 4.1 ± 1.2 mm in the enamel matrix derivative group and 3.7 ± 1.3 mm in the enamel matrix derivative + bone mineral-derived xenograph group. The radiographic bone fill of the intrabony component was 77 ± 19% in the modified minimally invasive surgical technique control group, 71 ± 18% in the enamel matrix derivative group and 78 ± 27% in the enamel matrix derivative + bone mineral-derived xenograph group. This initial controlled study had the power to detect a true difference of 0.96 mm in clinical attachment levels among treatment groups. However, the fact that the outcomes among the three groups could not be discriminated raises a series of hypotheses that focus on the intrinsic healing potential of a wound when ideal conditions are provided with the surgical approach. In other words, the outcomes of this study challenge clinicians with the possibility to obtain substantial clinical improvements without the use of products or materials. An independent study (143) reported similar outcomes, with no difference between a single flap approach only and a single flap approach plus a bioresorbable barrier and hydroxyapatite. However, larger studies are needed to confirm the reported outcomes.

Surgical and postsurgical events

Clinicians are interested in information about the surgical and postsurgical period, such as chair-time required for the surgical procedure, postsurgical complications, and pain and painkiller consumption after the procedure. From the very beginning of the 'guided tissue regeneration era' the frequent occurrence of complications was apparent, in particular exposure of barriers. It arose in almost 100% of cases in the pre-papilla preservation techniques period (1, 2, 14–16, 39, 40, 45, 82, 89, 119, 140) and was reportedly reduced to a small number of cases (6–50%) when papilla preservation flaps were adopted (19, 20, 22, 25, 26, 28, 29, 78, 90, 134–136). A consistent decrease in complications was observed when barriers were not incorporated into the surgical procedure. In particular, the adoption of amelogenins largely reduced the prevalence of complications (44, 134). A comparative study between barriers and amelogenins clearly demonstrated such a striking difference, reporting a complication rate of 100% in the barrier-treated sites compared with 6% in the amelogenin-treated sites (110).

Table 2 documents some surgical and postsurgical parameters from three studies. Two studies were performed applying the traditional papilla preservation flaps with bioresorbable barriers (28) and amelogenins (135). The third study was performed using the minimally invasive surgical technique in combination with amelogenins (33). A historical comparison highlights clear differences in some parameters among the three studies. Surgical chair-time was the longest when large papilla preservation flaps and barriers were applied, shorter when large papilla preservation flaps were combined with amelogenins and by far the shortest when the minimally invasive surgical technique and amelogenins were used. The number of subjects reporting postoperative pain was similar in the two papilla preservation flap studies and much reduced in the minimally invasive surgical technique study, as was pain intensity and consumption of painkillers (Table 2). The reported outcomes indicate that use of different materials (barriers or amelogenins) applied in combination with a similar surgical approach results in similar postoperative pain, whereas a more user-friendly, shorter chair-time, minimally invasive surgery is associated with less postoperative pain. In other words, postoperative pain apparently is not influenced by the type of regenerative material but by the type of surgical approach. The minimal amount of complications and postoperative problems associated with application of minimally invasive surgical technique and amelogenins was recently confirmed by the same group in another study on multiple defects (34) and by an independent controlled study (108). These considerations suggest that clinicians should adopt tissue-friendly approaches whenever possible.

Clinical potential and limits for regeneration

From the very beginning of modern periodontal regeneration therapy it was apparent that periodontal tissues could express a surprising regenerative potential under particular circumstances. Sparse case reports demonstrated that very deep defects, reaching the apical third of the root, could be substantially filled with new bone and new clinical attachment (1, 14, 102). Larger studies suggested that in deeper defects a greater amount of clinical improvements is generally obtained (46, 124, 131). These observations raised questions about the 'potential' for regeneration: is the potential greater in deeper defects? A multicenter randomized controlled study demonstrated that deep and shallow defects have the 'same potential' for

Table 2. Surgical and postsurgical parameters reported from three studies

References	Regenerative approach	No. of patients	Chair time (min)	Subjects with postoperative pain (%)	Pain intensity	No. of painkillers
Cortellini et al. (28)	Simplified papilla preservation flap/modified papilla preservation technique + bioresorbable barriers	56	99 ± 46	46	28.1 ± 2.5	4.1 ± 2.5
Tonetti et al. (137)	Simplified papilla preservation flap/modified papilla preservation technique + amelogenins	83	80 ± 34	50	28.0 ± 20.0	4.3 ± 4.5
Cortellini & Tonetti (33)	Minimally invasive surgical technique + amelogenins	40	58 ± 11	30	19.0 ± 10.0	1.1 ± 2.0

The chair-time was measured from the delivery of anesthesia to completion of the regenerative surgical procedures; the number of subjects reporting some postoperative pain was determined at the 1-week recall visit; the intensity of pain was measured using a visual analogue scale; and the number of painkillers was those taken in addition to the two compulsory painkillers administered at the end of surgery.

regeneration (23). The cited study reported similar amounts of attachment gain in defects presenting with an intrabony component of ≤ 3 mm (76% defect resolution) and defects of ≥ 4 mm (77% defect resolution), indicating that the potential for regeneration is similar in either shallow or deep intrabony components. The conclusions of this study are indirectly supported by the results of large controlled clinical trials performed with the application of different successful regenerative approaches (19, 22, 28, 134–136). Unpublished subanalyses of these experimental populations, in which the treated defects have been clustered according to defect depth, show that clinical attachment gain is obtained in all defects from shallow to deep, but deeper defects gain more attachment (in millimetres) than do shallow defects. In other words, regeneration seems to express its potential as much as the 'container' allows it to do so and irrespective of the 'regenerative approach' chosen, within the panel of the regenerative approaches tested. A recent controlled study has challenged the limits of the periodontium to repair or regenerate (38). The aim of this randomized, long-term clinical trial was to compare clinical and patient-based outcomes following periodontal regeneration or extraction and replacement of 'hopeless' teeth with attachment loss to or beyond the apex. Twenty-five hopeless teeth were treated with the application of a regenerative strategy. Most of the treated teeth had a periodontal lesion exceeding the apex of the tooth and involving three to four sides of the root (Figs. 7A–F and 8A–G). Twenty-three of the 25 regenerated teeth experienced extensive clinical improvements. The average clinical attachment level gain was 7.7 ± 2.8 mm, the radiographic bone gain was 8.5 ± 3.1 mm and the probing pocket-depth reduction was 8.8 ± 3.0 mm. Most of the regenerated teeth showed a decrease in tooth mobility. Only two teeth showed unsatisfactory outcomes and these were extracted at 1 year. The 23 (92%) successfully regenerated teeth were in good health and function at the 5-year examination visit and 84% did not develop biologic complications during the recall period. The authors concluded that regenerative therapy can be successfully applied, even at hopeless teeth, and has the potential to change their prognosis. It should be clearly shared that the reported outcomes were obtained in a carefully selected patient population, after applying 'state of the art' regenerative therapy by very experienced clinicians, within a high-quality program of periodontal and dental therapy and a strict periodontal supportive-care program. In other words, it is apparent from the cited studies that to succeed in extreme conditions a sound strategy has to be adopted.

Clinical strategies

Scientific background

In summary, periodontal regeneration in intrabony defects has been successfully attempted with a variety of different regenerative materials and surgical approaches. As discussed, meta-analyses of randomized controlled clinical trials, as well as human and animal histologic findings, support the potential of barrier membranes (50, 97), demineralized freeze-dried bone allograft (6–8), combinations of barrier membranes and grafts (11, 87) and the use of enamel matrix derivative (86, 147) or growth factors (65) to induce periodontal regeneration. Controlled clinical trials report that the above-mentioned approaches provide added benefits, in terms of

