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Biomaterials for promoting periodontal regeneration in human intrabony defects: a systematic review

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The main goals of periodontal treatment are the elimination of infection and the resolution of chronic inflammation in order to arrest disease progression and prevent its recurrence. This is manifested clinically by an absence of bleeding on probing and the presence of shallow probing pocket depths (≤4 mm) (15, 47). In contrast, the persistence of residual periodontal pockets of >5 mm following completion of active periodontal therapy is associated with an increased risk for disease progression (i.e. further loss of attachment) and tooth loss, irrespective of the presence or absence of bleeding on probing (15, 47). Increased probing depths following treatment are often related to the presence of intrabony (angular) periodontal defects, a feature of periodontitis and, in turn, intrabony defects have been shown to worsen the long-term prognosis for teeth (62). The rationale behind the treatment of intrabony defects is therefore to reduce residual probing depths to improve tooth prognosis. During the last three decades, various treatment approaches involving nonsurgical techniques, as well as conservative, resective and regenerative surgical techniques, have been employed for the treatment of intrabony defects and have achieved variable success (12, 16, 17, 37, 45, 59, 65, 69, 74, 82, 88, 97, 98).

Clinical studies have provided evidence indicating that conventional periodontal surgery, comprising various types of access flaps and/or resective techniques, may result in probing depth reduction, hard tissue fill or even the elimination of the intrabony component (37, 62, 65). However, residual pockets often persist following nonsurgical periodontal therapy or the use of access flaps, and resective techniques are associated with substantial loss of attachment and increases in soft-tissue recession (37, 62, 65, 69). Furthermore, despite the fact that such techniques may improve clinical outcomes, healing is predominantly characterized by repair (i.e. formation of a long junctional epithelium) and no, or very limited, regeneration (i.e. formation of root cementum with functionally oriented inserting periodontal ligament fibers connected to new alveolar bone) (14).

Thus, the optimal outcome of periodontal treatment is considered as the absence of bleeding on probing, the presence of shallow pockets associated with periodontal regeneration and limited/no soft-tissue recession. Since the early 1970s, a plethora of different surgical techniques, often including implantation of various types of bone grafts and/or substitutes, root surface demineralization, guided tissue regeneration, growth and differentiation factors, enamel matrix proteins or various combinations thereof, have been employed, aiming to achieve predictable periodontal regeneration (16, 17, 38, 45, 52, 74, 99). Systematic reviews of clinical trials have shown that some of these materials, when used in conjunction with surgical approaches designed to facilitate maximal preservation of soft and hard tissues, may indeed result in superior clinical outcomes in terms of probing depth reduction, clinical attach-

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Table 1. Search terms used to identify the relevant studies

Search terms
(“periodontal defect” OR “periodontal lesion” OR “periodontal osseous defect” OR “intraosseous defect” OR “intra-osseous defect” OR “intrabony defect” OR “intra-bony defect” OR “infrabony defect” OR “infra-bony defect” OR “angular defect” OR “bony defect” OR “osseous defect” OR “crater”)
AND
(“guided tissue regeneration” OR “GTR” OR “membrane” OR “barrier” OR “periodontal regeneration” OR “bone graft” OR “bone replacement graft” OR “bone substitute” OR “osseous graft” OR “bone transplantation” OR “bone regeneration” OR “bone matrix” OR “autograft” OR “autogenous bone graft” OR “allogenic bone graft” OR “allograft” OR “freeze dried bone allograft” OR “demineralized freeze-dried bone allograft” OR “decalcified freeze-dried bone allograft” OR “bovine bone” OR “xenograft” OR “xenogenic graft” OR “synthetic graft” OR “alloplastic graft” OR “alloplastic material” OR “polymer” OR “ceramic graft” OR “bioactive ceramic graft” OR “bioglass” OR “biomaterial” OR “bioceramic” OR “hydroxyapatite” OR “calcium phosphate” OR “tricalcium phosphate” OR “beta-tricalcium phosphate” OR “tricalcium phosphate” OR “ceramic” OR “calcium carbonate” OR “calcium sulfate” OR “Plaster of Paris” OR “Emdogain” OR “EMD” OR “enamel matrix derivative” OR “biomimetic substances” OR “growth factors” OR “graft” OR “grafting” OR “regenerative material”)
AND
(“human study” OR “clinical study” OR “patient” OR “human” OR “case” OR “report”)
AND
(“histological study” OR “histology” OR “histomorphometrical study” OR “histomorphometry” OR “electron microscopy study” OR “biopsy” OR “block section” OR “histological evaluation’ OR “histomorphometrical evaluation”)

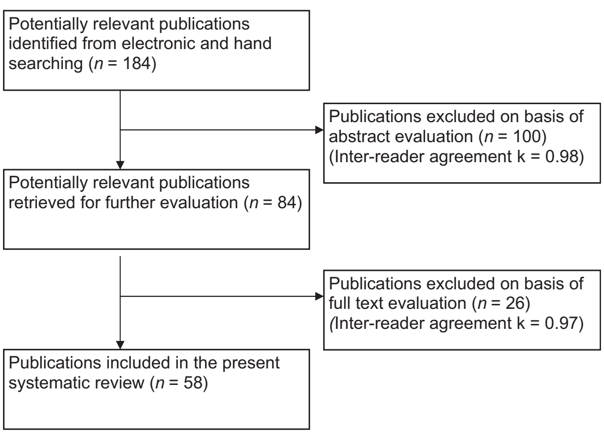

Fig. 1. Flow chart of the screened relevant publications.

Table 2. Studies excluded (n = 26)

Authors (year) (ref. no.)	**Reason for exclusion**
Bosshardt et al. (2005) (3)	Short healing
Sculean et al. (2003) (76)	Repeated data
Sculean et al. (2003) (81)	Unrelated data
Sculean et al. (2002) (85)	Unrelated data
Sculean et al. (2001) (75)	Unrelated data
Heijl (1997) (32)	Unrelated data
Reynolds & Bowers (1996) (67)	Repeated data
Guillemin et al. (1993) (29)	Unrelated data
Stahl & Froum (1991) (92)	Unrelated data
Galgut et al. (1990) (26)	Unrelated data
Wachtel et al. (1990) (104)	Language
Saffar et al. (1990) (72)	Unrelated data
Bowers et al. (1989) (6)	Unrelated data
Issahakian et al. (1989) (35)	Language
Mattout et al. (1988) (46)	Unrelated data
Ganeles et al. (1986) (27)	Unrelated data
Sapkos (1986) (73)	Unrelated data
Bowers et al. (1986) (8)	Unrelated data
Stahl & Froum (1986) (91)	Unrelated data
Baldock et al. (1985) (2)	Unrelated data
Rühling & Plagman (2001) (71)	Language
Kenney et al. (1986) (39)	Unrelated data
Dragoo & Kaldahl (1983) (18)	Language
Busschop & De Boever (1983) (9)	Unrelated data
Froum et al. (1982) (24)	Unrelated data
Hiatt et al. (1978) (33)	Mixed data

ment gain and hard tissue fill when compared with access flaps alone (38). Moreover, numerous studies have shown that the results obtained can be sustained long term, up to 15 years, provided that optimal plaque control and maintenance are evident (17, 74, 77, 82, 89, 98).

