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Wound healing following surgical and regenerative periodontal therapy

Cristiano Susin, Tiago Fiorini, Jaebum Lee, Jamie A. De Stefano, Douglas P. Dickinson & Ulf M. E. Wikesjö

Clinical studies have evaluated the effect of conventional periodontal surgical therapy (29, 30, 48). In general, although some clinical gain in tissue support may be attained, these therapies do not support regeneration of the periodontal attachment. Even though the biological possibility of periodontal regeneration has been demonstrated (68, 99, 113), the clinical application of this intrinsic potential appears difficult to harness; thus also conceptually most intriguing candidate protocols face clinical challenges (12, 86). In this review, we explore the bioclinical principles, *condiciones sine quibus non*, that unleash the innate potential of the periodontium to achieve clinically meaningful periodontal regeneration (i.e. space-provision, wound stability and conditions for primary intention healing) (Fig. 1). Moreover, limiting factors and detrimental practices that may compromise clinical and biological outcomes are reviewed, as well as tissue management in clinical settings.

Surgical site preparation

Periodontal surgery has long been associated with invasive protocols, including excision of soft tissues, resection of alveolar bone and even aspects of the periodontal ligament. However, as preservation of soft and hard tissues has become a prerequisite for regenerative periodontal therapy, novel surgical techniques, devices, implants and instruments are being developed to meet these changing demands. Following a trend in medicine, minimally invasive clinical procedures have been explored, including minimally invasive surgery (27, 28, 107), preservation techniques (16, 17, 101) and microsurgical approaches (13, 14, 91), yielding reduced surgical trauma for the benefit of periodontal wound healing/regeneration.

In perspective, standard periodontal surgical techniques may cause disruption of the vascular support in periodontal wounds. Retzepi et al. (75) observed a major decrease in flap perfusion postsurgery with a compensatory increase in blood flow on day 1, which subsided on day 2 and lasted until day 7. Baseline perfusion was only achieved by day 15. It is important to note that vertical incisions, periosteal fenestration and other surgical maneuvers that may further compromise perfusion were not performed in this study. In a small clinical trial, the same investigators compared the effect of minimally invasive procedures on vascular support to periodontal flaps (76). Blood flow returned significantly faster (day 4) to baseline levels when a minimally invasive surgical approach was used compared with standard techniques (day 7). In other studies, Burkhardt & Lang (6) evaluated the effect of microsurgery on vascularization of connective tissue grafts. A significant improvement in vascularization was observed with the use of microsurgical techniques. The favorable effect also included clinical outcomes including root coverage. These studies indicate that minimally invasive periodontal surgical procedures appear conducive to improved clinical outcomes more so than might be expected following standard surgical techniques. Whereas localized defects can be treated using limited incisions and minimal flap reflection, clinicians may desire greater access to treat large or multiple periodontal defects. The same guiding principles probably benefit periodontal wound healing/regeneration also in large, more challenging, defects and should be further explored.

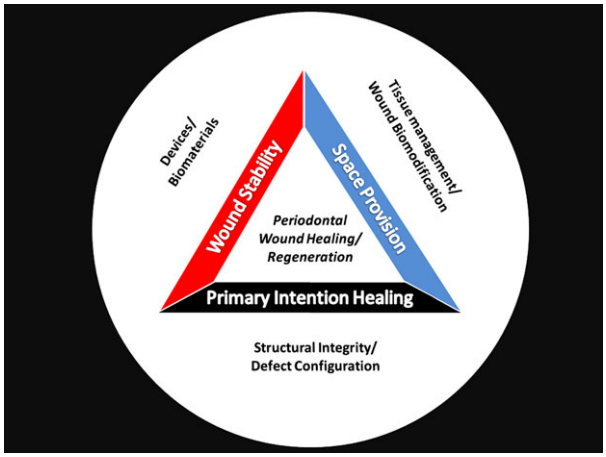

Fig. 1. Bioclinical principles for periodontal wound healing/regeneration.

As periodontitis progresses, the periodontium increasingly accumulates inflammatory cells in a fibrous connective tissue (3). A key component in the preparation of a surgical site for periodontal wound healing/regeneration is the complete removal of this granulation tissue to expose critical tissue resources in the residual alveolar bone and periodontal ligament. However, a need to decorticate the alveolar bone to access tissue resources, including mesenchymal cells, bone cell precursors and vascular elements, to enhance periodontal wound healing/regeneration does not appear critical (25).

Root surface cleansing, detoxification or biomodification encompasses a final step in surgical site preparation. Several protocols, including manual and power-driven scalers, air polishing devices and lasers to remove biofilm, bacterial endotoxins and dental calculus, have been introduced and evaluated. Systematic reviews have not been able to discern significant differences among methods (108); thus, a treatment protocol using one or a combination of methods remains at the discretion of the clinician.

Interest in a concept of root-surface conditioning, in particular to enhance periodontal wound healing/regeneration, emerged in the 1970s with the discovery that demineralized bone (109) and dentin (2, 74) harbor biologically active agents that may induce bone formation. Subsequent *in-vivo* and *in-vitro* studies have shown that root-surface conditioning using demineralizing or chelating agents produces a number of effects that conceptually appear beneficial to periodontal wound healing/regeneration, including elution of endotoxin from bacterially contaminated cementum/dentin (21), enhanced adsorption/adhesion of extracellular matrix components and fibroblast migration/attachment (113, 115). Perhaps most significant, removal of a root-surface smear layer exposing the dentin or cementum collagen matrix, thereby enhancing adsorption/adhesion of a fibrin clot to the exposed root surface and anchorage of new collagen fibrils (73, 82, 88). A large number of animal and clinical studies have used a saturated citric acid solution as conditioning agent, but other agents, including EDTA and tetracycline-HCl preparations, have also been used. In spite of favorable biological observations, a systematic review including 34 clinical studies failed to conclude significant clinical benefits of root-surface conditioning (i.e. attachment-level gain and probing-depth reduction) (58).

In summary, optimized site preparation should adopt surgical techniques and procedures that assure removal of granulation tissue and biofilm; minimize tissue invasion, loss and trauma; and secure rapid (re-)vascularization.