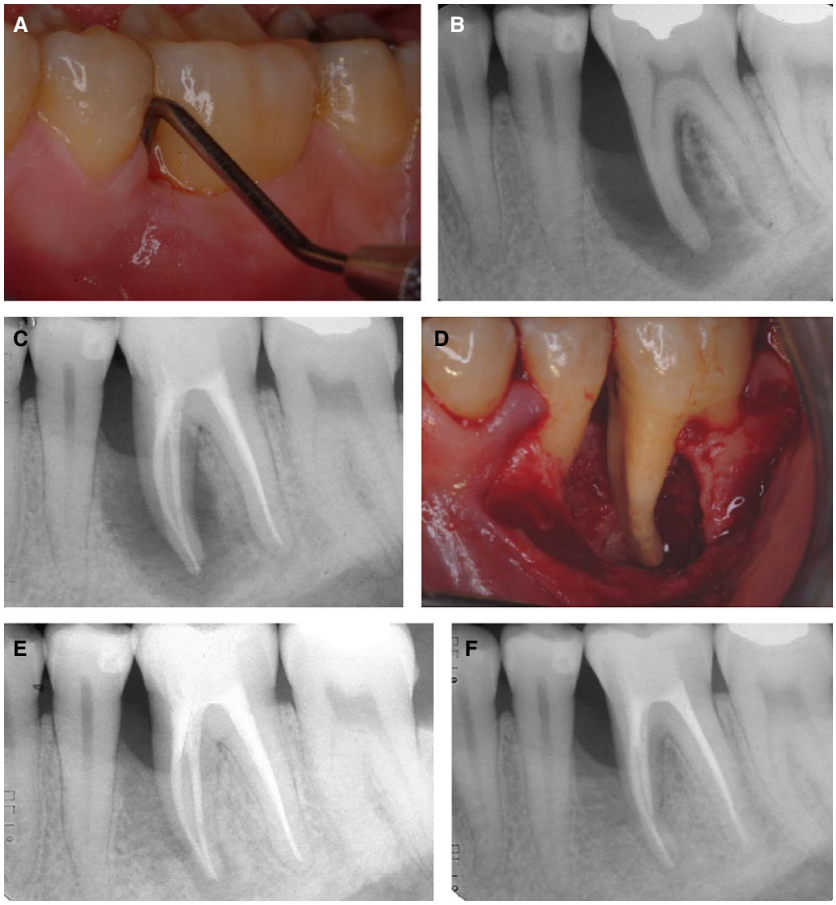

Fig. 7. (A) Baseline image of a 16-mm pocket mesial to the first lower-left molar. (B) The baseline radiograph shows evidence of a very deep intrabony defect extending beyond the apex of the mesial root. The tooth was vital and the furcation was not involved. (C) The vital tooth was endodontically treated 3 months before regeneration. The reason for the endodontic treatment of this vital tooth was the need to instrument the apex of the mesial root involved by the severe periodontal defect. (D) The mesial root is involved by an all-around periodontal defect and appears to be hanging in the space of a complex intrabony defect associated with a complete buccal bone dehiscence. The defect was treated with amelogenins. (E) The 1-year radiograph shows almost complete resolution of the defect associated with the mesial root. (F) Stability of the radiological outcomes after 5 years.

clinical attachment level gain, compared with open flap debridement alone [Trombelli et al. (141), Murphy & Gunsolley (90), Giannobile & Somerman (47), Esposito et al. (43, 44), Needleman et al. (91)]. Comparisons among some of the cited regenerative approaches failed to demonstrate clear superiority of one of the tested materials (44, 47, 90, 106, 142, 145). Therefore, the existing evidence does not support the choice of a single approach among the different regenerative possibilities. In addition, all the cited studies have shown a substantial degree of variability, in terms of clinical attachment level gains, reporting failures or unsatisfactory outcomes in part of the treated population. This trend is not unexpected because each patient randomly entered in each study presents with unique individual characteristics and each defect presents with very different and unique anatomies. The outcomes of each randomized study clearly demonstrate that none of the regenerative approaches can resolve all the different patient and defect presentations. It is therefore essential to build up a clinical decision tree that allows clinicians to apply the appropriate regenerative strategy to each individual case.

The body of evidence discussed above has already been utilized in recent years to develop an evidence-based regenerative strategy to guide clinicians through a decision-making process aimed at optimizing the clinical outcomes of periodontal regeneration in intrabony defects (26, 31). The performance of this clinical strategy has been assessed in a 40-patient consecutive case series treated with the papilla preservation techniques (31). Based on defect anatomy, nonresorbable titanium-reinforced expanded-polytetrafluoroethylene barrier membranes or bioresorbable membranes supported with a bone-replacement graft were used in 23 one- to two-wall-wide intrabony defects, bioresorbable barriers alone were used in seven narrow two-wall defects and amelogenins were applied to 10 defects with a prevalent three-wall component. Flap primary closure was obtained in 100% of the patients at completion of surgery and was maintained in 93% of the sites during the entire early healing phase. The 1-year clinical attachment gain was 6 ± 1.8 mm. No sites gained < 4 mm of clinical attachment; 77.5% gained ≥ 5 mm and 40% gained > 6 mm. Residual probing depths were 2.7 ± 0.6 mm, and the increase in gingival recession

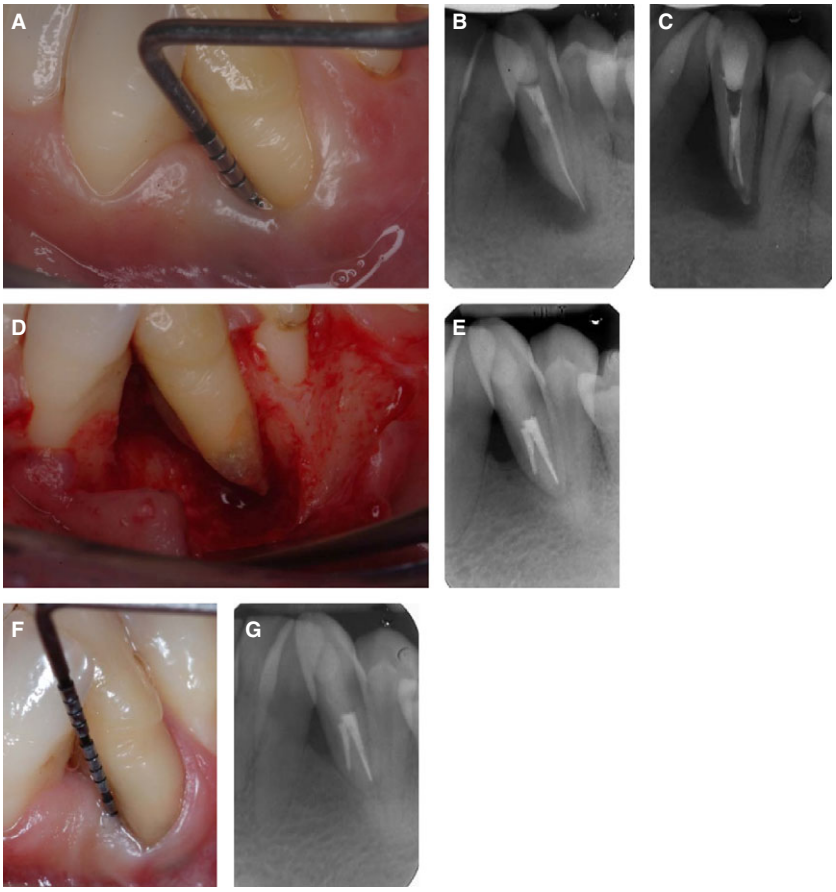

Fig. 8. (A) Lower-left cuspid. The tooth is associated with a pocket of 10 mm and clinical attachment loss of 16 mm. (B) The baseline radiograph highlights the presence of a very deep mesial defect, extending beyond the apex of the tooth, associated with an incomplete endodontic treatment. (C) The endodontic treatment has been improved. However, after 4 months, no radiologic improvements of the lesion were detected. (D) After debridement, calculus covering the root apes is evident. The mesial intrabony defect is associated with complete buccal dehiscence, extending beyond the apex. The area was treated with amelogenins. (E) The 1-year radiograph shows almost complete resolution of the defect. (F) The 5-year clinical image shows a 3-mm normal sulcus associated with clinical attachment level gain of 6 mm. (G) The 5-year radiograph shows the stability of the regenerated bone.

was minimal between baseline and 1 year (0.1 ± 0.7 mm). The four approaches resulted in 88–95% resolution of the original depth of the intrabony component of the defect (31). The outcomes of the reported study clearly demonstrate that the application of a sound strategy can help clinicians to optimize the clinical outcomes in each single case.

A novel, more comprehensive, clinical strategy has been developed to improve the clinical capacity further, to ensure optimal therapy for each given patient and defect site. This approach takes into account the relevance of the patient characteristics, as previously reported in this review, and bases its foundations on the need to satisfy the three major contributors to periodontal regeneration: (i) the need for space for the formation of the blood clot at the interface between the flap and the root surface (19, 20, 55, 67, 121, 133, 151); (ii) the need for stability of the blood clot to maintain continuity with the root surface, avoiding formation of a long junctional epithelium (55, 64, 75, 150); and (iii) the need for soft-tissue protection of the treated area to avoid bacterial contamination (39, 40, 94, 95, 104, 110, 119).

Space and blood-clot stability are self-provided in the so-called 'containing defects', the narrow three-wall defects in particular (24, 48, 74, 112, 120, 144). The 'noncontaining defects' – the large one- or two-wall defects – require an intervention to supplement the deficient anatomy (45, 131, 132, 135–137). The intervention can be based on the use of biomaterials, such as exoskeleton-like barriers or endoskeleton-like grafts, which are able to support the soft tissues and to stabilize the blood clot, or a combination of both approaches. In other words, the anatomic deficiencies of the defects have to be supplemented by the additional use of biomaterials. The same goal may also be achieved by adopting particular surgical strategies in which tissues are minimally elevated to increase their stability (the minimally invasive surgical technique and the modified minimally invasive surgical technique approaches) (32–37). Blood-clot stability is also clearly influenced by tooth mobility: splinting teeth with Miller grade II or grade III mobility is essential to avoid the disruption of the blood clot in the early healing phase (28, 138).