A recent systematic review has analyzed the regenerative potential of various available techniques and materials, either alone or in different combinations, for the treatment of periodontal intrabony defects, as assessed in pre-clinical *in vivo* studies that provide histological evidence (36). There was substantial heterogeneity in the studies with

Table 3. The 58 human histologic studies included for analysis, categorized into seven biomaterial groups (note that some studies are cited in more than one group)

Biomaterial group and author (year) (reference)	Biomaterials
Autogenous bone grafts (10 studies)	
Zubery et al. (1993) (109)	Autograft
Stahl et al. (1983) (95)	Autograft
Froum et al. (1983) (25)	Autograft
Evans (1981) (20)	Autograft
Listgarten & Rosenberg (1979) (42)	Autograft
Moskow et al. (1979) (53)	Autograft
Hawley & Miller (1975) (31)	Autograft
Dragoo & Sullivan (1973) (19)	Autograft
Nabers (1972) (55)	Autograft
Ross & Cohen (1968) (70)	Autograft
Allogeneic bone grafts (7 studies)	
Koylass et al. (2012) (41)	Mineralized human cancellous bone allograft
Mellonig (2006) (49)	Decalcified freeze-dried bone allograft or allogenic demineralized bone matrix
Froum (1996) (23)	Freeze-dried bone allograft
Bowers et al. (1991) (4)	Decalcified freeze-dried bone allograft
Bowers et al. (1989) (6)	Decalcified freeze-dried bone allograft
Stahl et al. (1983) (95)	Decalcified freeze-dried bone allograft
Listgarten & Rosenberg (1979) (42)	Decalcified freeze-dried bone allograft
Xenogeneic bone grafts (5 studies)	
Hartman et al. (2004) (30)	Bovine-derived xenograft
Sculean et al. (2003) (84)	Bovine-derived xenograft
Nevins et al. (2003) (57)	Bovine-derived xenograft
Camelo et al. (1998) (11)	Bovine-derived xenograft
Louise et al. (1992) (43)	Coralline
Alloplastic bone grafts (10 studies)	
Horváth et al. (2013) (34)	Hydroxyapatite
Mellonig et al. (2010) (51)	Calcium phosphate
Stavropoulos et al. (2010) (100)	Beta-tricalcium phosphate
Sculean et al. (2005) (86)	Bioglass
Nevins et al. (2000) (58)	Bioglass
Froum (1996) (23)	HTR
Stahl et al. (1990) (93)	HTR
Carranza et al. (1987) (13)	Hydroxyapatite
Stahl & Froum (1987) (94)	Hydroxyapatite

Table 3. (*Continued*)

Biomaterial group and author (year) (reference)	Biomaterials
Froum & Stahl (1987) (22)	Tricalcium phosphate
Barriers (9 studies)	
Windisch et al. (2002) (106)	Guided tissue regeneration
Sculean et al. (1999) (79)	Guided tissue regeneration
Sculean et al. (1999) (80)	Guided tissue regeneration
Windisch et al. (1999) (105)	Guided tissue regeneration
Parodi et al. (1997) (63)	Guided tissue regeneration
Stahl et al. (1990) (93)	Guided tissue regeneration
Gottlow et al. (1986) (28)	Guided tissue regeneration
Nyman et al. (1982) (60)	Guided tissue regeneration
Feingold et al. (1977) (21)	Guided tissue regeneration
Proteins/growth factors/chemical factors (8 studies)	
Majzoub et al. (2005) (44)	Enamel matrix derivative
Sculean et al. (2003) (87)	Enamel matrix derivative
Windisch et al. (2002) (106)	Enamel matrix derivative
Parodi et al. (2000) (64)	Enamel matrix derivative
Sculean et al. (2000) (78)	Enamel matrix derivative
Yukna et al. (2000) (108)	Enamel matrix derivative
Mellonig (1999) (48)	Enamel matrix derivative
Sculean et al. (1999) (80)	Enamel matrix derivative
Combinations (20 studies)	
Stavropoulos et al. (2011) (99)	Recombinant human growth and differentiating factor-5 + beta-tricalcium phosphate
Stavropoulos et al. (2011) (96)	Bovine-derived xenograft + guided tissue regeneration
Sculean et al. (2008) (88)	Bicalcium phosphate + enamel matrix derivative
Sculean et al. (2008) (77)	Bovine-derived xenograft + enamel matrix derivative
Ridgway et al. (2008) (68)	Beta-tricalcium phosphate + platelet-derived growth factor
Sculean et al. (2005) (86)	Bioglass + enamel matrix derivative
Sculean et al. (2004) (83)	Bovine-derived xenograft + guided tissue regeneration
Hartman et al. (2004) (30)	Bovine-derived xenograft + guided tissue regeneration
Nevins et al. (2003) (56)	Decalcified freeze-dried bone allograft + platelet-derived growth factor
Sculean et al. (2003) (84)	Bovine-derived xenograft + enamel matrix derivative
Nevins et al. (2003) (57)	Bovine-derived xenograft + guided tissue regeneration
Yukna et al. (2002) (107)	Bovine-derived xenograft + synthetic peptide (P-15)
Paolantonio et al. (2001) (61)	Bovine-derived xenograft + guided tissue regeneration
Camelo et al. (2001) (10)	Autograft + bovine-derived xenograft + guided tissue regeneration

Table 3. (*Continued*)

Biomaterial group and author (year) (reference)	Biomaterials
Mellonig (2000) (50)	Bovine-derived xenograft + guided tissue regeneration
Camelo et al. (1998) (11)	Bovine-derived xenograft + guided tissue regeneration
Bowers et al. (1991) (4)	Decalcified freeze-dried bone allograft + osteogenin
Stahl & Froum (1991) (90)	Decalcified freeze-dried bone allograft + guided tissue regeneration
Bowers et al. (1985) (7)	Decalcified freeze-dried bone allograft + guided tissue regeneration
Moskow & Lubarr (1983) (54)	Autograft + hydroxyapatite

HTR (synthetic bone polymer combining polymethylmethacralate (polyhydroxyethylmethacralate and calcium.

respect to species (i.e. dog or monkey), study design (i.e. parallel, split-mouth or mixed), materials utilized (i.e. resorbable and nonresorbable membranes, different types of bone grafts/bone substitutes, biologics and various combinations thereof), defect and tooth type, selected outcome measures and healing periods employed. Overall, the results demonstrated that flap surgery, in conjunction with most of the biomaterials evaluated, either alone or in various combinations, promoted periodontal regeneration to a greater extent than did flap surgery without biomaterials. Nevertheless, despite these positive observations in animal models and the successful outcomes reported for many of the available regenerative techniques and materials in patients, the extent to which clinical improvements reflect histologically proven periodontal regeneration remains to be determined. This paper presents a systematic summary of the available histological evidence on the effects of reconstructive periodontal surgery to enhance periodontal wound healing/regeneration in human intrabony defects.

Methods

Development of a protocol

A protocol covering all aspects of the systematic review methodology was developed before starting the review. The protocol included: the definition of a focused question; a defined search strategy; study inclusion criteria; the determination of outcome measures; screening methods, data extraction and analysis; and data synthesis.

Defining the focused question

The focused question was defined as: 'What is the regenerative effect obtained by using several biomaterials as adjuncts to surgery in the treatment of periodontal intrabony defects as evaluated in human histological studies?'

Search strategy

The literature was searched for articles published up to and including June 2014 using the MEDLINE database. Combinations of search terms were employed to identify appropriate studies (Table 1). Reference lists of review articles were also scanned manually. In addition, the reference lists of articles selected for inclusion in the present review were screened. Finally, a hand search of the *Journal of Dental Research*, the *Journal of Clinical Periodontology*, the *Journal of Periodontology*, the *Journal of Periodontal Research* and *The International Journal of Periodontics and Restorative Dentistry* was performed.

Criteria for study selection and inclusion

The study selection was limited to human histological studies evaluating the effect of nonsurgical or surgical treatment, with or without the adjunctive use of potentially regenerative materials (i.e. barrier membranes, grafting materials, growth factors/proteins and combinations thereof), for the treatment of periodontal intrabony defects. A time limitation of a minimum of 6 weeks for the postoperative evaluation period was applied. Only studies published in English were considered.