Space-provision

Early reports stress the critical importance of space-provision for bone regeneration (5, 54, 64). Periodontal-regenerative strategies have focused on the use of barrier devices or membranes for guided tissue regeneration, grafts or the use of bone biomaterials. Membranes were originally intended to provide tissue separation by diverting gingival epithelial and connective tissue cell migration/proliferation, whilst preferentially supporting migration/proliferation of cells from the periodontal ligament and alveolar bone into the wound site (37). With few exceptions, current membrane technologies do not possess necessary structural integrity to withstand compressive forces from, or transmitted through, the mucogingival flaps to provide or maintain space provision; thus, they commonly collapse or are compressed into the defects. The biological significance of this event has been demonstrated in studies utilizing the critical-size, supraalveolar periodontal defect model (26, 70, 72, 92). Healing following periodontal reconstructive surgery showed a significant correlation between space-provision by the membrane and regeneration of alveolar bone. Thus, sites experiencing membrane collapse or compression onto the root surface exhibited limited, if any, regeneration (26). In parallel studies using reinforced space-providing membranes, clinically relevant regeneration of alveolar bone and cementum including a functionally oriented periodontal ligament was observed following an 8-week healing interval in sites where space-provision was maintained (92). Polimeni et al. (70, 72)

specifically studied the relationship between space-provision and periodontal regeneration. Their analyses showed a significant, positive correlation between wound area underneath a membrane and newly formed periodontal tissues (72). These biologic studies clearly substantiate the contention that devices or biomaterials intended for periodontal regenerative therapy must be designed for unobtruded space-provision (Fig. 2).

A number of cadaver-sourced allogeneic or xenogeneic (bone derivatives) or synthetic (bone substitutes) biomaterials have been explored in support of periodontal wound healing/regeneration. Bone biomaterials have been used as stand-alone protocols and in combination with barrier devices and autologous bone, and as carriers for matrix, growth and differentiation factors. However, the use of biomaterials as stand-alone therapies carries limited clinical significance. Notably, as the formative phase of periodontal wound healing/regeneration appears complete within few weeks (19), an increasing body of evidence suggests that slowly resorbing biomaterials delay, interfere with or even obstruct, bone formation (94, 97) and periodontal wound healing/regeneration (38, 39, 105), compromising space-provision as a result of slow biodegradation rates. Moreover, the biodegradation process of implanted biomaterials *per se* may interfere with local bone formation and maintenance (1). Thus, the use of cadaver-sourced or synthetic bone biomaterials as stand-alone strategies, or in combination with barrier devices to enhance periodontal regeneration, remains to be justified. Nevertheless, carefully selected biomaterials may serve as carriers for matrix, growth and differentiation factors, with the growth factor potentially enhancing periodontal wound healing/regeneration, while at the same time accelerating biodegradation of the carrier (45).

Systematic reviews conclude that improved clinical outcomes in the treatment of intrabony defects should be expected following the use of membranes alone or in combination with biomaterials compared with standard surgical techniques (such as open flap debridement) (63, 65). Clinical attachment gain and radiographic defect fill obtained with biomaterials appeal to clinicians due to the illusion of clinical improvement provided by their obturation of periodontal defects. However, these conclusions should be interpreted with caution in light of the limited biological evidence for a regenerative effect of these biomaterials and high risk of bias for the available evidence. In perspective the reader should consider the observations from the randomized controlled trial of Cortellini & Tonetti (15),

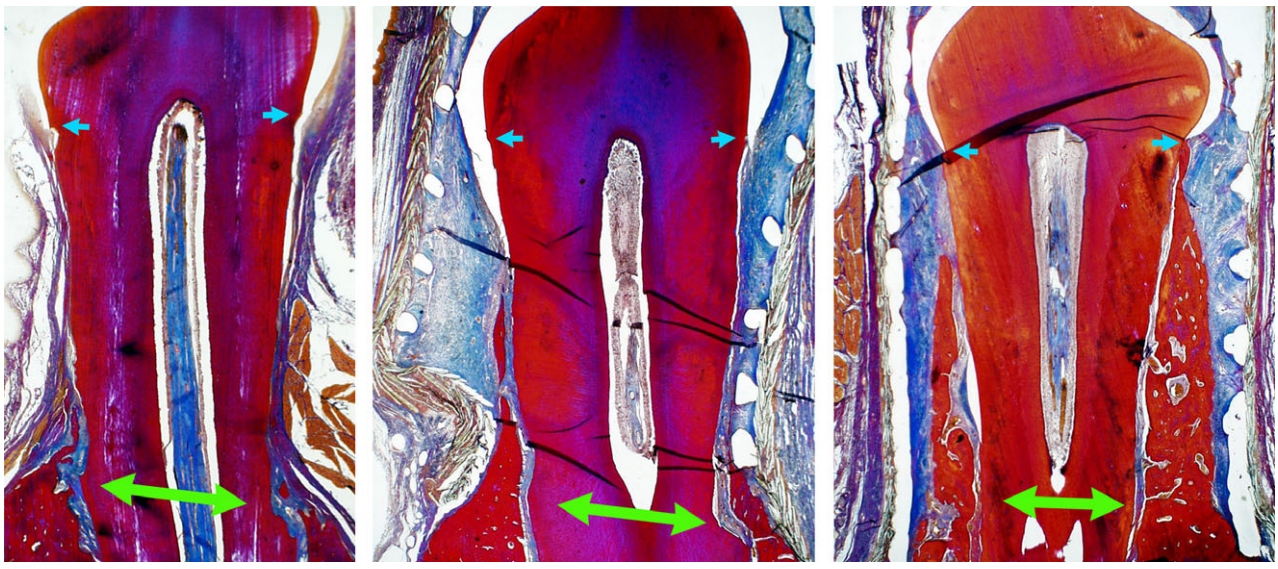

Fig. 2. Representative photomicrographs showing periodontal wound healing/regeneration in the critical-size, supraalveolar periodontal defect model following an 8-week healing interval. Staining was perfomed with Ladewig's connective tissue stain modified by Mallory. Sites receiving gingival flap surgery show only limited regeneration (left); sites showing collapse/compression of the reinforced space-providing expanded polytetrafluoroethylene membrane display compromised regeneration (center); and sites showing uncompromised space-provision provide evidence of the native regenerative potential of the periodontium (right). The green arrows show the apical extent of the supra-alveolar defect and the blue arrows show the coronal extent (i.e. the cemento–enamel junction). The distance between the arrows (the defect height) is approximately 5 mm (unpublished photomicrographs from Sigurdsson et al. 1994; ref 89).

which evaluated a minimally invasive surgical technique as a stand-alone approach, or combined with an enamel matrix derivative and a bovine bone biomaterial, in the management of advanced intrabony defects. No significant differences among interventions were observed, with the minimally invasive surgery without additions achieving substantial clinical attachment gain (4.1 ± 1.2 mm) and radiographic bone fill (77 ± 19%), suggesting that added protocols – in this case the enamel matrix derivative and the bovine bone biomaterial – may be of limited, if any, benefit. Still others have found similar results following minimally invasive procedures combined or not with guided tissue regeneration and a hydroxyapatite biomaterial (106, 107).