Protection of the regenerating area should be provided through the adoption of specifically designed surgical approaches. The different surgical approaches developed over time incorporate clear

differences in terms of flap design and suturing technique. In addition to their ability to provide protection to the regenerating area, they may make different contributions to improve one or more of the many processes potentially relevant to overall wound healing. The traditional papilla-preservation flaps (20, 25) were designed as wide and very mobile flaps in order to allow perfect visibility of the defect area, easy application of biomaterials and for the coronal positioning of the buccal flap to cover barriers and biomaterials. In other words, the papilla preservation flaps did not incorporate the mechanical characteristics to improve wound stability and the independent capacity to create space for regeneration. In contrast, the minimally invasive surgical technique (32, 33) was designed to reduce flap extension and mobility as much as possible, and to increase the ability for primary wound closure and blood-clot stability. This potential was partly highlighted in two studies that demonstrated a reduced impact of the number of residual bony walls and of the defect width on the outcomes obtained with amelogenins under a minimally invasive surgical technique (34, 35) and was recently confirmed in a comparative study demonstrating similar outcomes between the minimally invasive surgical technique alone and the minimally invasive surgical technique plus amelogenins (107).

A further development of this surgical approach ended in the modified minimally invasive surgical technique approach (36, 37). This advanced flap design further enhances the potential of the flap to provide space and stability for regeneration by leaving the interdental papillary soft tissues attached to the root surface of the crest-associated tooth and by avoiding any palatal flap elevation. The interdental soft tissues are the stable 'roof' of a room where the blood fills in and forms a clot. In addition, the hanging papilla prevents the collapse of the soft tissues, maintaining space for regeneration: the anatomic bone deficiencies are potentially supplemented by the specific flap design that provides additional 'soft-tissue walls' to the missing bony walls, thus improving stability: the walls of the 'room' are the residual bony walls, the root surface and the buccal/lingual soft tissues. The minimal flap extension and elevation also greatly reduces the damage to the vascular system. It is clear that such a flap is not designed to allow for the positioning of a barrier, but easily allows for the use of biologicals or grafts. The clinical flow charts presented here (Fig. 9–14) were developed also taking into account the scientific contributions on surgical and postsurgical events, such as chair-time, side effects and postoperative pain.

Clinical flow charts

The step-by-step clinical approach to the treatment of intrabony defects includes two presurgical flow charts dealing with patient and local factors (Flow charts 1 and 2) and four surgical flow charts (surgical nodes) (Flow charts 3–6). The build-up of the surgical nodes was driven by the willingness to treat a given defect using the procedure judged as fastest, easiest, less burdened by side effects and best tolerated by patients. Lastly, suggestions for postoperative care are discussed.

The step-by-step approach starts with the control of patient-associated characteristics (Flow chart 1): low levels of plaque and residual infection; high levels of compliance; and absence of adverse conditions such as smoking habit, stress and uncontrolled diabetes or other systemic diseases have to be well established.

A few conditions, such as the endodontic condition, local contamination and mobility of the involved tooth, have to be controlled before surgery (Flow chart 2). Endodontic diagnosis and eventual treatment should be performed well in advance of the regenerative surgery (27). Vital teeth should preferably be kept vital, the only exception being involvement of the apex of a tooth by the periodontal lesion (38). Nonvital teeth have to be successfully treated with root canal therapy. Existing root canal therapies should be carefully evaluated, and inadequate treatments should be redone. Local contamination of the defect-associated pocket should be as low as possible (62). The presence of bleeding on probing (i.e. bacteria) should be controlled with additional gentle root-surface debridement and eventually with the additional use of local antimicrobials (54, 146) a few weeks before regeneration (38). Teeth with a mobility

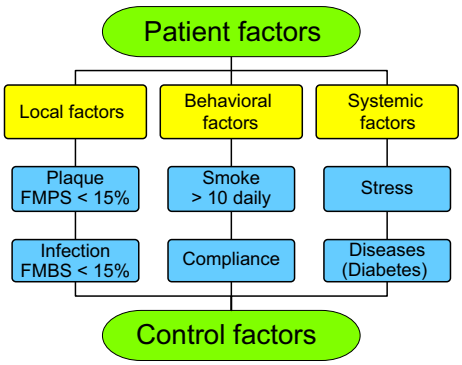

Fig. 9. Presurgical control of patient-associated characteristics. FMBS, full mouth bleeding score; FMPS, full mouth plaque score.

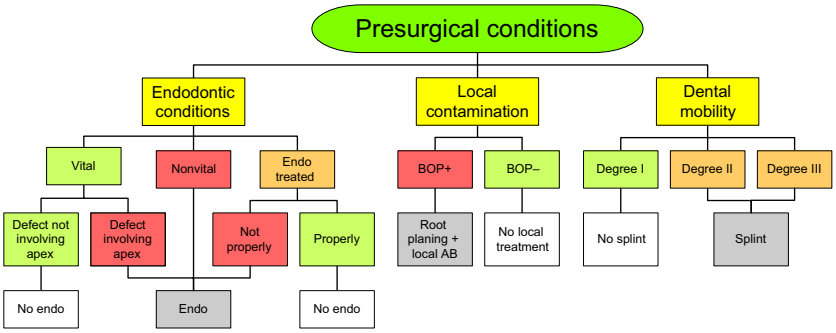

Fig. 10. Presurgical control of patient conditions. AB, antibiotic; BOP, bleeding on probing; Endo, endodontic treatment.

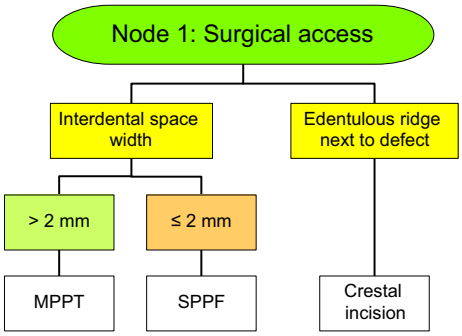

Fig. 11. Node 1: surgical access. MPPT, modified papilla preservation technique; SPPF, simplified papilla preservation flap.

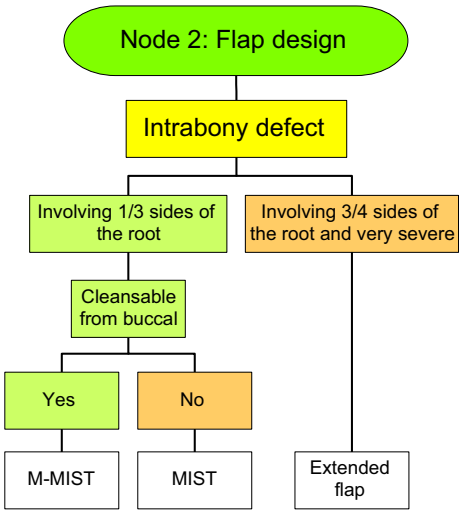

Fig. 12. Node 2: flap design. MIST, minimally invasive surgical technique; M-MIST, modified minimally invasive surgical technique.

of Miller grade II or grade III should be splinted before or immediately following the surgical procedure (28, 139). Tooth mobility should be re-evaluated during the early healing phase and any increase in mobility should be detected and managed appropriately.

Surgical access to the intrabony defects is selected from three different surgical approaches: the simplified papilla preservation flap (25); the modified papilla preservation technique (20); and the crestal incision (26) (Flow chart 3, Node 1). The simplified papilla preservation flap is chosen whenever the width of the interdental space is 2 mm or less, as measured at the level of the supracrestal portion of the papilla. The modified papilla preservation technique is used at sites with an interdental width of > 2 mm; and the crestal incision is applied next to an edentulous area.

Surgical node 2 (Flow chart 4) deals with the choice of the flap design. Whenever a defect involves one or two sides of a root and is cleansable from a tiny buccal window, the modified minimally invasive surgical technique is applied (36). In some instances the modified minimally invasive surgical technique approach can be applied to both the interdental spaces neighboring the defect-associated tooth, allowing for instrumentation of a defect involving up to three sides of a root. If the defect is not cleansable from the buccal window, the interdental papilla is elevated, applying a minimally invasive surgical technique approach (32, 34). A large flap, extended to the neighboring teeth and including also an eventual periosteal incision and/or vertical-releasing incisions, will be chosen in the presence of a very severe and deep defect, involving three or four sides of the root, requiring ample visibility for instrumentation and the use of either endoskeletons or exoskeletons (20, 25).

Selection of the regenerative material is based on the defect anatomy and on the flap design chosen to expose the defect (Flow chart 5, node 3). If a modified minimally invasive surgical technique approach is applied, amelogenins or no regenerative materials are the elective choices (36, 37). If a minimally invasive surgical technique approach is applied, amelogenins can be used alone in containing defects or in combination with a filler in noncontaining defects (32–35, 107). If a large flap is elevated, stability to the area should be provided by applying barriers or fillers, combinations of barriers and fillers, or combinations of amelogenins/growth factors and fillers. Amelogenins alone are preferred in defects with a prevalent three-wall morphology or in well-supported two-wall defects.