Outcome measure determination

The primary outcome of interest was the formation of periodontal tissues following the use of regenerative materials, as evaluated histologically/histomorphometrically. The development of periodontal ligament, cementum and bone, given as linear measurements (in mm) or as a percentage of the instrumented root

Table 4. Histologic results of human periodontal defects treated using autogenous bone grafts (10 studies)

Study: authors (year) (ref. no.)	No. of patients No. of defects	Defect type Defect depth	Healing time Graft type	Healing type	Histological results
Ross & Cohen (1968) (70)	1 1	1-3 osseous walls 6 mm	8 months Mandible Alveolar crest	Regeneration	Notch was placed at the most apical extent of the defect. New cellular cementum, new bone formation and new periodontal ligament fibers running parallel to the tooth were present. Bone formation and fibrous tissue around autograft particles that were under remodeling. Complete defect resolution was reported
Nabers et al. (1972) (55)	1 1	3 osseous walls –	57 months –	Regeneration	The termination of the root planing was used as mark. New cellular cementum, new bone formation and new periodontal ligament fibers were present. The graft was fully replaced. Complete defect resolution was reported
Dragoo & Sullivan (1973) (19)	4 12	1-2 osseous walls 1.4 mm	2–8 months Iliac crest Cancellous	Regeneration	One notch at the level of alveolar crest was placed. New cellular cementum, new bone formation and new periodontal ligament fibers running oblique to the tooth were present. Autograft particles were not fully replaced. Complete defect resolution was reported
Hawley & Miller (1975) (31)	1 1	3 osseous walls –	28 months Maxilla Alveolar crest	Regeneration	The termination of the root planing was used as mark. New cellular cementum, bone formation and new fibers of oblique orientation were observed. Partial defect resolution was reported
Moskow et al. (1979) (53)	1 1	1-3 osseous walls –	28 weeks Mandible Alveolar crest	Long junctional epithelium Osseous repair	No notches were placed. Epithelium downgrowth and encapsulated graft particles by connective tissue and epithelium were noticed. Active osteogenesis opposite to tooth surface was seen. Complete defect resolution was reported
Listgarten & Rosenberg (1979) (42)	6 6	1-3 osseous walls 3–7 mm	6–12 months Alveolar crest Cancellous	Long junctional epithelium Regeneration	Notch was placed at the bottom of the defect. Epithelium downgrowth and encapsulated graft particles by epithelium (exfoliation) were noticed. Signs of new cementum and bone formation were seen. Partial defect resolution was reported
Evans (1981) (20)	1 1	2-3 osseous walls –	24 months Alveolar crest Coagulum	Regeneration	Notch at the level of alveolar crest was used. Fully regeneration of periodontal tissues was noticed. The graft was fully replaced. Complete defect resolution was reported

Table 4. (*Continued*)

Study: authors (year) (ref. no.)	No. of patients No. of defects	Defect type Defect depth	Healing time Graft type	Healing type	Histological results
Froum et al. (1983) (25)	1 3	2-3 osseous walls 2–5 mm	24 weeks Alveolar Osseous Coagulum	Long junctional epithelium Regeneration	Two notches at the level of alveolar crest and the base of the defect were placed. Citric acid was applied on the teeth. Long junctional epithelium coronally and regeneration of periodontal tissues apically were noticed. Encapsulated graft particles were seen. Partial defect resolution was reported
Stahl et al. (1983) (95)	1 4	1-3 osseous walls	12 months Maxillary Osseous Coagulum	Long junctional epithelium Regeneration	No notches were placed. Citric acid was applied on the teeth. Long junctional epithelium coronally and regeneration of periodontal tissues apically were observed. Limited osteogenesis of the graft particles was seen. Partial defect resolution was reported
Zubery et al. (1993) (109)	1 1	1-3 osseous walls 5 mm	8 months Maxillary Osseous Swaged graft	Long junctional epithelium Connective tissue adhesion Osseous repair	No notches were placed. Tetracycline was applied on the tooth surface. Long junctional epithelium coronally, connective tissue adhesion apically and osteogenesis were found. Encapsulated graft particles were observed. Partial defect resolution was reported

length (%), were analyzed. Postsurgical alterations in the periodontal defect size, based upon histomorphometric measurements, were also considered. When specific data were reported, defect resolution and fill were calculated. The nature of healing associated with defect resolution was recorded (i.e. complete regeneration, long junctional epithelium, connective tissue attachment, connective tissue adhesion or osseous repair).

Screening method

The titles and abstracts of the selected studies were independently screened by two reviewers (D.N. and G.N.). The screening of titles and abstracts was based on the following question: 'Was the study conducted in humans and did it present histological treatment outcomes in periodontal intrabony lesions following the use of regenerative biomaterials, at least 6 weeks postoperatively?' The full text of each article was obtained if the response was 'yes' or 'uncertain' to the screening question. Disagreement regarding inclusion was resolved by discussion between authors. The level of agreement between reviewers was determined by kappa scores. The authors of the studies were contacted to provide missing data where this was necessary and feasible.

Data extraction and analysis

Data were extracted on the following: the general characteristics (authors and year of publication); study characteristics (number of patients and defects, tooth type, defect characteristics, intervention strategies, evaluation period and outcome measures); methodological characteristics (study design and methodological quality); and conclusions. There was substantial heterogeneity in the data collected regarding study design, defect type, materials used, evaluation methods, outcome measures and observation periods. Additionally, most of the studies had either a split-mouth or a mixed design and did not provide data on intra-individual variance. Weighted mean differences could not be calculated, and consequently it was not possible to perform a quantitative data synthesis leading to a meta-analysis. Therefore, the mean and standard deviation, the 95% confidence intervals and the statistical significance were determined and extracted from the reviewed articles. These data were summarized in separate tables based upon the various types of biomaterials/interventions employed. Furthermore, the results of the studies that used similar outcome measurements were combined and the data are presented graphically.

Table 5. Histomorphometric results of human periodontal defects treated using autogenous bone grafts

Study*	Tooth notation	Defect depth (mm)	Osseous walls	Collagen fibers on tooth surface (mm)	New cementum (mm)	New bone (mm)	Long junctional epithelium (mm)	Healing type
Dragoo & Sullivan (1973) (19)	4 teeth	1.4	1-2	3.1	3.1	2.1	1.3	Regeneration
		1.4	1-2	3.1	3.1	2.1	1.3	Regeneration
		1.4	1-2	3.1	3.1	2.1	1.3	Regeneration
		1.4	1-2	3.1	3.1	2.1	1.3	Regeneration
Listgarten & Rosenberg (1979) (42)	Mesial	5.2	1	0	0	0.8	3.6	Long junctional epithelium
	43 mesial	3.4	2	1.5	1	2.6	2.2	Long junctional epithelium, regeneration
	14 mesial	1.4	1-2	0	0	0	2.4	Long junctional epithelium
	33 mesial	2.1	1-2	1.2	0.4	1.1	3.5	Long junctional epithelium, regeneration
	43 mesial	7.5	1-2	4.2	3.8	3.8	2.8	Long junctional epithelium, regeneration
	13 distal	4.5	3	1.4	0.9	2.0	3.5	Long junctional epithelium, regeneration
Mean	–	3.0 ± 2.1	–	2.1 ± 1.5	1.9 ± 1.5	1.9 ± 1.0	2.3 ± 1.0	80% regeneration

*Only 2 of the 10 studies given in Table 4 provided appropriate histomorphometric data, which are presented here.

Results

Data extraction after literature searching

The MEDLINE literature search resulted in 162 potentially relevant publications (Fig. 1). After the first selection step, based upon the title and abstract of the collected studies, 62 articles were included for further analysis (inter-reader agreement $k = 0.98$). Hand searching identified a further 22 articles that were added at this stage, resulting in a total of 84 selected studies. Finally, based upon text screening, 26 articles were excluded (the reasons for exclusion are presented in Table 2), and 58 publications, completely fulfilling the inclusion criteria, were selected for the review (inter-reader agreement $k = 0.97$). These are presented in Table 3 and grouped according to relevant biomaterial/intervention.

Data analysis of the regenerative effect of each biomaterial/intervention

Seven biomaterial/intervention types – autografts, allogenic bone, xenogeneic bone, alloplastic materials, barriers, growth factors and biological factors (and combinations thereof) – were reported. Autografts are tissues transferred from one part of a person's body to another part of the same person; allogenic bone is bone harvested from genetically distinct individuals within the same species; xenogeneic bone is that harvested from a species genetically different from humans; and alloplastic materials are biological materials that are synthesized or chemically processed.