Providing and maintaining a space to allow regeneration from specific tissue sources and preventing scar formation is one basic tenet of tissue engineering. Whereas barrier devices and biomaterials are unlikely to improve the innate regenerative potential of the periodontium, they should not jeopardize periodontal wound healing/regeneration by obstructing tissue formation. In perspective, the use of devices or biomaterials for space-provision in contained periodontal defects seems redundant.

Wound biomodification

Defect configuration dictates the presence and nature of cellular resources available to support periodontal wound healing/regeneration. Healing of deep three-wall intrabony defects is facilitated by the presence of vascular and cellular resources circumscribing the defect. Moreover, the defect architecture provides space and wound stability, further facilitating healing. Clinical studies have shown that the innate regenerative potential in deep intrabony periodontal defects appears quite substantial (15, 18, 106, 107). In contrast, the presence and availability of regenerative resources in two- and one-wall intrabony defects, Class II and III furcation defects, and supra-alveolar defects is dramatically reduced. Under such clinical scenarios, wound biomodification using matrix, growth and differentiation factors may prove an attractive avenue to enhance the innate regenerative potential. Several such factors have been evaluated in attempts to enhance periodontal wound healing/regeneration (reviewed by Lee et al. (50)). Table 1 summarizes the outcomes of selected pre-clinical studies to illustrate research in the area. Only a few candidate matrix, growth and differentiation factors have produced sufficiently enhanced periodontal regeneration in pre-clinical settings to deserve clinical scrutiny.

Initial studies, using growth factors to enhance periodontal regeneration in humans, evaluated a platelet-derived growth factor–insulin-like growth factor construct (35). Thirty-eight patients with bilateral intrabony and furcation defects participated in a Phase I/II clinical trial. Defect sites received recombinant human platelet-derived growth factor-BB combined with recombinant human insulin-like growth factor I (50/50 or 150/150 μg/ml) in a gel carrier, and were compared with carrier or sham-surgery controls. Subjects receiving recombinant human platelet-derived growth factor-BB/recombinant human insulin-like growth factor I (50/50 μg/ml) showed similar bone fill in experimental and control sites at 9 months, whereas subjects receiving recombinant human platelet-derived growth factor-BB/recombinant human insulin-like growth factor I (150/150 μg/ml) showed statistically significant increased mean bone fill (2.1 mm), corresponding to 42% fill, compared with 0.8 mm or 19% fill for the controls. Noteworthy, despite these apparently encouraging observations, further evaluation of the recombinant human platelet-derived growth factor-BB/recombinant human insulin-like growth factor I combination has not been reported.

A multicenter Phase III randomized controlled clinical trial evaluated the safety and efficacy of recombinant human platelet-derived growth factor-BB in a β-tricalcium phosphate carrier (66). One-hundred and eighty subjects with deep intrabony periodontal defects were randomized to receive recombinant human platelet-derived growth factor-BB (0.3 or 1.0 mg/ml) or carrier control. Whereas limited, statistically significant, clinical attachment gain was observed for sites receiving recombinant human platelet-derived growth factor-BB (0.3 mg/ml) compared with the control at 3 months (3.8 ± 0.2 mm vs. 3.3 ± 0.2 mm, respectively [mean ± SE]), no significant differences were observed at 6 months (3.8 ± 0.2 mm vs. 3.5 ± 0.2 mm, respectively [mean ± SE]). Recombinant human platelet-derived growth factor-BB (1.0 mg/ml) exhibited no noteworthy or significant differences compared with control or recombinant human platelet-derived growth factor-BB (0.3 mg/ml). In a separate study, histological evaluation of human intrabony periodontal defects treated with recombinant human platelet-derived growth factor-BB (0.3 or 1.0 mg/ml) in the β-tricalcium phosphate carrier showed limited regeneration in 12 of 16 defects (range: 0.3–1.6 mm) following a healing interval of 6 months or longer (78). A majority

of the defect sites displayed residual β-tricalcium phosphate that appeared to obstruct bone formation and periodontal regeneration.

Enamel matrix derivatives represent an extensively evaluated technology intended for periodontal wound healing/regeneration. A systematic review combined the results of nine randomized clinical trials with at least 1 year of follow-up (20). Compared with the control, intrabony defects treated with enamel matrix derivative showed significant clinical

Table 1. Effect of growth/differentiation factors on periodontal wound healing/regeneration in pre-clinical studies