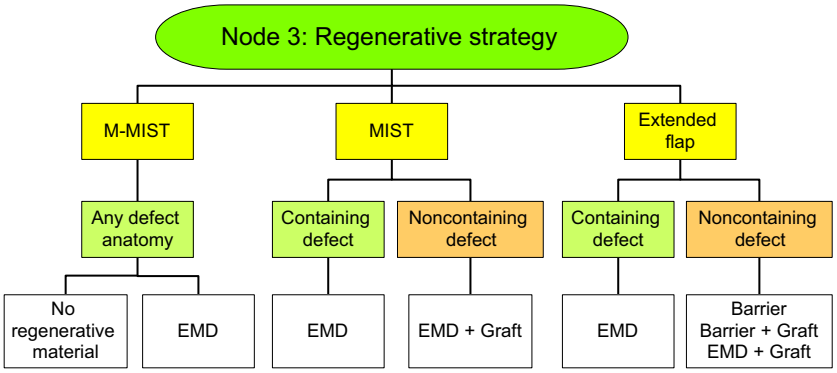

Fig. 13. Node 3: regenerative strategy. EMD, enamel matrix derivative; MIST, minimally invasive surgical technique; M-MIST, modified minimally invasive surgical technique.

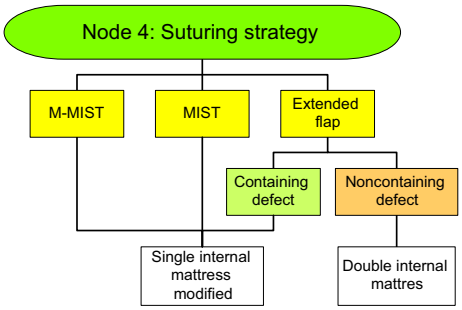

Fig. 14. Node 4: suturing strategy. MIST, minimally invasive surgical technique; M-MIST, modified minimally invasive surgical technique.

The suturing approach is chosen according to the type of regenerative strategy applied (Flow chart 6, node 4). It will consist of a single internal modified mattress suture when a modified minimally invasive surgical technique or a minimally invasive surgical technique approach is chosen and amelogenins alone are applied (32, 34, 36). It consists of the combination of two internal mattress sutures applied at the defect-associated interdental area to reach primary closure of the papilla in the absence of any tension when a large flap with a periosteal incision is used in association with a barrier or a graft or a combination thereof (19, 20, 22, 24, 26, 31). The surgical procedure is preferably performed with the aid of magnification, such as loops or an operating microscope (29, 31, 148). Microsurgical instruments and materials should be utilized to complement the normal periodontal set.

Postsurgical and early home-care protocols are directly derived from the experiences developed by running several controlled clinical trials (19, 22, 23, 28, 134–136). For the control of bacterial contamination, an empirical protocol consisting of doxycycline (100 mg twice daily for 1 week), 0.12% chlorhexidine mouth rinsing three times daily and weekly prophylaxis is prescribed. Sutures are removed after 1 week. Patients are requested to avoid normal brushing, flossing and chewing in the treated area for periods of 6–10 weeks. A postsurgical soft toothbrush soaked in chlorhexidine is adopted from week 1 to gently wipe the treated area. Nonresorbable membranes are removed after 6 weeks. Patients can resume full oral hygiene and chewing function in the treated area 2–4 weeks after membrane removal or when the bioresorbable membranes are fully resorbed. Patients treated with amelogenins resume full oral hygiene after a period of 4–5 weeks. At the end of the 'early healing phase', patients are placed on a 3-month recall system. A general suggestion to avoid any invasive clinical maneuver, such as hard subgingival instrumentation, restorative dentistry, orthodontics and additional surgery, for a period of about 9 months is also part of a strategy that is aimed at optimizing the clinical outcomes of periodontal regeneration.

Long-term effects and benefits of regeneration

A pertinent question with respect to regenerative treatment is whether the attachment level and bone gains achieved can be maintained over an extended period of time. In a long-term follow-up study, Gottlow et al. (51) assessed the stability of new attachment gained through guided tissue-regeneration procedures in 39 patients. The results of this study and those of other trials indicate that attachment gain obtained following guided tissue-regeneration treatment can be maintained on a long-term basis (2, 83).

An investigation on intrabony defects demonstrated that the stability of sites treated with guided tissue regeneration was dependent on patients' participation in a recall program, and on the absence of bacterial plaque, bleeding on probing and reinfection with periodontal pathogens in the treated sites (17). In addition, the susceptibility to disease recurrence at sites treated with nonbioabsorbable barrier membranes was assessed in a study comparing long-term changes in attachment levels at regenerated and non-regenerated sites in the same patient (21). The results

indicated a high degree of concordance in the clinical outcomes (stability compared with recurrence of attachment loss) within the same patient, suggesting that patient factors, rather than site factors, are associated with disease recurrence. Among patient factors, compliance with oral hygiene, smoking habits and susceptibility to disease progression, rather than the treatment modality employed, were the major determinants of stability of the treated sites. Other long-term studies show that if the patient participates in a professionally delivered supportive periodontal care program and maintains good oral hygiene, the regenerated attachment can be maintained long-term (13, 41, 93, 96, 105, 117, 124).

One investigation has looked at the long-term effects of periodontal regeneration on tooth survival. Cortellini & Tonetti (30) performed a Kaplan–Meier analysis of tooth survival following periodontal-regenerative treatment in a sample of 175 patients followed up for 2–16 years (average 8 $\pm$ 3.4 years) in a specialist environment. In this study, 96% of teeth treated by periodontal regeneration survived. Of interest was the observation that tooth loss was observed only among the 32% of the population that was smoking (tooth survival was 89% among smokers and 100% among nonsmokers). Clinical attachment levels were located at the same level or coronal to the pretreatment levels in 92% of patients up to 15 years after treatment.

Conclusions

Periodontal regeneration with many different regenerative materials, including barrier membranes, grafts, active biological compounds and combinations of those, demonstrated significant clinical improvements in intrabony defects, far beyond those achieved with debridement only. Different surgical approaches have been proposed and tested in combination with the various regenerative materials, but none has demonstrated clear superiority over the others. Although all proposed regenerative approaches showed a high degree of clinical variability in terms of clinical attachment level gain, none demonstrated the capacity to solve all the different and unique patient/defect presentations. It is therefore necessary to choose a regenerative strategy out of a panel of options to treat a given defect. The adoption of a clinical strategy for optimal application of materials and surgical approach could increase the efficacy of periodontal regeneration and provide improved clinical outcomes. The potential for periodontal regeneration can be expressed in defects from very shallow to very deep, up to extreme conditions in which the application of regenerative therapy can change the prognosis of a hopeless tooth into a maintainable unit. Clinical outcomes obtained with periodontal regeneration can be stably maintained on a long-term basis, provided that good oral hygiene and infection control within a stringent recall program are enforced. Current data indicate that, in patients participating in a supportive periodontal-care program, 96% of teeth with severe intrabony defects and treated with a periodontal-regenerative procedure could be retained for a period of up to 15 years.

References

1. Becker W, Becker BE, Berg L, Pritchard J, Caffesse R, Rosenberg E. New attachment after treatment with root isolation procedures: report for treated class III and class II furcations and vertical osseous defects. *Int J Periodontics Restorative Dent* 1988: **8**: 2–16.
2. Becker W, Becker B. Treatment of mandibular three-wall intrabony defects by flap debridement and expanded polytetrafluoroethylene barrier membranes. Long term evaluation of 32 treated patients. *J Periodontol* 1993: **64**: 1138–1144.
3. Blumenthal NM. The use of collagen membranes to guide regeneration of new connective tissue attachment in dogs. *J Periodontol* 1988: **59**: 830–836.
4. Blumenthal MN. A clinical comparison of collagen membranes with e-PTFE membranes in the treatment of human mandibular buccal class II furcation defects. *J Periodontol* 1993: **64**: 925–933.
5. Bosshardt DD. Biological mediators and periodontal regeneration: a review of enamel matrix proteins at the cellular and molecular levels. *J Clin Periodontol* 2008: **35** (Suppl. 8): 87–105.
6. Bowers GM, Chadroff B, Carnevale R, Mellonig J, Corio R, Emerson J, Stevens M, Romberg E. Histologic evaluation of new attachment apparatus formation in humans. Part I. *J Periodontol* 1989: **60**: 664–674.
7. Bowers GM, Chadroff B, Carnevale R, Mellonig J, Corio R, Emerson J, Stevens M, Romberg E. Histologic evaluation of new human attachment apparatus formation in humans. Part II. *J Periodontol* 1989: **60**: 675–682.
8. Bowers GM, Chadroff B, Carnevale R, Mellonig J, Corio R, Emerson J, Stevens M, Romberg E. Histologic evaluation of a new attachment apparatus formation in humans. Part III. *J Periodontol* 1989: **60**: 683–693.
9. Caffesse RG, Nasjleti CE, Morrison EC, Sanchez R. Guided tissue regeneration: comparison of bioabsorbable and non-bioabsorbable membranes. Histologic and histometric study in dogs. *J Periodontol* 1994: **65**: 583–591.
10. Camargo PM, Lekovic V, Weinlander M, Nedic M, Vasilic N, Wolinsky LE, Kenney EB. A controlled re-entry study on the effectiveness of bovine porous bone mineral used in combination with a collagen membrane of porcine origin