The nature of healing in the treated defects following regenerative therapy was defined as follows: long junctional epithelium (epithelial downgrowth covering the treated tooth surface); connective tissue attachment (new cementum with inserting collagen fibers on the treated tooth surface but not connected to new bone); connective tissue adhesion (connective tissue contact to the root without apparent cementum formation); regeneration (new cementum with inserting fibers functionally oriented and new bone); and osseous repair (bone formation opposite the tooth surface, leading to defect filling but without apparent periodontal ligament formation). Histologic data were included in tables, reporting, for each selected study, the following items: author name, publication date, number of patients, number of defects, number of osseous walls, defect depth, healing period, biomaterial type, healing type and histological results (Tables 4–18). Additionally, histomorphometric data were analyzed by reporting the

Table 6. Histologic results of human periodontal defects treated using allogeneic bone grafts (7 studies)

Study: authors (year) (ref. no.)	No. of patients No. of defects	Defect type Defect depth	Healing time Graft type	Healing type	Histological results
Listgarten & Rosenberg (1979) (42)	6 6	1-3 osseous walls 3–6.5 mm	6–12 months –	Long junctional epithelium Connective tissue attachment Osseous repair	Notch was placed at the bottom of the defect. Epithelium downgrowth, new cementum and new bone formation were present. Graft particles encapsulated by epithelium or connective tissue were reported. Partial defect resolution was noticed
Stahl et al. (1983) (95)	1 1	2 osseous walls –	12 months Decalcified freeze-dried bone allograft	Long junctional epithelium Regeneration	Notches at the gingival margin and the most apical extent of calculus. Long junctional epithelium was present. Apically, new cementum, new periodontal ligament and limited osteogenesis were noticed. Graft particles encapsulated by epithelium were seen. Partial defect resolution was reported
Bowers et al. (1989) (5, 6)	12 32	1-3 osseous walls 6.9 mm	6 months Decalcified freeze-dried bone allograft	Regeneration (68%) Connective tissue attachment Long junctional epithelium	Notches at the level of alveolar crest and the most apical extent of calculus. New cellular cementum, parallel or perpendicular new periodontal ligament fibers and bone formation were noticed. Graft particles were not often observed
Bowers et al. (1991) (4)	6 11	1-3 osseous walls 5.3 mm	6 months Decalcified freeze-dried bone allograft	Regeneration (95%) Long junctional epithelium	Notches at the level of alveolar crest and the most apical extent of calculus. New cellular cementum, periodontal ligament fibers and bone formation were observed
Froum (1996) (23)	1 3	Circumferential 3–4 mm	30 months Decalcified freeze-dried bone allograft	Long junctional epithelium	Notches at the gingival margin and the most apical extent of calculus. Long junctional epithelium was present. No evidence of graft particles and osteogenesis. Partial defect resolution was reported

Table 6. (*Continued*)

Study: authors (year) (ref. no.)	No. of patients No. of defects	Defect type Defect depth	Healing time Graft type	Healing type	Histological results
Mellonig (2006) (49)	3 3	2-3 osseous walls –	6 months Allogenic demineralized bone matrix	Regeneration Connective tissue adhesion	Notch at the most apical extent of calculus. New cellular cementum, parallel or perpendicular new periodontal ligament fibers and bone formation were noticed. Connective tissue adherence, in one case, was seen. Partial defect resolution was reported
Koylass et al. (2012) (41)	4 4	–	6 months Mineralized human cancellous bone allograft	Regeneration, Connective tissue attachment, Long junctional epithelium	Notch at the apical extent of the calculus. New cellular cementum, parallel or perpendicular new periodontal ligament fibers and bone formation were noticed

amount of new cementum and bone formation, and also collagen fibers and junctional epithelium on the tooth surface, as linear measurements (in mm).

Regenerative effect of autogenous bone

Ten studies providing data on the effect of autogenous bone grafting in intrabony defects were identified. Histologic and histomorphometric results of these studies are summarized in Tables 4 and 5, respectively. Five of the 10 studies reported complete regeneration of the periodontal tissues (i.e. the entire defect) (19, 20, 31, 55, 70), three studies noticed a long junctional epithelium (25, 42, 95) coronally and periodontal regeneration apically and two studies observed only long junctional epithelium and osseous repair (53, 109). Autograft particles were not fully replaced in six studies and were encapsulated in bone or connective tissue in five studies depending upon the healing observation period, graft source and type of healing (Tables 4 and 5). Fifty percent of studies revealed complete defect resolution, and no remarkable inflammation was described. However, in five studies, root notches were not placed before treatment, and the absence of a fixed landmark may call into question the results because the histological region of interest was not necessarily identifiable (Tables 4 and 5). Only two of the 10 studies provided histomorphometric data and included a total of 10 defects; in 80% of the defects partial or complete regeneration was observed. The mean defect depth was 3.0 mm, and both cementum and bone formation were 1.9 mm when calculated in a linear manner (along the tooth surface).

Regenerative effect of allogeneic bone

Seven studies providing data on the effect of allogeneic bone grafting in intrabony defects were identified. The histologic and histomorphometric results of these studies are summarized in Tables 6 and 7, respectively. Two studies reported almost complete periodontal regeneration (4, 49); six studies observed a combination of long junctional epithelium and periodontal regeneration/connective tissue attachment (4–6, 41, 42, 95); and one study observed a reparative type of healing characterized by long junctional epithelium (23). Two of the studies reported that graft particles were not completely replaced and remained encapsulated within connective tissue. No histological study to date has demonstrated or reported complete defect resolution, but equally none has reported any significant inflammation. Five studies provided histomorphometric data involving a total of 31 defects; 70% of the defects demonstrated signs of partial periodontal regeneration but none reported complete regeneration. The mean defect depth at surgery was 6.0 mm, and cementum and bone formation (given in linear measurements) of 1.3 and 1.8 mm, respectively, were noted.

Table 7. Histomorphometric results of human periodontal defects treated using allogeneic bone grafts

Study*	Tooth notation	Defect depth (mm)	Osseous walls	Collagen fibers on tooth surface (mm)	New cementum (mm)	New bone (mm)	Junctional epithelium (mm)	Healing type
Listgarten & Rosenberg (1979) (42)	43 mesial	5	3	1.4	0.8	2.1	2.9	Long junctional epithelium, connective tissue attachment, regeneration
	43 mesial	3	2	0	0	2.6	2.5	Long junctional epithelium, osseous repair
	21 mesial	4.5	2	0.8	0.2	1.4	2.4	Long junctional epithelium, connective tissue attachment, regeneration
	24 mesial	5	2-3	1.5	0.9	0	1.0	Long junctional epithelium, connective tissue attachment
	14 mesial	6.5	1-3	2.4	2.1	0.8	2.0	Long junctional epithelium, connective tissue attachment, regeneration
	45 mesial	6	1-3	2.1	2.2	1.4	4.5	Long junctional epithelium, connective tissue attachment, regeneration
Bowers et al. (1989) (6)	*12x*	6.9	–	0.1	1.2	1.8	1.2	Long junctional epithelium, connective tissue attachment, regeneration
Bowers et al. (1991) (4)	*6x*	5.3	1-3	0	1.7	2.5	0.6	Long junctional epithelium, regeneration
Mellonig (2006) (49)	13 distal	–	2-3	3.1	1.3	2.3	–	Connective tissue attachment, regeneration
	45 mesial	–	–	0.6	2.9	3.5	–	Connective tissue attachment, regeneration
	23 distal	–	–	0	0	0	–	Long junctional epithelium
Koylass et al. (2012) (41)	42 mesial	–	–	–	2.85	2.59	–	Regeneration
	42 mesial	–	–	–	1.53	2.02	–	Regeneration
	12 mesial	–	–	–	0	0	–	Long junctional epithelium
	42 distal	–	–	–	2.38	0	–	Connective tissue attachment
Mean (in mm)		6.0 ± 1.1	1–3	0.5 ± 0.9	1.3 ± 0.7	1.8 ± 0.9	1.4 ± 0.9	70% regeneration

12x and *6x* are given in italics because there were 12 and 6 defects, respectively. As the author reported mean values and not single values per defect, the mean value contributed in a 12-fold and 6-fold manner, respectively, to the final result.
*Five of the 7 studies given in Table 6 provided appropriate histomorphometric data, which are presented here.