Factor	Model/Platform	Dose; Carrier; Healing interval	Major observation	References
Platelet-derived growth factor	Fenestration/dog	10 μg/ml; topical application; 1, 3 and 7 days	Increased fibroblast proliferation	(110)
	Class III furcation/dog	0.5 μg/ml; topical application; 5, 8 and 11 weeks	Favorable periodontal regeneration including bone fill	(8, 69)
	Chronic periodontitis/ nonhuman primate	10 μg; methylcellulose gel; 4 and 12 weeks	Increased new attachment and bone fill	(24)
Fibroblast growth factor	Three-wall intrabony/ dog	30, 40 and 50 μg; fibrin gel; 6 weeks	Dose-dependent periodontal regeneration	(61)
	Intrabony and Class II furcation/ nonhuman primate	30, 40 and 50 μg; fibrin gel; 8 weeks	Dose-dependent periodontal regeneration	(61)
	Class II furcation/dog	30 μg; topical application; 6 weeks	Increased periodontal ligament, bone formation	(62)
	Class II furcation/ nonhuman primate	0.1 and 0.4%; gelatin; 8 weeks	Dose-dependent bone and cement regeneration	(100)
	Class III furcation/dog	0.5 and 1.0 mg; topical application; 90 days	Low dose: enhanced cement and bone formation	(83)
	Re-implanted incisor/ dog	0.1 and 1.5 μg; collagen gel; 4 and 8 weeks	Enhanced cementum formation, periodontal ligament	(87)
Transforming growth factor-β	Class II furcation/ sheep	80 μg/ml; 25% pluronic F-127; 6 weeks	Transforming growth factor-β1 + guided tissue regeneration enhanced bone formation over transforming growth factor-β1 alone	(59)
	Supraalveolar/dog	20 μg; $CaCO_3$ composite; 4 weeks	Limited cementum and bone formation	(102, 122) (45, 60, 121)
	Class II furcation/ nonhuman primate	1.5 and 2.5 μg; gelatinous, heterotopic induced ossicles, minced muscle tissue; 8 weeks	Enhanced vascularity, substantial regeneration	(103)
Recombinant human platelet-derived growth factor/Insulin-like growth factor I	Periodontitis defects/ dog	1/1 μg; aqueous gel; 2 and 5 weeks	Enhanced bone and cementum formation	(56, 57)
	Chronic intrabony defects/nonhuman primate	10/10 μg; methylcellulose gel; 4 and 12 weeks	Enhanced periodontal regeneration	(24)

Table 1. (*Continued*)

Factor	Model/Platform	Dose; Carrier; Healing interval	Major observation	References
Bone morphogenetic protein-2	Supraalveolar/dog	0.05–0.4 mg/ml; poly (lactic-co-glycolic acid), demineralized bone matrix, polylactic acid, absorbable collagen sponge, calcium phosphate cement, hyaluronan sponge; 8 and 24 weeks	Significant bone and cementum formation; no periodontal ligament; root resorption/ankylosis	(90, 93, 94, 96, 117–120, 123)
	Supraalveolar/dog	0.4 mg/ml; gelatin/poly (lactic-co-glycolic acid); 12 weeks	Enhanced bone, cementum and periodontal ligament	(42)
	Three-wall intrabony/ nonhuman primate	0.4 mg/ml; absorbable collagen sponge, α-BSM; 16 weeks	Enhanced periodontal regeneration	(4)
	Three-wall intrabony/ dog	0.2 mg/ml; absorbable collagen sponge; 8 and 24 weeks	Enhanced bone but not cementum formation	(9)
	Supraalveolar/dog	0.1 mg/ml; gelatin sponge, spacer membrane; 12 weeks	Spacer eliminated root resorption/ankylosis but reduced bone formation	(84)
Bone morphogenetic protein-7/Osteogenic protein-1	Class II furcation/ nonhuman primate	0, 100 and, 500 μg/g type I collagen; 8 weeks	Enhanced cementogenesis and periodontal ligament	(79)
	Class II furcation/ nonhuman primate	0.5 and 2.5 mg/g type I collagen; 24 weeks	Enhanced periodontal ligament and alveolar bone formation	(81)
	Class III furcation/dog	0.75, 2.5 and 7.5 mg/g type I collagen; 8 weeks	Enhanced periodontal regeneration	(23)
	Class II furcation/ nonhuman primate	100 μg/g type I collagen; 8 weeks	Recombinant human osteogenic protein-1: enhanced cementogenesis	(80)
Bone morphogenetic protein-12/ Growth differentiation factor-7	Supraalveolar/dog	Growth differentiation factor-7, 0.04, 0.1 and 0.2 mg/ml; absorbable collagen sponge; 8 weeks	Growth differentiation factor-7: periodontal ligament regeneration	(117)
Bone morphogenetic protein-14/Growth differentiation factor-5	One-wall intrabony/ dog	20 μg/site; β-tricalcium phosphate; 8 weeks	Enhanced bone and cementum formation, periodontal ligament	(51)
	One-wall intrabony/ dog	1, 20 and 100 μg/site; absorbable collagen sponge; 8 weeks	Enhanced bone and cementum formation, periodontal ligament	(41)
	Supraalveolar/dog	500 μg/g β-tricalcium phosphate; poly(lactic-co-glycolic acid); 8 weeks	Enhanced cementum and bone formation, periodontal ligament	(46)
	Dehiscence/dog	93 μg/site; poly(lactic-co-glycolic acid); 2, 4, 6 and 8 weeks	Accelerated bone regeneration	(47)

attachment gain (mean = 1.1 mm; 95% CI: 0.6–1.6) and significant probing-depth reduction (mean = 0.9 mm; 95% CI: 0.3–0.8). However, some studies included in this primary analysis were at a high risk of bias, which could inflate the results. It is noteworthy that when a separate analysis for trials at low risk of bias was conducted, the mean effect on clinical attachment gain was reduced to 0.6 mm (95% CI: 0.3–1.0).

Initial pre-clinical and clinical (43) studies evaluating fibroblast growth factor for periodontal wound healing/regeneration showed promise and were followed by a large multicenter, randomized, double-blind, placebo-controlled clinical trial, including 253 subjects, that evaluated the effect of recombinant human basic fibroblast growth factor (0.2, 0.3 and 0.4%) in a hydroxypropylcellulose gel carrier in two- or three-wall intrabony defects (44). A significant increase in radiographic bone fill was observed at 9 and 18 months for all doses of recombinant human basic fibroblast growth factor compared with controls. Recombinant human basic fibroblast growth factor (0.3%) appeared to be the preferred concentration, reaching 52.2 ± 38.1% (mean±SD) radiographic bone fill compared with 15.9 ± 22.1% for controls. Notably, no significant differences in clinical attachment gain were observed among experimental groups.

A Phase IIa randomized, controlled, clinical and histological trial including 20 patients evaluated the effect of recombinant human growth/differentiation factor-5 in a β-tricalcium phosphate carrier on periodontal regeneration (98, 124). The recombinant human growth/differentiation factor-5/β-tricalcium phosphate construct, compared with open flap debridement, was applied into intrabony defects at teeth treatment planned for extraction. The results following a 6-month healing interval suggested that recombinant human growth/differentiation factor-5/β-tricalcium phosphate is safe; no significant adverse events were noted. Although not statistically significant, less gingival recession and almost two-fold greater clinical attachment gain were observed for the recombinant human growth/differentiation factor-5/β-tricalcium phosphate-treated sites compared with control (mean ± sd: 3.2 ± 1.7 mm vs. 1.7 ± 2.2 mm). The histological evaluation suggested that recombinant human growth/differentiation factor-5/β-tricalcium phosphate favorably affects bone formation and may substantially support periodontal regeneration. These findings should be interpreted in light of the limited sample size and unfavorable defect characteristics of eligible hopeless teeth. Larger clinical trials are necessary to assess the clinical relevance of this treatment.