in the treatment of intrabony defects in humans. *J Clin Periodontol* 2000: **27**: 889–896.
11. Camelo M, Nevins ML, Schenk RK, Simion M, Rasperini G, Lynch SE, Nevins M. Clinical radiographic, and histologic evaluation of human periodontal defects treated with Bio-Oss® and Bio-Gide. *Int J Periodontics Restorative Dent* 1998: **18**: 321–331.
12. Caton J, Greenstein G, Zappa U. Synthetic bioabsorbable barrier for regeneration in human periodontal defects. *J Periodontol* 1994: **65**: 1037–1045.
13. Christgau M, Schamlz G, Wenzel A, Hiller KA. Periodontal regeneration of intrabony defects with resorbable and non-resorbable membranes: 30 month results. *J Clin Periodontol* 1997: **24**: 17–27.
14. Cortellini P, Pini-Prato G, Baldi C, Clauser C. Guided tissue regeneration with different materials. *Int J Periodontics Restorative Dent* 1990: **10**: 136–151.
15. Cortellini P, Pini-Prato G, Tonetti M. Periodontal regeneration of human infrabony defects. I. Clinical measures. *J Periodontol* 1993: **64**: 254–260.
16. Cortellini P, Pini-Prato G, Tonetti M. Periodontal regeneration of human infrabony defects. II. Reentry procedures and bone measures. *J Periodontol* 1993: **64**: 261–268.
17. Cortellini P, Pini-Prato G, Tonetti M. Periodontal regeneration of human infrabony defects. V. Effect of oral hygiene on long term stability. *J Clin Periodontol* 1994: **21**: 606–610.
18. Cortellini P, Pini-Prato G, Tonetti M. Interproximal free gingival grafts after membrane removal in GTR treatment of infrabony defects. A controlled clinical trial indicating improved outcomes. *J Periodontol* 1995: **66**: 488–493.
19. Cortellini P, Pini-Prato G, Tonetti M. Periodontal regeneration of human infrabony defects with titanium reinforced membranes. A controlled clinical trial. *J Periodontol* 1995: **66**: 797–803.
20. Cortellini P, Pini-Prato G, Tonetti M. The modified papilla preservation technique. A new surgical approach for interproximal regenerative procedures. *J Periodontol* 1995: **66**: 261–266.
21. Cortellini P, Pini-Prato G, Tonetti M. Long term stability of clinical attachment following guided tissue regeneration and conventional therapy. *J Clin Periodontol* 1996: **23**: 106–111.
22. Cortellini P, Pini-Prato G, Tonetti M. Periodontal regeneration of human intrabony defects with bioresorbable membranes. A controlled clinical trial. *J Periodontol* 1996: **67**: 217–223.
23. Cortellini P, Carnevale G, Sanz M, Tonetti MS. Treatment of deep and shallow intrabony defects. A multicenter randomized controlled clinical trial. *J Clin Periodontol* 1998: **25**: 981–987.
24. Cortellini P, Tonetti M. Radiographic defect angle influences the outcome of GTR therapy in intrabony defects. *J Dent Res* 1999: **78**: 381 (abstract).
25. Cortellini P, Pini-Prato G, Tonetti M. The simplified papilla preservation flap. A novel surgical approach for the management of soft tissues in regenerative procedures. *Int J Periodontics Restorative Dent* 1999: **19**: 589–599.
26. Cortellini P, Tonetti MS. Focus on intrabony defects: guided tissue regeneration (GTR). *Periodontol 2000* 2000: **22**: 104–132.
27. Cortellini P, Tonetti M. Evaluation of the effect of tooth vitality on regenerative outcomes in intrabony defects. *J Clin Periodontol* 2000: **28**: 672–679.
28. Cortellini P, Tonetti MS, Lang NP, Suvan JE, Zucchelli G, Vangsted T, Silvestri M, Rossi R, McClain P, Fonzar A, Dubravec D, Adriaens P. The simplified papilla preservation flap in the regenerative treatment of deep intrabony defects: clinical outcomes and postoperative morbidity. *J Periodontol* 2001: **72**: 1701–1712.
29. Cortellini P, Tonetti MS. Microsurgical approach to periodontal regeneration. Initial evaluation in a case cohort. *J Periodontol* 2001: **72**: 559–569.
30. Cortellini P, Tonetti MS. Long-term tooth survival following regenerative treatment of intrabony defects. *J Periodontol* 2004: **75**: 672–678.
31. Cortellini P, Tonetti MS. Clinical performance of a regenerative strategy for intrabony defects: scientific evidence and clinical experience. *J Periodontol* 2005: **76**: 341–350.
32. Cortellini P, Tonetti MS. A minimally invasive surgical technique (MIST) with enamel matrix derivate in the regenerative treatment of intrabony defects: a novel approach to limit morbidity. *J Clin Periodontol* 2007: **34**: 87–93.
33. Cortellini P, Tonetti MS. Minimally invasive surgical technique (M.I.S.T.) and enamel matrix derivative (EMD) in intrabony defects. (I) Clinical outcomes and intra-operative and post-operative morbidity. *J Clin Periodontol* 2007: **34**: 1082–1088.
34. Cortellini P, Nieri M, Pini Prato GP, Tonetti MS. Single minimally invasive surgical technique (MIST) with enamel matrix derivative (EMD) to treat multiple adjacent intrabony defects. Clinical outcomes and patient morbidity. *J Clin Periodontol* 2008: **35**: 605–613.
35. Cortellini P, Tonetti MS. Minimally invasive surgical technique and enamel matrix derivative (EMD) in intrabony defects: 2. Factors associated with healing outcomes. *Int J Periodontics Restorative Dent* 2009: **29**: 256–265.
36. Cortellini P, Tonetti MS. Improved wound stability with a modified minimally invasive surgical technique in the regenerative treatment of isolated interdental intrabony defects. *J Clin Periodontol* 2009: **36**: 157–163.
37. Cortellini P, Tonetti MS. Clinical and radiographic outcomes of the modified minimally invasive surgical technique with and without regenerative materials: a randomized- controlled trial in intra-bony defects. *J Clin Peridontol* 2011: **38**: 365–373.
38. Cortellini P, Stalpers G, Mollo A, Tonetti MS. Periodontal regeneration versus extraction and prosthetic replacement of teeth severely compromised by attachment loss to the apex: 5-year results of an ongoing randomized clinical trial. *J Clin Periodontol* 2011: **38**: 915–924.
39. DeSanctis M, Clauser C, Zucchelli G. Bacterial colonization of barrier material and periodontal regeneration. *J Clin Periodontol* 1996: **23**: 1039–1046.
40. DeSanctis M, Zucchelli G, Clauser C. Bacterial colonization of bioabsorbable barrier material and periodontal regeneration. *J Periodontol* 1996: **67**: 1193–1200.
41. Eickholz P, Krigar DM, Kim TS, Reitmeir P, Rawlinson A. Stability of clinical and radiographic results after guided tissue regeneration in infrabony defects. *J Periodontol* 2007: **78**: 37–46.