Regenerative effect of xenogeneic bone

At the time of writing, five studies had provided data on the effect of xenogeneic bone grafting in intrabony defects. The histologic and histomorphometric results of these studies are summarized in Tables 8 and 9, respectively. Of the five studies, three reported periodontal regeneration (11, 57, 84), one described the combination of long junctional epithelium and periodontal regeneration/connective tissue attachment (30), and one, using coralline hydroxyapatite, did not provide any information on the healing outcome (43). Graft particles were partially encapsulated within connective tissue in two studies, and all studies reported bone formation in contact with graft particles. None of the studies described or reported complete defect resolution, and no information on the degree of inflammation was provided. Four studies provided histomorphometric data for a total of 11 defects. Partial periodontal regeneration was observed in 73% of the defects, but none showed complete

Table 8. Histologic results of human periodontal defects treated using xenogeneic bone grafts (5 studies)

Study: authors (year) (ref. no.)	No. of patients No. of defects	Defect type Defect depth	Healing time Graft type	Healing type	Histological results
Hartman et al. (2004) (30)	2 5	1-2 osseous walls 5–8 mm	6 months Bone mineral matrix	Long junctional epithelium Connective tissue attachment Regeneration	Notch was placed at the apical extent of calculus. Root conditioning (tetracycline) was applied. New cellular cementum was noticed. Encapsulated biomaterial particles and/or bone formation around biomaterial particles was seen. All types of healing were observed
Sculean et al. (2003) (84)	1 1	1-2 osseous walls –	6 months Bone mineral matrix	Regeneration	Notches were placed at the most apical extent of the defect and at the level of the alveolar crest. New cellular cementum with inserting collagen fibers was reported. Bone formation around graft particles was found
Nevins et al. (2003) (56)	2 2	2-3 osseous walls 5–6 mm	9 months Bone mineral matrix	Regeneration	Notch was placed at the apical extent of calculus. Root conditioning (tetracycline) was applied. Bone formation around graft particles was evident. New cellular cementum was noticed
Camelo et al. (1998) (11)	2 2	3 osseous walls 6 mm	6–9 months Bone mineral matrix	Regeneration	No notches were placed. New cellular cementum was noticed. Encapsulated biomaterial particles and/or bone formation around biomaterial particles was seen
Louise et al. (1992) (43)	4 4	2-3 osseous walls Moderate/severe intrabony defects	6–36 months Coralline hydroxyapatite	Not reported	No notches were placed. New bone formation around biomaterial particles was reported

regeneration. The mean defect depth was 6.6 mm, and cementum and bone formation (expressed in linear measurements) were 2.4 and 2.3 mm, respectively.

Regenerative effect of alloplastic substitutes

Ten studies provided data on the effect of the implantation of alloplastic materials in intrabony defects. The histologic and histomorphometric results of these studies are summarized in Tables 10 and 11, respectively. The healing was predominantly characterized by a long junctional epithelium and connective tissue encapsulation of the graft particles. Periodontal or cementum regeneration was usually limited to the apical parts of the defects (Fig. 2). Despite prolonged healing periods of 18–30 months, all studies reported the presence of biomaterial/graft particles, which were surrounded by fibrous tissue. Bone formation around graft particles was very limited and only occasionally reported. No studies reported complete defect resolution, and remarkably little inflammation was described. Additionally, notches were employed for reference in all studies except one. Five studies provided histomorphometric data on 23 defects. Partial periodontal regeneration was observed in 34% of the defects, but none demonstrated complete regeneration. The mean defect depth was >4 mm at baseline, with linear measurements of cementum and bone formation being 0.6 and 0.4 mm, respectively.

Regenerative effect of guided tissue regeneration

We identified nine studies presenting data on the effect of guided tissue regeneration in intrabony defects, and the histologic and histomorphometeric

Table 9. Histomorphometric results of human periodontal defects treated using xenogeneic bone grafts

Study*	Tooth notation	Defect depth (mm)	Osseous walls	Collagen fibers on tooth surface (mm)	New cementum (mm)	New bone (mm)	Junctional epithelium mm)	Healing type
Hartman et al. (2004) (30)	31 mesial	7	1	1.6	0.6	0	1	Connective tissue attachment
	41 distal	7	1	0	0	0	2.3	Long junctional epithelium
	16 mesial	8	2	3.3	0.8	2.7	0.9	Regeneration
	45 mesial	7	2	0.7	0.6	0	0.9	Connective tissue attachment
	36 mesial	7	1	1.5	2.5	2	0.8	Regeneration
Sculean et al. (2003) (84)	46 distal	–	1-2	–	2.5	2.1	–	Regeneration
Nevins et al. (2003) (56)	24 mesial	6	2-3	–	3.4	4.1	–	Regeneration
	44 distal	5	2	–	3.7	3.1	–	Regeneration
Camelo et al. (1998) (11)	23 mesial	6	3	–	5.2	4.8	–	Regeneration
	32 distal	6	3	–	5.1	4.2	–	Regeneration
Mean (in mm)	–	6.6 ± 0.9	–	1.4 ± 1.2	2.4 ± 1.9	2.3 ± 1.8	1.2 ± 0.6	70% regeneration

*Four of the 5 studies given in Table 8 provided appropriate histomorphometric data, which are presented here.

results of these studies are summarized in Tables 12 and 13, respectively. Five of the nine studies reported periodontal regeneration (63, 79, 80, 105, 106) (Fig. 3), one described a combination of long junctional epithelium formation and connective tissue attachment (93) and three provided evidence of new connective tissue attachment (21, 28, 60). Interestingly, all studies that employed collagen barriers reported periodontal regeneration, and no collagen barrier remnants were observed after 5 months of healing. Two studies revealed complete defect resolution, but no data were reported on inflammatory reactions or infiltrates. Additionally, in two studies, notches were not placed. Eight studies provided histomorphometric data encompassing a total of 20 defects. Seventy-five percent of the defects showed either partial or complete periodontal regeneration. The mean defect depth was 5.1 mm, with 2.6 and 1.7 mm of cementum and bone formation respectively (as expressed by linear measurements).

Regenerative effect of biological agents

Of the eight studies reporting data on the efficacy of biological factors in intrabony defects, all utilized enamel matrix derivative (Tables 14 and 15). The histologic and histomorphometric results of these studies are summarized in Tables 14 and 15, respectively. All studies except one reported a healing characterized predominantly by periodontal regeneration and/or connective tissue attachment (44, 48, 78, 80, 87, 106, 108) (Fig. 4), while one study showed the formation of a long junctional epithelium (64). No studies reported complete defect resolution, and no remarkable inflammation was described. In all studies, reference notches were placed on the root surfaces. Six studies provided histomorphometric data for a total of 29 defects; in 45% of the defects, partial periodontal regeneration was observed. The mean defect depth was 4.8 mm, and cementum and bone formation (as expressed in linear measurements) were 1.3 and 0.6 mm, respectively.