Wound biomodification using matrix, growth and differentiation factors may represent a viable avenue to enhance/accelerate the innate regenerative potential of the periodontium. Several laboratories have evaluated matrix, growth and differentiation factors in pre-clinical settings and reported favorable results; however, the clinical efficacy thus far appears insufficient to warrant wider clinical use. Further studies, first explaining functionality and then optimizing dosing and delivery strategies, will hopefully produce second-generation technologies that may result not only in clinically relevant periodontal wound healing/regeneration but also facilitate overall clinical protocol.

Wound stability

A fibrin clot is formed immediately upon wound closure in the space between the mucogingival flap and the root surface through the conversion of fibrinogen to fibrin, serving to seal the wound and provide an initial matrix for cell migration (116). However, the stability of this fragile matrix is continuously challenged by functional mechanical forces acting on the wound margins. Early observations suggest that lack of stability of the root surface–mucogingival flap complex may compromise the root surface-adhering fibrin clot and in turn affect periodontal wound healing, leading to the formation of a long junctional epithelium rather than a new connective tissue attachment and periodontal regeneration (31, 55).

To illustrate the concept of wound stability, we experimentally compromised the adsorption/adhesion of the fibrin clot in the root surface–mucogingival flap interface by impregnating the root surface with a heparin solution (26, 112, 114). The hypothesis for these studies was that if adsorption of plasma proteins and/or subsequent adhesion of the establishing fibrin clot to the tooth is compromised, this would allow progressive migration/proliferation of the gingival epithelial tissues until contact inhibition by an intact fibrin-clot adhering to the root surface. Root surfaces treated in this manner exhibited formation of a long junctional epithelium, whereas the epithelium was arrested at, or immediately apical to, the cemento–enamel junction in control defects (114). However, apical aspects of the heparin-compromised defects matured into a new connective tissue attachment, suggesting that local factors had a stronger

impact than the heparin treatment on wound healing, the wound environment in the apical aspects being sheltered from functional forces acting on the wound margins.

We then tested the hypothesis that if the mucogingival flaps in heparin-compromised periodontal defects were stabilized by mechanical means, even a compromised fibrin clot would mature into a new connective tissue attachment. Thus, in subsequent studies, the mucogingival flaps were supported/stabilized using a polylactic acid matrix (112) or expanded polytetrafluoroethylene membranes (26). The new connective-tissue attachment extended coronal to the polylactic acid matrix or expanded polytetrafluoroethylene membranes in most situations, with the epithelium arrested immediately apical to the cemento–enamel junction at some distance from the matrix or the membrane. Apparently, these devices stabilized the mucogingival flaps and absorbed or deflected wound-rupturing forces that otherwise would have been transmitted to the fragile maturing fibrin clot at the root surface–mucogingival flap interface. These observations indicate wound stability as a critical element of periodontal wound healing/regeneration and show that epithelial apical migration or formation of a long junctional epithelium is not an inevitable fate, but rather an expression of wound failure.

The structural integrity – the resilience of the wound (the adaption/adhesion of the periodontal flap to the root surface mediated by the maturing fibrin clot) to withstand wound-rupturing forces – appears an important aspect of wound stability. The tensile strength of the mucogingival flap–tooth surface interface, a measure of structural integrity, increases significantly from approximately 200 g within days of wound closure to reach 340 g at 7 days, and may exceed 1,700 g (resisting wound-rupturing challenges) at 14 days in limited experimental periodontal defects (31, 85). However, the volume, morphology and wound closure are significantly larger/more complex in clinical periodontal wounds, thus reaching structural integrity may take even longer. Evidently, periodontal wounds need extended protection from physiologic masticatory and induced (oral hygiene and others) forces that otherwise may jeopardize early events of periodontal wound healing/regeneration.

During the early events of healing, the structural integrity of the wound relies almost completely on sutures. Hence, the strategic placement and purpose of sutures needs to be carefully negotiated. Högström and coworkers studied suture-holding strength in intestinal and laparotomy wounds and showed a decreased suture-holding strength at 24 and 48 hours postincision (33, 34). Aggregation of an inflammatory infiltrate extending up to 3 mm from the incision line compromised the integrity of the sutures (32). Therefore, relying on sutures placed within or close to the inflammatory zone to maintain wound closure and stability may not be advisable owing to the reduced suture-holding strength of the tissues, increasing the risk for wound rupture and suture-line dehiscences. However, suturing may be manipulated to improve wound stability using holding sutures (e.g. vertical and horizontal mattress sutures) placed outside the inflammatory zone to abate tensile forces acting on the wound margins. Holding sutures are intended to provide passive adaption and approximation of the mucogingival flap margins that allow uneventful wound closure through placement of simple closing sutures within the inflammatory zone; such mattress-suture techniques encompass larger amounts of tissue, promoting wound closure by distribution of tensile forces acting on the wound margins (95, 125).

Primary intention healing

Wound closure for primary intention healing appears to be an absolute prerequisite for periodontal wound healing/regeneration, avoiding exposure of newly formed and maturing tissues to the oral environment. Wound closure of periodontal defects is challenging because of the position, rigidity and mineralized avascular nature of the root surface, as well as the confined spaces between teeth and tensile forces acting on the wound margins challenging flap adaption, frequently leading to suture-line exposures and compromised outcomes (26, 86, 92, 104). Passive flap adaptation thus appears to be a provision for successful wound closure for primary intention healing initiated already in the planning and execution of the primary and secondary incisions setting off the surgical procedure as well as achieved by flap-advancement techniques using periosteal fenestration, sharp/blunt dissection(s) and releasing incisions. During these surgical maneuvers, care should be exercised to avoid excessive compromise of the vascular support and the integrity of the mucogingival flap.