42. Ehmke B, Rudiger SG, Hommens A, Karch H, Flemmig FD. Guided tissue regeneration using a polylactic acid barrier. Part II: predictors influencing treatment outcome. *J Clin Periodontol* 2003: **30**: 368–374.
43. Esposito M, Grusovin MG, Coulthard P, Worthington HV. Enamel matrix derivative (Emdogain) for periodontal tissue regeneration in intrabony defects. *Cochrane Database Syst Rev* 2005: **19**: CD003875.
44. Esposito M, Grusovin MG, Papanikolaou N, Coulthard P, Worthington HV. Enamel matrix derivative (Emdogain) for periodontal tissue regeneration in intrabony defects. A Cochrane Systematic Review. *Eur J Oral Implantol* 2009: **2**: 247–266.
45. Falk H, Laurell L, Ravald N, Teiwik A, Persson R. Guided tissue regeneration therapy of 203 consecutively treated intrabony defects using a bioabsorbable matrix barrier. Clinical and radiographic findings. *J Periodontol* 1997: **68**: 571–581.
46. Garrett S, Loos B, Chamberlain D, Egelberg J. Treatment of intraosseous periodontal defects with a combined therapy of citric acid conditioning, bone grafting and placement of collagenous membranes. *J Clin Periodontol* 1988: **15**: 383–389.
47. Giannobile WV, Somerman MJ. Growth and amelogenin-like factors in periodontal wound healing. A systematic review. *Ann Periodontol* 2003: **8**: 193–204.
48. Goldman H, Cohen DW. The infrabony pocket: classification and treatment. *J Periodontol* 1958: **29**: 272–291.
49. Gottlow J, Nyman S, Karring T, Lindhe J. New attachment formation as the result of controlled tissue regeneration. *J Clin Periodontol* 1984: **11**: 494–503.
50. Gottlow J, Nyman S, Lindhe J, Karring T, Wennström J. New attachment formation in the human periodontium by guided tissue regeneration. *J Clin Periodontol* 1986: **13**: 604–616.
51. Gottlow J, Nyman S, Karring T. Maintenance of new attachment gained through guided tissue regeneration. *J Clin Periodontol* 1992: **19**: 315–317.
52. Gottlow J, Laurell L, Lundgren D, Mathiesen T, Nyman S, Rylander H, Bogentoft C. Periodontal tissue response to a new bioresorbable guided tissue regeneration device. A longitudinal study in monkeys. *Int J Periodontics Restorative Dent* 1994: **14**: 437–449.
53. Guillemin M, Mellonig J, Brunswold M. Healing in periodontal defects treated by decalcified freeze-dried bone allografts in combination with e-PTFE membranes. (I) Clinical and scanning electron microscope analysis. *J Clin Periodontol* 1993: **20**: 528–536.
54. Hanes PJ, Purvis JP. Local anti-infective therapy: pharmacological agents. A systematic review. *Ann Periodontol* 2003: **8**: 79–98.
55. Haney JM, Nilveus RE, McMillan PJ, Wikesjo UME. Periodontal repair in dogs: expanded polytetrafluorethylene barrier membranes support wound stabilisation and enhance bone regeneration. *J Periodontol* 1993: **64**: 883–890.
56. Harrel SK, Rees TD. Granulation tissue removal in routine and minimally invasive surgical procedures. *Compend Contin Educ Dent* 1995: **16**: 960–967.
57. Harrel TK, Nunn ME. Longitudinal comparison of the periodontal status of patients with moderate to severe periodontal disease receiving no treatment, non-surgical treatment, and surgical treatment utilizing individual sites for analysis. *J Periodontol* 2001: **72**: 1509–1519.
58. Harrel SK, Wilson TG Jr, Nunn ME. Prospective assessment of the use of enamel matrix proteins with minimally invasive surgery. *J Periodontol* 2005: **76**: 380–384.
59. Heden G, Wennström J, Lindhe J. Periodontal tissue alterations following Emdogain treatment of periodontal sites with angular bone defects. A series of case reports. *J Clin Periodontol* 1999: **26**: 855–860.
60. Heden G. A case report study of 72 consecutive Emdogain-treated intrabony periodontal defects: clinical and radiographic findings after 1 year. *Int J Periodontics Restorative Dent* 2000: **20**: 127–139.
61. Heden G, Wennstrom JL. Five-year follow-up of regenerative periodontal therapy with enamel matrix derivative at sites with angular bone defects. *J Periodontol* 2006: **77**: 295–301.
62. Heitz-Mayfield L, Tonetti MS, Cortellini P, Lang NP, European Research Group on Periodontology (ERGOPERIO). Microbial colonization patterns predict the outcomes of surgical treatment of intrabony defects. *J Clin Periodontol* 2006: **33**: 62–68.
63. Heijl L, Heden G, Svärdström C, Ostgren A. Enamel matrix derivate (EMDOGAIN®) in the treatment of intrabony periodontal defects. *J Clin Periodontol* 1997: **24**: 705–714.
64. Hiatt WH, Stallard RE, Butler ED, Badget B. Repair following mucoperiosteal flap surgery with full gingival retention. *J Periodontol* 1968: **39**: 11–16.
65. Howell TH, Fiorellini JP, Paquette DW, Offenbacher S, Giannobile WV, Lynch S. A phase I/II clinical trial to evaluate a combination of recombinant human platelet-derived growth factor-BB and recombinant human insulin-like growth factor-I in patients with periodontal disease. *J Periodontol* 1997: **68**: 1186–1193.
66. Hürzeler MB, Quinones CR, Caffesse RG, Schupback P, Morrison EC. Guided periodontal tissue regeneration in interproximal intrabony defects following treatment with a synthetic bioabsorbable barrier. *J Periodontol* 1997: **68**: 489–497.
67. Kim CS, Choi SH, Chai JK, Cho KS, Moon IS, Wikesjo UME, Kim CK. Periodontal repair in surgically created intrabony defects in dogs. Influence of the number on bone walls on healing response. *J Periodontol* 2004: **75**: 229–235.
68. Kitamura M, Nakashima K, Kowashi Y, Fujii T, Shimauchi H, Sasano T, Furuuchi T, Fukuda M, Noguchi T, Shibutani T, Iwayama Y, Takashiba S, Kurihara H, Ninomiya M, Kido J, Nagata T, Hamachi T, Maeda K, Hara Y, Izumi Y, Hirofuji T, Imai E, Omae M, Watanuki M, Murakami S. Periodontal tissue regeneration using fibroblast growth factor-2: randomized controlled phase II clinical trial. *PLoS ONE* 2008: **3**: e2611.
69. Kitamura M, Akamatsu M, Machigashira M, Hara Y, Sakagami R, Hirofuji T, Hamachi T, Maeda K, Yokota M, Kido J, Nagata T, Kurihara H, Takashiba S, Sibutani T, Fukuda M, Noguchi T, Yamazaki K, Yoshie H, Ioroi K, Arai T, Nakagawa T, Ito K, Oda S, Izumi Y, Ogata Y, Yamada S, Shimauchi H, Kunimatsu K, Kawanami M, Fujii T, Furuichi Y, Furuuchi T, Sasano T, Imai E, Omae M, Yamada S, Watanuki M, Murakami S. FGF-2 stimulates periodontal regeneration: results of a multi-center randomized clinical trial. *J Dent Res* 2011: **90**: 35–40.