Regenerative effect of combination techniques

Twenty studies provided data on the effect of using a combination of regenerative techniques. The histologic and histomorphometeric results of these studies are summarized in Tables 16 and 17, respectively. In most studies the healing was predominantly characterized by periodontal regeneration and/or formation of a connective tissue attachment. Formation

Table 10. Histologic results of human periodontal defects treated using alloplastic materials (10 studies)

Study: authors (year) (ref. no.)	No. of patients No. of defects	Defect type Defect depth	Healing time Graft type	Healing type	Histological results
Froum & Stahl (1987) (22)	1 5	1-2 walls 3–9 mm	13–18 months Tricalcium phosphate	Long junctional epithelium	Notches were placed at the gingival margin and at the most apical extent of calculus. Long junctional epithelium was evident. Graft particles were limited and surrounded by fibrous tissue. Minimal cementogenesis and ostogenesis were seen
Stahl & Froum (1987) (94)	3 12	1-2 walls 4–11 mm	3–12 months Hydroxyapatite	Long junctional epithelium	Notches were placed at the gingival margin and at the most apical extent of calculus. Long junctional epithelium was reported with minimal cementogenesis observed apically in a few cases. Graft particles were present and surrounded by fibrous tissue with peripheral bone formation
Carranza et al. (1987) (13)	2 2	1-2 walls Deep	5–6 months Hydroxyapatite	Long junctional epithelium Regeneration	No notches were placed. Long junctional epithelium was established. New cementum formation and collagen fibers were observed in one case. Graft particles were present and surrounded by fibrous tissue with peripheral bone formation
Stahl et al. (1990) (93)	5 11	1-2 walls 2–9 mm	4–26 weeks HTR	Long junctional epithelium	Notches were placed at the gingival margin and at the most apical extent of calculus. Long junctional epithelium was reported and minimal cementogenesis was seen apically in a few cases. Graft particles were surrounded by fibrous tissue with occasionally peripheral bone formation
Froum (1996) (23)	1 3	Circumferential 4–5 mm	30 months HTR	Long junctional epithelium	Notches were placed at the gingival margin and at the most apical extent of calculus. Long junctional epithelium was seen. No cementogenesis was observed. Graft particles were surrounded by fibrous tissue with peripheral bone formation occasionally. Partial defect resolution was noticed
Nevins et al. (2000) (58)	5 5	1-2 osseous walls ≥3 mm	6 months Bioglass	Long junctional epithelium	Notch at the most apical part of the defect was placed. Minimal biomaterial resorption and encapsulated graft particles were found. Long junctional epithelium with no signs of regeneration was reported

Table 10. (*Continued*)

Study: authors (year) (ref. no.)	No. of patients No. of defects	Defect type Defect depth	Healing time Graft type	Healing type	Histological results
Sculean et al. (2005) (86)	3 3	1-3 walls 4–6 mm	6 months Bioglass	Long junctional epithelium Regeneration	Notch was placed at the most apical extent of calculus. Epithelial downgrowth, connective tissue encapsulation of the graft material and limited regeneration at the most apical part of one defect were reported
Stavropoulos et al. (2010) (100)	5 5	1-2 osseous walls ≥4 mm	6 months Beta-tricalcium phosphate	Regeneration Connective tissue adhesion	Notch was placed at the most apical part of the defect. New cellular cementum with inserting, functionally oriented collagen fibers were reported. Connective tissue adhesion in three cases and new bone formation in four cases were noticed. Graft particles were present, surrounded by fibrous tissue with peripheral bone formation occasionally
Mellonig et al. (2010) (51)	4 4	Vertical osseous defects >4 mm	6 months Calcium phosphate cement	Long junctional epithelium	Notch was placed at the most apical extent of calculus. Poor wound healing was observed. Encapsulated graft particles with no regeneration were reported
Horvath et al. (2013) (34)	6 6	1-2 osseous walls 3.5 mm	7 months Nano-hydroxyapatite	Long junctional epithelium	Notch at the base of the defect. No ankylosis or root resorption. Graft particles were surrounded by connective tissue in two of six specimens. Limited regeneration

HTR, synthetic bone polymer combining polymethylmethacralate, polyhydroxyethylmethacralate and calcium.

of long junctional epithelium was limited and generated located to the most coronal part of the defect. The most frequently utilized graft material was of bovine origin. Graft particles were present in all studies, irrespective of the healing period (up to 5 years) employed, and were surrounded by either bone or fibrous tissue (Fig. 5). Bone formation around the graft particles varied widely among the studies and was related to the type of graft material used. No study reported complete defect resolution, and no inflammation worthy of note was described. Additionally, notches were used in all studies except four. Thirteen studies provided histomorphometric data for a total of 74 defects. In 75% of the defects, partial periodontal regeneration was observed. The mean defect depth was more than 6.1 mm at baseline, and formation of cementum and bone (analyzed by linear measures) was 1.9 mm for both parameters.

Data synthesis

In order to derive summary measures of the histological outcomes of studies employing the same methods of evaluation and similar outcome measures, the data from these studies were collated and averaged and are illustrated in Table 18 and Figs 6 and 7. The mean values of periodontal regeneration, expressed as 'means of new cementum with inserting periodontal ligament fibres' and 'new bone', were calculated as linear measurements based upon relevant data. The length of any new cementum layer, presented as a percentage of the defect depth for comparison, ranged from 15% to 63%. The linear length of new bone also ranged from 13% to 63%. Additionally, the percentage of treated defects demonstrating complete or partial regeneration was estimated (Tables 4–17) and is presented in Fig. 7. Periodontal regeneration, in

Table 11. Histomorphometric results of human periodontal defects treated using alloplastic materials

Study*	Tooth notation	Defect depth (mm)	Osseous walls	Collagen fibers on tooth surface (mm)	New cementum (mm)	New bone (mm)	Junctional epithelium (mm)	Healing type
Nevins et al. (2000) (58)	46 distal	3	2 walls	–	0	0	–	Long junctional epithelium
	13 mesial	4	Circumferential	–	0	0	–	Long junctional epithelium
	37 mesial	>4	Wide	–	0	0	–	Long junctional epithelium
	24 mesial	–	1-2	–	0	0	–	Long junctional epithelium
	15 mesial	–	Circumferential	–	0	0	–	Long junctional epithelium
Sculean et al. (2005) (86)	36	4	3 walls	–	1.2	1.4	–	Regeneration
	15	6	1-2 walls	–	0	0	–	Long junctional epithelium
	16	4	1-2 walls	–	0	0	–	Long junctional epithelium
Stavropoulos et al. (2010) (100)	44 mesial	≥4	1-2 walls	–	1.2	1.1	–	Regeneration
	34 mesial	≥4	1-2 walls	–	1.8	1.9	–	Regeneration
	16 distal	≥4	1-2 walls	–	1.5	0.7	–	Regeneration
	46 mesial	≥4	1-2 walls	–	3.0	0.0	–	Connective tissue attachment
	44 mesial	≥4	1-2 walls	–	1.8	1.3	–	Regeneration
Mellonig et al. (2010) (51)	45 mesial	>4	–	0.0	0.0	0.0	2.4	Long junctional epithelium
	44 distal	>4	–	0.2	0.2	0.0	3.1	Long junctional epithelium
	23 mesial	>4	–	0.1	0.1	0.0	4.7	Long junctional epithelium
	45 mesial	>4	–	0.0	0.0	0.0	1.4	Long junctional epithelium
Horvath et al. (2013) (34)	–	4	1-2 walls	–	0	0	–	Long junctional epithelium
	–	4	1-2 walls	–	0.86	0.86	–	Regeneration
	–	4	1-2 walls	–	0	0	–	Long junctional epithelium
	–	3	1-2 walls	–	0.53	1.02	–	Regeneration
	–	3	1-2 walls	–	0.79	1.33	–	Regeneration
	–	3	1-2 walls	–	0	0	–	Long junctional epithelium
Mean (in mm)		>4		0.1 ± 0.1	0.6 ± 0.8	0.4 ± 0.6	2.9 ± 1.4	34% regeneration

*Five of the 10 studies given in Table 10 provided appropriate histomorphometric data, which are presented here.

terms of the percentage of maximal potential treatment outcomes, ranged from 34% to 80%.

Discussion

The results of the present systematic review indicate that periodontal regeneration in human intrabony defects can be achieved to a variable extent using a variety of methods and materials. Periodontal regeneration was observed after the use of a variety of bone grafts and substitutes, guided tissue regeneration, biological factors and combinations thereof. Surprisingly, the amount of periodontal regeneration (i.e. new cementum with inserting fibers functionally oriented and new bone) was rather similar among the various treatments (the average values ranged from 1.3 to 2.3 mm), except for alloplastic materials and biological factors used as monotherapies, which showed limited amounts of periodontal regeneration (the average values were 0.4 and 0.6 mm, respectively), despite a large range (3.0–6.6 mm) in pretreatment intrabony defect depth. This finding appears to contrast with observations made in clinical studies on regenerative periodontal therapy, in which deep defects showed more clinical attachment gain and radiographic bone fill compared with shallow defects (18).