Maintained wound closure for primary intention healing depends, to a large extent, on the strategy and materials used for suturing, as discussed above. Ideally, the suture material should maintain adequate tensile strength during the critical period of healing

and induce minimal tissue reactions. However, the oral cavity is characterized by a moist environment with a high infection potential, and placement of sutures elicits an inflammatory reaction regardless of the presence of anti-infective therapy. Differences in biological responses to the suture material appear to be related to the physicochemical properties of the suture material and characteristics of the sutured tissues.

Although critically important to maintain wound stability and structural integrity, evaluations of tissue reactions to suture materials have been infrequent. Silk sutures have been used in oral and periodontal surgery because of ease of handling and cost. However, several studies indicate that this braided material causes a more extensive inflammatory reaction, greater bacterial influx and a more pronounced epithelialization around the suture channels than do most relevant alternatives (52, 53, 89). Moreover, silk sutures show a significantly higher degree of slack of the suture loop compared with expanded polytetrafluoroethylene sutures after 7 days, indicating that silk sutures may not provide adequate wound stability over a healing interval of 7 days or longer. Comparison of tissue reactions to silk and expanded polytetrafluoroethylene sutures in the presence and absence of anti-infective therapy in Beagle dogs showed that silk suture specimens were characterized by keratinization of the perisutural epithelium and aggregations of rods and fusiform bacteria, as well as bacterial aggregates between the silk fibrils at 14 days, indicating maturation of a potentially pathogenic biofilm (53). This bacterial contamination appears of particular concern when the surgical protocol also incudes implanted devices and biomaterials. Overall, these observations, combined with those showing that tensile strength is insufficient at 7 days and increases dramatically 10 days after wounding, with no additional major increases the next few days (11, 111), suggest a period of 10–12 days for suture removal. It is important to remember that the timing of suture removal also depends on the use of devices and biomaterials that may compromise wound stability. Bioresorbable natural and synthetic-fiber sutures (e.g. polyglycolic acid) have gained popularity as a result of their ease of use. However, it is important to acknowledge that the tensile strength of these suture materials decreases substantially faster than their resorption rate would imply; therefore, clinicians should use sutures of known tensile strength before committing to their use. Moreover, limited data are available on bacterial infiltration and inflammatory reactions to bioresorbable sutures in an oral environment (40).

Early and late healing events

Wound closure is not the end – not even the beginning of the end – but the end of the beginning of a sequence of well-orchestrated innate temporal and spatial events that dictate the outcomes of wound healing, in this context either periodontal regeneration or periodontal repair. Basic cellular events of wound healing in humans have been documented (10, 36); the events follow a dynamic complex biomolecular path involving cell migration/proliferation, angiogenesis and extracellular matrix formation/remodeling. For periodontal wound healing/regener-

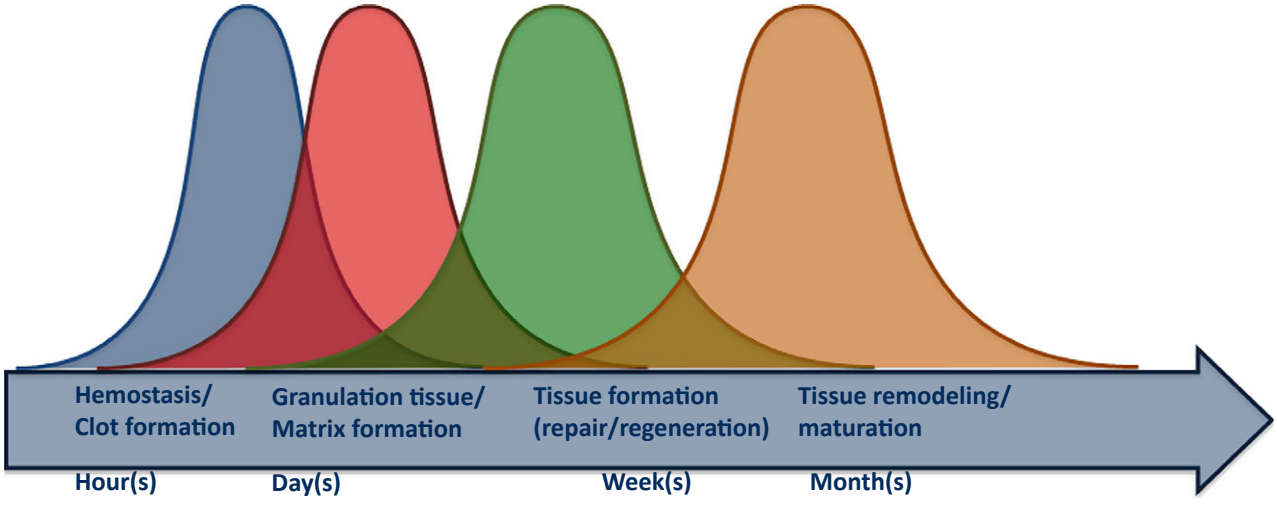

Fig. 3. Timetable depicting early and late phases of wound healing/regeneration.