70. Kwok V, Caton J. Prognosis revisited: a system for assigning periodontal prognosis. *J Periodontol* 2007: **78**: 2063–2071.
71. Jayakumar A, Rajababu P, Rohini S, Butchibabu K, Naveen A, Krishnajaneya Reddy P, Vidyasagar S, Satyanarayana D, Pavan Kumar S. Multi-centre, randomized clinical trial on efficacy and safety of recombinant human platelet-derived growth factor with b-tricalcium phosphate in human intra-osseous periodontal defects. *J Clin Periodontol* 2011: **38**: 163–172.
72. Lang NP, Tonetti MS. Periodontal diagnosis in treated periodontitis. Why, when and how to use clinical parameters. *J Clin Periodontol* 1996: **23**: 240–250.
73. Laurell L, Falk H, Fornell J, Johard G, Gottlow J. Clinical use of a bioresorbable matrix barrier in guided tissue regeneration therapy. Case series. *J Periodontol* 1994: **65**: 967–975.
74. Linares A, Cortellini P, Lang NP, Suvan J, Tonetti MS, European Research Group on Periodontology (ErgoPerio). Guided tissue regeneration/deproteinized bovine bone mineral or papilla preservation flaps alone for treatment of intrabony defects. II: radiographic predictors and outcomes. *J Clin Periodontol* 2006: **33**: 351–358.
75. Linghorne WJ, O'Connel DC. Studies in the regeneration and reattachment of supporting structures of teeth. I. Soft tissue reattachment. *J Dental Res* 1950: **29**: 419–428.
76. Lundgren D, Slotte C. Reconstruction of anatomically complicated periodontal defects using a bioresorbable GTR barrier supported by bone mineral. A 6-month follow-up study of 6 cases. *J Clin Periodontol* 1999: **26**: 56–62.
77. Machtei E, Cho M, Dunford R, Norderyd J, Zambon J, Genco R. Clinical, microbiological, and histological factors which influence the success of regenerative periodontal therapy. *J Periodontol* 1994: **65**: 154–161.
78. Machtei E. The effect of membrane exposure on the outcome of regenerative procedures in humans: a meta-analysis. *J Periodontol* 2001: **72**: 512–516.
79. Magnusson I, Nyman S, Karring T, Egelberg J. Connective tissue attachment formation following exclusion of connective tissue and epithelium during healing. *J Periodont Res* 1985: **20**: 201–208.
80. Magnusson I, Batich C, Collins BR. New attachment formation following controlled tissue regeneration using biodegradable membranes. *J Periodontol* 1988: **59**: 1–6.
81. Matuliene G, Pjetursson BE, Salvi GE, Schmidlin K, Brägger U, Zwahlen M, Lang NP. Influence of residual pockets on progression of periodontitis and tooth loss: results after 11 years of maintenance. *J Clin Periodontol* 2008: **35**: 685–695.
82. Mayfield L, Söderholm G, Hallström H, Kullendorff B, Edwardsson S, Bratthall G, Brägger U, Attström R. Guided tissue regeneration for the treatment of intraosseous defects using a bioabsorbable membrane. A controlled clinical study. *J Clin Periodontol* 1998: **25**: 585–595.
83. McClain P, Schallhorn RG. Long term assessment of combined osseous composite grafting, root conditioning and guided tissue regeneration. *Int J Periodontics Restorative Dent* 1993: **13**: 9–27.
84. McGuire MK, Nunn ME. Prognosis versus actual outcome. II. The effectiveness of clinical parameters in developing an accurate prognosis. *J Periodontol* 1996: **67**: 658–665.
85. McGuire MK, Nunn ME. Prognosis versus actual outcome. III. The effectiveness of clinical parameters in accurately predicting tooth survival. *J Periodontol* 1996: **67**: 658–665.
86. Mellonig JT. Enamel matrix derivate for periodontal reconstructive surgery: Technique and clinical and histologic case report. *Int J Periodontics Restorative Dent* 1999: **19**: 9–19.
87. Mellonig JT. Human histologic evaluation of a bovine-derived bone xenograft in the treatment of periodontal osseous defects. *Int J Periodontics Restorative Dent* 2000: **20**: 18–29.
88. Miller SC. *Textbook of periodontia (Oral Medicine)*, 2nd edn. Philadelphia: The Blakiston Co., 1943: 103.
89. Murphy K. Post-operative healing complications associated with Gore-tex periodontal material. Part 1. Incidence and characterization. *Int J Periodontics Restorative Dent* 1995: **15**: 363–375.
90. Murphy KG, Gunsolley JC. Guided tissue regeneration for the treatment of periodontal intrabony and furcation defects. A systematic review. *Ann Periodontol* 2003: **8**: 266–302.
91. Needleman IG, Worthington HV, Giedrys-Leeper E, Tucker RJ. Guided tissue regeneration for periodontal infra-bony defects. *Cochrane Database Syst Rev* 2006: **19**: CD001724.
92. Nevins M, Giannobile WV, McGuire MK, Kao RT, Mellonig JT, Hinrichs JE, McAllister BS, Murphy KS, McClain PK, Nevins ML, Paquette DW, Ham TJ, Reddy MS, Lavin PT, Genco RJ, Lynch SE. Platelet-derived growth factor stimulates bone fill and rate of attachment level gain: results of a large multicenter randomised controlled trial. *J Periodontol* 2005: **76**: 2205–2215.
93. Nickles K, Ratka-Kruger P, Neukranz E, Raetzke P, Eickholz P. Open flap debridement and guided tissue regeneration after 10 years in infrabony defects. *J Clin Periodontol* 2009: **36**: 976–983.
94. Nowzari H, Slots J. Microorganisms in polytetrafluoroethylene barrier membranes for guided tissue regeneration. *J Clin Periodontol* 1994: **21**: 203–210.
95. Nowzari H, Matian F, Slots J. Periodontal pathogens on polytetrafluoroethylene membrane for guided tissue regeneration inhibit healing. *J Clin Periodontol* 1995: **22**: 469–474.
96. Nygaard-Østby P, Bakke V, Nesdal O, Susin C, Wikesjö UME. Periodontal healing following reconstructive surgery: effect of guided tissue regeneration using a bioresorbable barrier device when combined with autogenous bone grafting. A randomized controlled trial 10-year follow-up. *J Clin Periodontol* 2010: **37**: 366–373.
97. Nyman S, Lindhe J, Karring T, Rylander H. New attachment following surgical treatment of human periodontal disease. *J Clin Periodontol* 1982: **9**: 290–296.
98. Palmer RM, Cortellini P, Group B of European Workshop on Periodontology. Periodontal tissue engineering and regeneration: consensus Report of the Sixth European Workshop on Periodontology. *J Clin Periodontol* 2008: **35** (Suppl. 8): 83–86.
99. Paolantonio M, Scarano A, Di Placido G, Tumini V, D'Archivio D, Piattelli A. Periodontal healing in humans using anorganic bovine bone and bovine peritoneum-derived collagen membrane: a clinical and histologic case report. *Int J Periodontics Restorative Dent* 2001: **21**: 505–515.

100. Paul BF, Mellonig JT, Towle HJ III, Gray JL. Use of collagen barrier to enhance healing in human periodontal furcation defects. *Int J Periodontics Restorative Dent* 1992: **12**: 123–131.
101. Pietruska MD. A comparative study on the use of Bio-Oss and enamel matrix derivative (Emdogain) in the treatment of periodontal bone defects. *Eur J Oral Sci* 2001: **109**: 178–181.
102. Pini Prato GP, Cortellini P, Clauser C. Fibrin and fibronectin sealing system in a guided tissue regeneration procedure. A case report. *J Periodontol* 1988: **59**: 679–683.
103. Pitaru S, Tal H, Soldinger M, Grosskopf A, Noff M. Partial regeneration of periodontal tissues using collagen barriers. Initial observations in the canine. *J Periodontol* 1988: **59**: 380–386.
104. Polimeni G, Xiropaidis VX, Wikesjo UME. Biology and principles of periodontal wound healing/regeneration. *Periodontol 2000* 2006: **41**: 30–47.
105. Pretzl B, Kim TS, Steinbrenner H, Dorfer C, Himmer K, Eickholz P. Guided tissue regeneration with bioabsorbable barriers III 10-year results in infrabony defects. *J Clin Periodontol* 2009: **36**: 349–356.
106. Reynolds MA, Aichelmann-Reidy ME, Branch-Mays GL, Gunsolley JC. The efficacy of bone replacement grafts in the treatment of periodontal osseous defects. A systematic review. *Ann Periodontol* 2003: **8**: 227–265.
107. Ribeiro FV, Casarin RC, Palma MA, Júnior FH, Sallum EA, Casati MZ. The role of enamel matrix derivative protein in minimally invasive surgery in treating intrabony defects in single rooted teeth: a randomized clinical trial. *J Periodontol* 2011: **82**: 522–532.
108. Ribeiro FV, Casarin RC, Palma MA, Júnior FH, Sallum EA, Casati MZ. Clinical and patient-centered outcomes after minimally invasive non-surgical or surgical approaches for the treatment of intrabony defects: a randomized clinical trial. *J Periodontol* 2011: **82**: 1256–1266.
109. Rosen PS, Reynolds MA, Bowers GM. The treatment of intrabony defects with bone grafts. *Periodontol 2000* 2000: **22**: 88–103.
110. Sanz M, Tonetti MS, Zabalegui I, Sicilia A, Blanco J, Rebelo H, Rasperini G, Merli M, Cortellini P, Suvan JE. Treatment of intrabony defects with enamel matrix proteins or barrier membranes: results from a multicenter practice-based clinical trial. *J Clin Periodontol* 2005: **75**: 726–733.
111. Sanders JJ, Sepe WW, Bowers GM, Koch RW, Williams JE, Lekas JS, Mellonig JT, Pelleu GB Jr, Gambill V. Clinical evaluation of freeze-dried bone allografts in periodontal osseous defects. Part III. Composite freeze-dried bone allografts with and without autogenous bone grafts. *J Periodontol* 1983: **54**: 1–8.
112. Schallhorn RG, Hiatt WH, Boyce W. Iliac transplants in periodontal therapy. *J Periodontol* 1970: **41**: 566–580.
113. Schallhorn RG, McClain PK. Combined osseous composite grafting, root conditioning, and guided tissue regeneration. *Int J Periodontics Restorative Dent* 1988: **4**: 9–31.
114. Sculean A, Donos N, Chiantella GC, Windisch P, Reich E, Brecx M. GTR with bioresorbable membranes in the treatment of intrabony defects: a clinical and histologic study. *Int J Periodontics Restorative Dent* 1999: **19**: 501–509.
115. Sculean A, Donos N, Windisch P, Brecx M, Gera I, Reich E, Karring T. Healing of human intrabony defects following treatment with enamel matrix proteins or guided tissue regeneration. *J Periodontal Res* 1999: **34**: 310–322.
116. Sculean A, Berakdar M, Chiantella GC, Donos N, Arweiler NB, Brecx M. Healing of intrabony defects following treatment with a bovine-derived xenograft and collagen membrane. A controlled clinical study. *J Clin Periodontol* 2003: **30**: 73–80.
117. Sculean A, Schwarz F, Miliauskaite A, Kiss A, Arweiler N, Becker J, Brecx M. Treatment of infrabony defects with an enamel matrix protein derivative or bioresorbable membrane: an 8-year follow-up split-mouth study. *J Periodontol* 2006: **77**: 1879–1886.
118. Sculean A, Kiss A, Miliauskaite A, Schwarz F, Arweiler NB, Hannig M. Ten-year results following treatment of intrabony defects with enamel matrix proteins and guided tissue regeneration. *J Clin Periodontol* 2008: **35**: 817–824.
119. Selvig K, Kersten B, Chamberlain A, Wikesjo UME, Nilveus R. Regenerative surgery of intrabony periodontal defects using e-PTFE barrier membranes. Scanning electron microscopic evaluation of retrieved membranes vs. clinical healing. *J Periodontol* 1992: **63**: 974–978.
120. Selvig K, Kersten B, Wikesjö UME. Surgical treatment of intrabony periodontal defects using expanded polytetrafluoroethylene barrier membranes: influence of defect configuration on healing response. *J Periodontol* 1993: **64**: 730–733.
121. Sigurdsson TJ, Hardwick R, Bogle GC, Wikesjo UME. Periodontal repair in dogs: space provision by reinforced ePTFE membranes enhances bone and cementum regeneration in large supraalveolar defects. *J Periodontol* 1994: **65**: 350–356.
122. Silvestri M, Ricci G, Rasperini G, Sartori S, Cattaneo V. Comparison of treatments of infrabony defects with enamel matrix derivate, guided tissue regeneration with a nonresorbable membrane and Widman modified flap. A pilot study. *J Clin Periodontol* 2000: **27**: 603–610.
123. Silvestri M, Sartori S, Rasperini G, Ricci G, Rota C, Cattaneo V. Comparison of infrabony defects treated with enamel matrix derivative versus guided tissue regeneration with a nonresorbable membrane. A multicenter controlled clinical trial. *J Clin Periodontol* 2003: **30**: 386–393.
124. Slotte C, Asklow B, Lundgren D. Surgical guided tissue regeneration treatment of advanced periodontal defects: a 5-year follow-up study. *J Clin Periodontol* 2007: **34**: 977–984.
125. Smith MacDonald E, Nowzari H, Contreras A, Flynn J, Morrison J, Slots J. Clinical evaluation of a bioabsorbable and a nonresorbable membrane in the treatment of periodontal intraosseous lesions. *J Periodontol* 1998: **69**: 445–453.
126. Stavropoulos A, Karring ES, Kostopoulos L, Karring T. Deproteinized bovine bone and gentamicin as an adjunct to GTR in the treatment of intrabony defects: a randomized controlled clinical study. *J Clin Periodontol* 2003: **30**: 486–495.
127. Stavropoulos A, Mardas N, Herrero F, Karring T. Smoking affects the outcome of guided tissue regeneration with bioresorbable membranes: a retrospective analysis of intrabony defects. *J Clin Periodontol* 2004: **31**: 945–950.
128. Stavropoulos A, Windisch P, Gera I, Capsius B, Sculean A, Wikesjo UME. A phase IIa randomized controlled clinical and histological pilot study evaluating rhGDF-5/b-TCP for