Another interesting observation made in this review is that, in the majority of cases, the amount

Table 12. Histologic results of human periodontal defects treated using guided tissue regeneration (9 studies)

Study: authors (year) (ref. no.)	No. of patients No. of defects	Defect type Defect depth	Healing time Graft type	Healing type	Histological results
Feingold et al. (1977) (21)	4 14	1-2 walls Shallow broad craters	12–28 weeks Scleral barrier	Connective tissue attachment	No notches were placed. Findings included new cementum at tooth nicks, dense connective tissue formation, no evidence of epithelial downgrowth or osteogenesis and complete repair
Nyman et al. (1982) (60)	1 1	1-3 walls 2 mm	3 months Teflon barrier	Connective tissue attachment	Notch at the level of alveolar crest was placed. New cementum and new inserting fibers, but no coronal bone overgrowth, were noticed. Complete defect resolution was reported
Gottlow et al. (1986) (28)	2 2	Extensive periodontal destruction	3 months Teflon barrier	Connective tissue attachment	Root-planed surface served as reference mark. New cementum and new fiber attachment were noticed. No bone regrowth was reported
Stahl et al. (1990) (93)	2 2	1-2 walls 4.5–5 mm	14–30 weeks Teflon barrier	Long junctional epithelium Connective tissue attachment	Two notches at the gingival margin and at the most apical extent of calculus were placed. Defect resolution was achieved by the combination of long junctional epithelium and new attachment apically
Parodi et al. (1997) (63)	1 1	Circumferential 3.5 mm	5 months Collagen barrier	Regeneration	Two notches at the gingival margin and at the level of the alveolar crest were placed. New cellular cementum, new periodontal ligament fibers perpendicular to tooth and bone formation were reported. No collagen traces were observed Complete defect resolution was seen
Sculean et al. (1999) (79)	2 2	Circumferential	6 months Collagen barrier	Regeneration	Two notches at the level of the alveolar crest and at the bottom of the defect were placed. New cellular cementum, new periodontal ligament fibers perpendicular to tooth and bone formation were reported
Sculean et al. (1999) (80)	7 7	Advanced angular defects	6 months Collagen barrier	Regeneration	Two notches at the level of the alveolar crest and at the most apical extent of calculus or the bottom of the defect were placed. New cellular cementum, new periodontal ligament fibers perpendicular to tooth and bone formation were reported

Table 12. (*Continued*)

Study: authors (year) (ref. no.)	No. of patients No. of defects	Defect type Defect depth	Healing time Graft type	Healing type	Histological results
Windisch et al. (1999) (105)	1 1	Advanced angular defect	6 months Collagen barrier	Regeneration	Notch at the bottom of the defect was placed. New cellular cementum, new periodontal ligament fibers and osteogenesis were reported
Windisch et al. (2002) (106)	4 4	1-3 walls 3–7 mm	6 months Collagen barrier	Regeneration	Two notches at the level of alveolar crest and at the most apical extent of calculus or the bottom of the defect were placed. New cellular cementum, new periodontal ligament fibers perpendicular to tooth and bone formation were reported

of new cementum formation, in terms of height, was either larger than or equal to that of the new bone (83 and 46, respectively, of 198 histological cases). Also, the observation that a long junctional epithelium was not necessarily formed in nonguided tissue regeneration cases supports the notion that new connective tissue attachment formation may not depend primarily on physical exclusion of epithelial downgrowth (e.g. the guided tissue regeneration principle) and that wound stability and space provision are of paramount importance for unfolding the innate regenerative potential of the periodontium. It also supports the notion that bone is the most 'sensitive' tissue within the periodontium, and that the majority of available bone grafts and substitute materials are merely osteocompatible rather than osteoconductive, in the sense of enhancing bone formation. Indeed, several preclinical *in vivo* experiments and human biopsies have shown that bone-substitute materials may actually delay, rather than accelerate, bone formation when space provision is assured (2, 13, 102, 103). On the other hand, a recent pre-clinical *in vivo* study indicated that given enough time for tissue remodeling and maturation, even very large periodontal defects grafted with a bone substitute by guided tissue regeneration showed complete regeneration (36). Thus, the reduced amount of bone formation observed in several biopsies may simply be the result of a less-than-adequate healing time.

Amongst the criteria established by the American Academy of Periodontology at the World Workshop in Periodontics in 1996 to evaluate regenerative periodontal procedures, was the existence of human histologic specimens, ideally obtained from randomized controlled trials and demonstrating the formation of cementum, bone and periodontal ligament coronal to a notch in the root, in calculus or at the gingival margin. The present systematic review identified only a single randomized controlled trial that provided human histologic data following regenerative treatment of intrabony defects with growth differentiation factor-5 coated onto beta-tricalcium phosphate particles (99), whereas the remainder of the reported studies utilized data obtained from only few patient case series. Theoretically, human histological data from randomized controlled trials should provide the most convincing evidence for the potential of a procedure to facilitate periodontal regeneration; however, such studies are extremely difficult and costly to conduct. Moreover, human histological studies may raise ethical concerns, given the need for biopsy in otherwise healthy/healing tissues and the fact that some form of patient compensation is provided, and this may be regarded as 'coercion' to participate. Patients participate in such studies after receiving verbal and written information about the aims and procedures involved and the consequences of those procedures, and after having provided written informed consent. Moreover, in most of the studies identified, ethical approval from the relevant authorities was obtained. In contrast to human studies, a large sector of society has difficulties understanding why it is more ethical to kill a dog or a monkey, animals unable to provide informed consent before participating in the 'experiment', to evaluate periodontal regeneration. Indeed, the core principle underpinning the

Table 13. Histomorphometric results of human periodontal defects treated using guided tissue regeneration

Study*	Tooth notation	Defect depth (mm)	Osseous walls	Collagen fibers on tooth surface (mm)	New cementum (mm)	New bone (mm)	Junctional epithelium (mm)	Healing type
Nyman et al. (1982) (60)	32 distal	2	1-3	7	7	2	–	Connective tissue attachment
Gottlow et al. (1986) (28)	24	9–11	Circumferential	4.3	4.3	0	–	Connective tissue attachment
	48	9	Circumferential	3.2	3.2	0	–	Connective tissue attachment
Stahl et al. (1990) (93)	34 distal	5.1	2	1.1	1.1	0	1.9	Long junctional epithelium, connective tissue attachment
	23 distal	3.4	1	0	0	0	2	Long junctional epithelium
Parodi et al. (1997) (63)	27 mesial	3.5	Circumferential	3.8	3.8	3.5	–	Regeneration
Sculean et al. (1999) (79)	35	5	2	2.4	2.4	2.3	–	Regeneration
	46 distal	–	1-3	3.1	3.1	3.0	–	Regeneration
Sculean et al. (1999) (80)	36	4	2	–	3.6	2.9	–	Regeneration
	35	–	–	–	0.6	0.2	–	Regeneration
	47	–	–	–	2.8	2.6	–	Regeneration
	46	3	2	–	1.7	1.2	–	Regeneration
	46	–	–	–	3.1	2.8	–	Regeneration
	25	8	1	–	2.5	2.4	–	Regeneration
	35	–	–	–	2.4	2.3	–	Regeneration
Windisch et al. (1999) (105)	25	–	–	–	2.9	2.5	–	Regeneration
Windisch et al. (2002) (106)	26	3	1	–	0.3	0.1	–	Regeneration
	12	3	2	–	1.5	1.0	–	Regeneration
	47	7	3	–	3.5	3.0	–	Regeneration
	35	6	3	–	2.8	2.5	–	Regeneration
Mean (in mm)	–	5.1 ± 2.5	–	3.1 ± 2.1	2.6 ± 1.6	1.7 ± 1.3	–	75% regeneration

*Eight of the 9 studies given in Table 12 provided appropriate histomorphometric data, which are presented here.

difference between 'experimentation' and 'research' is the importance of obtaining informed consent in the latter, something missing in the former. No animal models provide an anatomic and physiologic environment identical to that of the human, with variations between species, including anatomy and dimensions of the teeth and alveolar processes, amount and character of the gingiva, local physiologic environment, behavior, healing rate, as well as susceptibility to periodontitis. Therefore, translation of results from animal 'experiments' to the human situation is always problematic.