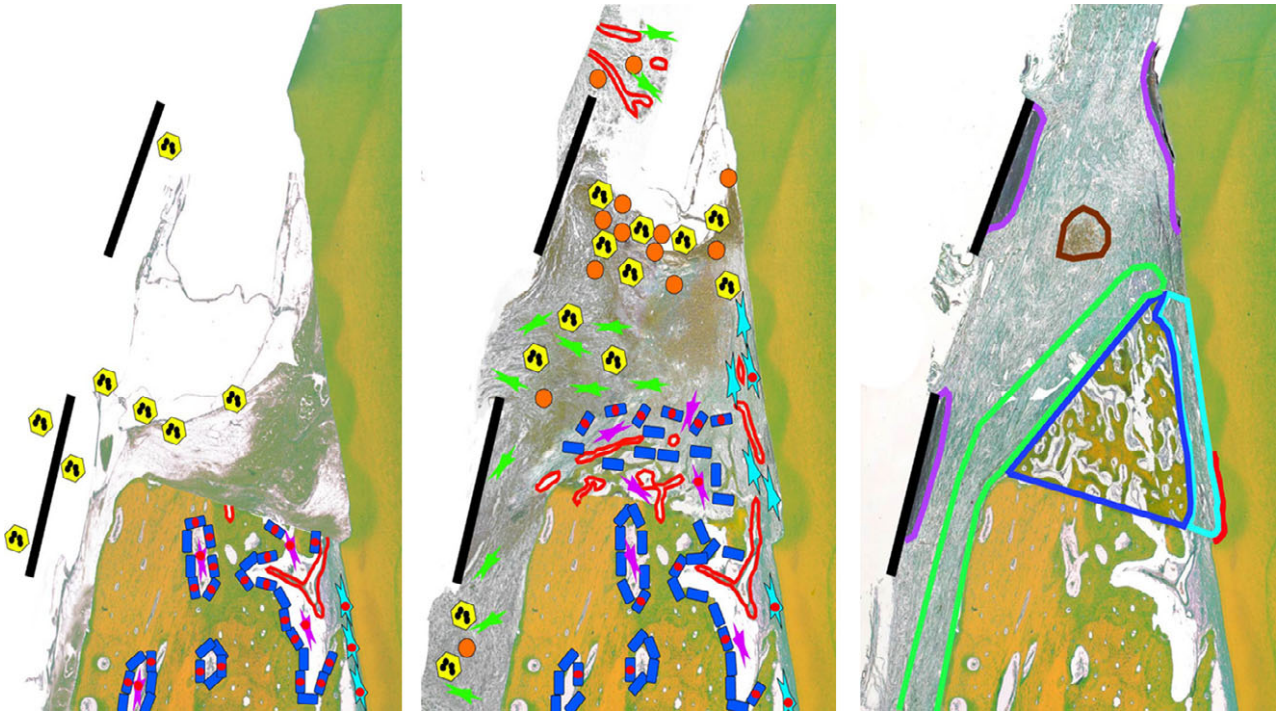

Fig. 4. Stylized representation of temporal and spatial events in periodontal wound healing/regeneration in the critical-size, supraalveolar periodontal defect model using a space-providing titanium mesh; staining performed using oxone-aldehyde fuschin-Halmi stain. Days 2–5 (left). An inhomogeneous fibrin clot fills the wound space. A red blood cell-rich fibrin matrix is deposited onto the base of the defect and onto the root surface. Above, threads of polymerized fibrin line a fluid-filled cell-poor space containing modest amounts of fibrin. By day 5, leucocytes, primarily neutrophils, largely confined to the vicinity of the fibrin matrix and the fibrin–fluid interface, enter the defect space. Few leucocytes are seen in the red blood cell-rich fibrin matrix adjacent to the bone. A high proportion of endosteal bone-lining, bone mesenchymal and periodontal ligament cells of different morphologies proliferate. Days 9–14 (center). Vascular outgrowth from the bone supports migrating cells that produce a new matrix. Dividing bone-lining cells migrate into the defect space. As they traverse the new matrix, the nondividing secretory osteoblasts left behind produce collagen-rich mineralizing osteoid. The volume of newly formed osteoid appears comparable with the eventual final volume of new bone. Few inflammatory cells are seen in the region below the fibrin clot. A new, vascularized periodontal ligament extends along the root surface, and a vascularized zone of connective tissue (future periosteum) overlies the osteoid. Above the red blood cell-rich region of the fibrin clot, monocytes and some macrophages have infiltrated, concentrated along the solid–fluid boundary of the fluid-filled cell-poor space. Granulation tissue, blood vessels, fibroblasts and inflammatory cells invade the defect space through the pores in the space-providing titanium mesh, competing with the osteogenic tissue for space. Weeks 4–8 (right). Trabecular bone fills the wound space in part and contacts the newly formed periodontal ligament. Vascularized periosteal tissues cover the newly formed bone and contact the periodontal ligament. Less well-vascularized fibrous connective tissue fills the remainder of the defect space, although some red blood cells and inflammatory cells remain. New cementum extends from the native cementum at the base of the defect. Oxytalan-rich fibrous tissue covers the titanium mesh and the coronal aspects of the root, reaching the periodontal ligament. Key for the left and center panels: dark blue, endosteal bone-lining cells; purple, bone mesenchymal cells; pale blue, periodontal ligament cells (various types); green, fibroblasts; yellow, polymorphonuclear leukocytes; orange, monocytes/macrophages; red nuclei, dividing cells; red lines, blood vessels. The position of a space-providing titanium mesh is denoted by black lines. Key for the right panel: dark blue lines, new bone; light blue lines, new periodontal ligament; green lines, new periosteum; red line, new cementum; purple lines, fibrous encapsulation tissue; brown line, residual clot material and inflammatory cells (from Dickinson et al. 2013; ref 19; copyrighted by and modified with permission from Wiley-Blackwell).

ation, the events also depend on clinical surgical strategies providing wound stability, space-provision and conditions for primary intention healing (71, 113), with intrinsic and acquired local and systemic host factors also playing a role, as well as factors presently less clearly defined. Although wound healing appears rather orderly, time needed for its completion varies depending on the size and volume of the wound, the availability of adjoining tissue resources and other factors. Based on structural observations in experimental primarily dermal incision and excision wounds, the sequence of wound healing is commonly

divided into distinct, but overlapping, phases: hemostasis/fibrin clot formation; granulation tissue/matrix formation; tissue formation; and tissue remodeling/maturation (Fig. 3).

The immediate tissue response to surgical or other injury includes vasoconstriction to limit/stop bleeding facilitating formation of a fibrin clot (49, 67). The fibrin clot provides an initial/provisional matrix/scaffold for cell migration/proliferation from adjoining tissues; in periodontal defects the periodontal ligament, alveolar bone and gingival mucosa (Fig. 4). Upon (and even in advance of) wound closure the surgically debrided periodontal defect becomes concealed by an irregular fibrin clot, with clot formation initiated as blood elements are expelled into, imposed onto and adsorb/adhere to the boundaries of the wound – cementum/dentin, alveolar bone, gingival connective tissue – as well as sealing any void space. As blood is extravasated, platelets activate and release soluble mediators as they express surface membrane coagulation factors and phospholipids. Platelets promote the activation of coagulation proenzymes, leading to an amplified clotting cascade culminating in the production of an insoluble fibrin polymer that, in addition to hemostasis, functions as a transitional matrix/scaffold for cells armed with fibrin receptors, while at the same time, recruiting fibroblasts and monocytes. In addition, platelets release a plethora of soluble factors: adhesive proteins including von Willebrand factor, fibrinogen, fibronectin and thrombospondin; chemotactic factors; and growth factors including platelet-derived growth factor, transforming growth factor-α and transforming growth factor-β. These have stimulatory effects on the migration and proliferation of osteoblasts, fibroblasts, smooth muscle cells, leukocytes, monocytes and neutrophils, as well as on periodontal cellular components. Hence, the unabated establishment of a fibrin clot provisional matrix appears a first and essential milestone toward periodontal wound healing/regeneration.