periodontal regeneration. *J Clin Periodontol* 2011: **38**: 1044–1054.
129. Steffensen B, Weber HP. Relationship between the radiologic periodontal defect angle and healing after treatment. *J Periodontol* 1989: **60**: 248–254.
130. Tanner MG, Solt CW, Vuddhakanok S. An evaluation of new attachment formation using a microfibrillar collagen barrier. *J Periodontol* 1988: **59**: 524–530.
131. Tonetti M, Pini-Prato G, Cortellini P. Periodontal regeneration of human infrabony defects. IV. Determinants of the healing response. *J Periodontol* 1993: **64**: 934–940.
132. Tonetti M, Pini-Prato G, Cortellini P. Effect of cigarette smoking on periodontal healing following GTR in infrabony defects. A preliminary retrospective study. *J Clin Periodontol* 1995: **22**: 229–234.
133. Tonetti M, Pini-Prato G, Cortellini P. Factors affecting the healing response of intrabony defects following guided tissue regeneration and access flap surgery. *J Clin Periodontol* 1996: **23**: 548–556.
134. Tonetti M, Cortellini P, Suvan JE, Adriaens P, Baldi C, Dubravec D, Fonzar A, Fourmosis I, Magnani C, Muller-Campanile V, Patroni S, Sanz M, Vangsted T, Zabalegui I, Pini Prato G, Lang NP. Generalizability of the added benefits of guided tissue regeneration in the treatment of deep intrabony defects. Evaluation in a multi-center randomized controlled clinical trial. *J Periodontol* 1998: **69**: 1183–1192.
135. Tonetti MS, Lang NP, Cortellini P, Suvan JE, Adriaens P, Dubravec D, Fonzar A, Fourmousis I, Mayfield L, Rossi R, Silvestri M, Tiedemann C, Topoll H, Vangsted T, Wallkamm B. Enamel matrix proteins in the regenerative therapy of deep intrabony defects. A multicenter randomized controlled clinical trial. *J Clin Periodontol* 2002: **29**: 317–325.
136. Tonetti MS, Cortellini P, Lang NP, Suvan JE, Adriaens P, Dubravec D, Fonzar A, Fourmousis I, Rasperini G, Rossi R, Silvestri M, Topoll H, Wallkamm B, Zybutz M. Clinical outcomes following treatment of human intrabony defects with GTR/bone replacement material or access flap alone. A multicenter randomized controlled clinical trial. *J Clin Periodontol* 2004: **31**: 770–776.
137. Tonetti MS, Fourmousis I, Suvan J, Cortellini P, Bragger U, Lang NP, European Research Group on Periodontology (ERGOPERIO). Healing, post-operative morbidity and patient perception of outcomes following regenerative therapy of deep intrabony defects. *J Clin Periodontol* 2004: **31**: 1092–1098.
138. Trejo PM, Weltman R, Caffesse R. Treatment of intraosseous defects with bioabsorbable barrier alone or in combination with decalcified freeze-dried bone allograft: a randomized clinical trial. *J Periodontol* 2000: **71**: 1852–1861.
139. Trejo PM, Weltman RL. Favourable periodontal regenerative outcomes from teeth with presurgical mobility: a retrospective study. *J Clin Periodontol* 2004: **75**: 1532–1538.
140. Trombelli L, Kim CK, Zimmerman GJ, Wikesjö UME. Retrospective analysis of factors related to clinical outcome of guided tissue regeneration procedures in intrabony defects. *J Clin Periodontol* 1997: **24**: 366–371.
141. Trombelli L, Heitz-Mayfield LJ, Needleman I, Moles D, Scabbia A. A systematic review of graft materials and biological agents for periodontal intraosseous defects. *J Clin Periodontol* 2002: **29**(Suppl. 3): 117–135.
142. Trombelli L, Farina R. Clinical outcomes with bioactive agents alone or in combination with grafting or guided tissue regeneration. *J Clin Periodontol* 2008: **35**(Suppl.): 117–135.
143. Trombelli L, Simonelli A, Pramstraller M, Wikesjo UME, Farina R. Single flap approach with and without guided tissue regeneration and a hydroxyapatite biomaterial in the management of intraosseous periodontal defects. *J Periodontol* 2010: **81**: 1256–1263.
144. Tsitoura E, Tucker R, Suvan J, Laurell L, Cortellini P, Tonetti M. Baseline radiographic defect angle of the intrabony defect as a prognostic indicator in regenerative periodontal surgery with enamel matrix derivative. *J Clin Periodontol* 2004: **31**: 643–647.
145. Tu Y-K, Woolston A, Faggion CM Jr. Do bone grafts or barrier membranes provide additional treatment effects for infrabony lesions treated with enamel matrix derivatives? A network meta-analysis of randomized-controlled trials. *J Clin Periodontol* 2010: **37**: 59–79.
146. Tunkel J, Heinecke A, Flemmig TF. A systematic review of efficacy of machine-driven and manual subgingival debridement in the treatment of chronic periodontitis. *J Clin Periodontol* 2002: **29**(Suppl. 3): 72–81.
147. Yukna R, Mellonig JT. Histologic evaluation of periodontal healing in humans following regenerative therapy with enamel matrix derivative. A 10-case series. *J Periodontol* 2000: **71**: 752–759.
148. Wachtel H, Schenk G, Bohm S, Weng D, Zuhr O, Hurzeler MB. Microsurgical access flap and enamel matrix derivative for the treatment of periodontal intrabony defects: a controlled clinical study. *J Clin Periodontol* 2003: **30**: 496–504.
149. Wang HL, O'Neal RB, Thomas CL, Shyr Y, Macneil RL. Evaluation of an absorbable collagen membrane in treating class II furcation defects. *J Periodontol* 1994: **65**: 1029–1036.
150. Wikesjo UME, Nilveus R. Periodontal repair in dogs: effect of wound stabilisation on healing. *J Periodontol* 1990: **61**: 719–724.
151. Wikesjo UME, Lim WH, Thomson RC, Cook AD, Hardwick WR. Periodontal repair in dogs: gingival tissue occlusion, a critical require- ment for guided tissue regeneration. *J Clin Periodontol* 2003: **30**: 655–664.