Within hours of wound closure, the early phase of inflammation becomes established as inflammatory cells – predominantly polymorphonuclear leukocytes (neutrophils), but also monocytes/macrophages – accumulate adjoining the fibrin clot. Within days, the late phase of inflammation emerges as monocytes/macrophages dominate the inflammatory events (77, 116); inflammation initiated concurrent with fibrin clot formation and platelet degranulation (49, 67). With increased vasodilation and capillary permeability, and the presence of platelet-derived growth factor and soluble mediators, inflammation abounds. Complement is activated, cascading to the formation of anaphylatoxin C5a, fibrinopeptides are released by thrombin-cleaved fibrinogen, fibrin-degradation products arise from plasmin-degraded fibrin and eventually bacterial proteins are cleaved into formyl methionyl peptides. All help to recruit polymorphonuclear leukocytes and macrophages (derived from monocytes) to the wound site. The proinflammatory cytokine, tumor necrosis factor-α, released by activated macrophages, stimulates neutrophils to marginate for egression out of circulation and promotes their activation. Interleukin-8, secreted by macrophages and fibroblasts, enhances macrophage and neutrophil chemotaxis. Interferon-γ, secreted by T-lymphocytes and macrophages, activates macrophages and neutrophils, retards collagen synthesis and cross-linking, and stimulates collagenase activity. Chemokines, a diverse group of heparin-binding proteins, act to regulate leukocyte trafficking and direct the recruitment and activation of neutrophils, lymphocytes, macrophages, eosinophils and basophils to the wound site. Neutrophils and macrophages phagocytose effete blood cells and tissue debris; release proteases such as collagenase and elastase, which degrade damaged matrix components; and secrete growth factors, including transforming growth factor-β, transforming growth factor-α, heparin-binding epidermal growth factor and basic fibroblast growth factor, further attracting macrophages and stimulating the migration of fibroblasts, epithelial cells and vascular endothelial cells. As the number of neutrophils decrease, having peaked at 24–48 hours, the number of macrophages steadily increases, as these cells carry the load of the degradative and reparative events. In addition to secreting cytokines that initiate angiogenesis, macrophages secrete growth factors that stimulate proliferation of fibroblasts, smooth muscle cells and endothelial cells.

As fibroblasts, endothelial cells and keratinocytes, and, in periodontal wounds, osteoblasts and fibroblast populations originating from the periodontal ligament and gingival connective tissue, direct the secretion of growth factors, wound healing progresses to a phase of cell proliferation and matrix formation/repair (49, 67). The provisional fibrin-, fibronectin-, tenasin- and vitronectin-rich matrix is slowly cleared of damaged proteins by mostly matrix metalloproteinases and neutrophil elastase and is then replaced with proteoglycans, elastin and collagen, as fibroblasts/osteoblasts populate this new matrix already by day 3. Metabolic demands of this proliferative stage are met by an increased vascularity stimulated by vascular endothelial growth factor

and other growth factors (7, 22). Endothelial cells penetrating the new extracellular matrix proliferate and elongate, constituting the initial vessels, which, upon interaction with pericytes and reconstitution of the basement membrane, acquire stability and later function.

Within a few weeks, synchronous with the conclusion of epithelialization, the phase of granulation tissue/matrix formation progresses into a phase of remodeling/maturation, characterized by the continuous build-up of matrix. Fibroblasts and vascular endothelial cells then undergo apoptosis, decreasing in number and visibly reducing the tissue volumes.

Re-epithelialization actually begins immediately following tissue injury, and involves epithelial cells at the edge of the wound loosening their cell–cell and cell–extracellular matrix contacts and taking on a migratory phenotype. Once the cells at the wound edge begin to migrate, epithelial cells following the edge populate the exposed site. Thus, periodontal wound healing/regeneration largely appears complete within 2–3 weeks of wound closure, to be followed by iterations of remodeling/tissue maturation to meet functional demands (19). In this context, stable biomaterials targeting regeneration have potentially outlived their purpose by 2–3 weeks, and could interfere with remodeling.

Concluding remarks

Clinical strategies for periodontal regeneration have included wound/root biomodification exemplified by the concept of root-surface demineralization, guided tissue regeneration, application of growth-stimulating factors, as well as implantation of autogenous bone, bone derivatives or substitutes. Optimal wound healing following periodontal reconstructive surgery should result in the formation of new cementum, periodontal ligament and alveolar bone, appropriately sealed by gingival tissue. While discriminating pre-clinical models have provided enhanced understanding of parameters of wound healing critical to periodontal repair or regeneration, and clinically relevant regeneration has been demonstrated, attempts at reconstruction of clinical defects have generally met limited success. The observations presented herein indicate that the often limited and variable clinical success may not be related to the native regenerative potential in a periodontal defect, as demonstrated under optimal conditions for wound healing, but, more likely, be a consequence of limitations in clinical behavior, including flap management and wound maintenance during the early healing sequence, common to all regenerative techniques.

Wound integrity during the early healing phase rests primarily with that offered by suturing, and the tooth–mucogingival flap interface is vulnerable to disruption by mechanical forces for a considerable time postsurgery. This knowledge underscores the importance of positioning and maintaining the mucogingival flaps to protect them from mechanical insult. It appears equally critical that procedures aimed at periodontal regeneration provide space for the formation of alveolar bone and for regeneration of the periodontal attachment. This may be accomplished by the adjunctive use of suitable devices or biomaterials. However, such devices or biomaterials must not impede, but rather reinforce, the healing process. Wound biomodification using matrix, growth and differentiation factors – at the optimal dose and using appropriate delivery strategies – may have the potential to amplify the innate regenerative potential of the periodontium to eventually enhance clinical protocol. Such strategies will need to address the temporal and spatial kinetics of delivery required to optimize cellular events during regeneration. Any wound modification intended to augment periodontal wound healing/regeneration should target early healing events because *de-novo* tissue formation appears to be complete within a matter of few weeks.

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