By contrast, it should be stressed that information obtained from human histological case reports should not be accepted without critical evaluation and appraisal, and the findings must always be interpreted with care. When evaluating the outcome of regenerative procedures in human biopsies, one has to bear in mind the fact that the harvesting procedure itself plays an important role in unbiased evaluation of the

Table 14. Histologic results of human periodontal infrabony defects treated using biological agents (8 studies)

Study: authors (year) (ref. no.)	No. of patients No. of defects	Defect type Defect depth	Healing time Factor type	Healing type	Histological results
Sculean et al. (1999) (80)	7 7	– –	6 months Enamel matrix derivative	Long junctional epithelium Connective tissue attachment Regeneration	Two notches at the most apical extent of the defect or calculus and at the level of the alveolar crest were placed. Root surface conditioning (ethylenediaminetetraacetic acid) was applied. New cellular cementum, new periodontal ligament and new bone were observed
Mellonig (1999) (48)	1 1	3 walls 5 mm	6 months Enamel matrix derivative	Regeneration	Notch at the most apical extent of calculus was placed. Root surface conditioning (ethylenediaminetetraacetic acid) was applied. New acellular cementum, new periodontal ligament and new bone were observed
Yukna & Mellonig (2000) (108)	8 10	1-3 walls 3–10 mm	6 months Enamel matrix derivative	Long junctional epithelium Connective tissue attachment Regeneration	Notches at the most apical extent of calculus, the defect base and the level of alveolar crest were placed. Root surface conditioning (citric acid) was applied. New cellular or accelular cementum, new periodontal ligament and new bone were observed
Sculean et al. (2000) (78)	2 2	1-2 walls –	6 months Enamel matrix derivative	Connective tissue attachment Regeneration	Notches at the most apical extent of defect and at the level of alveolar crest were placed. Root surface conditioning (ethylenediaminetetraacetic acid) was applied. New cellular or accelular cementum, new periodontal ligament and new bone were observed
Parodi et al. (2000) (64)	2 2	1-2 walls –	6–9 months Enamel matrix derivative	Long junctional epithelium	Notch at the most apical extent of defect was placed. Root surface conditioning (ethylenediaminetetraacetic acid) was applied. No regeneration was reported
Windisch et al. (2002) (106)	6 6	1-2 walls 3–8 mm	6 months Enamel matrix derivative	Long junctional epithelium Connective tissue attachment Regeneration	Notches at the most apical extent of defect or calculus and at the level of alveolar crest were placed. Root surface conditioning (ethylenediaminetetraacetic acid) was applied. New cementum, new periodontal ligament and new bone were observed
Sculean et al. (2003) (87)	10 10	– –	6 months Enamel matrix derivative	Long junctional epithelium Connective tissue attachment Regeneration	Notch at the most coronal level of gingival was placed. Root surface conditioning (ethylenediaminetetraacetic acid) was applied. Reparative cementum was reported
Majzoub et al. (2005) (44)	1 1	1-3 walls 8.5 mm	9 months Enamel matrix derivative	Regeneration	Notch at the most apical extent of calculus was placed. Orthophosphoric acid as root conditioner was applied. New cellular cementum, new periodontal ligament and new bone were observed

results. Obviously, with the exception of the dexterity and experience of the operating surgeon, anatomic (the presence of neighboring teeth and a narrow vs. a wide interproximal space), esthetic (anterior vs. posterior region) and prosthetic (need to extract vs. need to preserve part of the tooth) concerns dictate the spatial origin and volume (i.e. the biopsy represents all or part of the original intrabony defect, including the defect bone wall) and the amount of harvested periodontal tissue available for evaluation.

Table 15. Histomorphometric results of human periodontal infrabony defects treated using biological agents

Study*	Tooth notation	Defect depth (mm)	Osseous walls	Collagen fibers on tooth surface (mm)	New cementum (mm)	New bone (mm)	Junctional epithelium (mm)	Healing type
Sculean et al. (1999) (80)	37	–	–	4	2.3	0	–	Connective tissue attachment
	21	–	–	4	3.6	2.2	–	Regeneration
	21	–	–	2	1.6	0.5	–	Regeneration
	11	–	–	4	2.3	2.0	–	Regeneration
	16	–	–	2	1.5	0.5	–	Regeneration
	21	–	–	5	4.0	0	–	Connective tissue attachment
	11	–	–	2	0	0	–	Long junctional epithelium
	21 mesial	–	1-2	3	1.8	1.9	–	Regeneration
Sculean et al. (2000) (78)	11 mesial	–	1-2	5	4.8	0	–	New connective tissue attachment
	21 mesial	–	1-2	3	1.9	1.8	–	Regeneration
Parodi et al. (2000) (64)	–	–	–	–	0	0	–	Long junctional epithelium
	–	–	–	–	0	0	–	Long junctional epithelium
Windisch et al. (2002) (106)	11	3	1	2.7	1.5	0.2	–	Regeneration
	16	3	2	2.7	0	0	–	Long junctional epithelium
	37	6	2	2.7	2.3	0	–	Connective tissue attachment
	21	4	2	2.7	1.9	1.8	–	Regeneration
	21	3	2	2.7	1.6	0.5	–	Regeneration
	12	8	2	2.7	3.6	2.2	–	Regeneration
Sculean et al. (2003) (87)	–	–	–	3	0.5	0.3	–	Regeneration
	–	–	–	2	0	0	–	Long junctional epithelium
	–	–	–	2	0	0	–	Long junctional epithelium
	–	–	–	1	0	0	–	Long junctional epithelium
	–	–	–	–1	0	0	–	Long junctional epithelium
	–	–	–	2	0	0	–	Long junctional epithelium
	–	–	–	1	0	0	–	Long junctional epithelium
	–	–	–	2	0.4	0	–	Connective tissue attachment
	–	–	–	2	0.6	0	-	Connective tissue attachment

Table 15. (*Continued*)

Study*	Tooth notation	Defect depth (mm)	Osseous walls	Collagen fibers on tooth surface (mm)	New cementum (mm)	New bone (mm)	Junctional epithelium (mm)	Healing type
	–	–	–	3	0.2	0.2	–	Regeneration
Majzoub et al. (2005) (44)	46 distal	8.5	1-3	5	3.2	3.6	–	Regeneration
Mean (in mm)	–	4.8 ± 2.4	–	2.5 ± 1.6	1.3 ± 1.5	0.6 ± 1.0	–	45% regeneration

*Six of the 8 studies given in Table 14 provided appropriate histomorphometric data, which are presented here.

An important parameter that may introduce bias during the histological evaluation, and that needs consideration for a balanced interpretation of the healing outcome, and/or fair comparisons between treatment modalities, relates to the variation in morphological characteristics and dimensions of naturally developing periodontal defects. For instance, healing of deep three-walled intrabony lesions and deep dehiscence or gingival recession defects appears to be greatly influenced by vascular and cellular resources from the periodontal ligament, alveolar bone and gingiva circumscribing the defect (Fig. 8). In contrast, it is obvious that the distribution and contribution of tissue resources is dramatically altered and reduced in two- and one-walled intrabony defects, Class II and III furcations, and horizontal bone defects. Such an assumption is supported by observations made in an experimental study in dogs, using box-type intrabony defects, in which larger amounts of periodontal regeneration were observed as the number of bone walls increased (40).

Indeed, defect dimensions appear to be an important factor in predicting healing outcomes in the clinical situation, following both conventional surgical therapy, in which wide defects responded with less bone gain compared with narrow defects (101), as well as following periodontal regenerative surgery, in which better clinical outcomes (i.e. larger clinical attachment level gain and bone fill) are achieved in deep, narrow intrabony defects compared with wide, shallow defects (16). Regeneration in shallow defects may be antagonized to a greater extent by microbial factors or epithelial downgrowth than regeneration in deep defects. Nevertheless, it should also be recognized that the number of bone walls present preoperatively does not appear to influence the treatment outcomes, in terms of clinical measurements, following conventional (66) or regenerative (97, 98, 102, 103) periodontal surgery. Finally, irrespective of the type of periodontal defect, the amount and character of residual gingiva is critical for achieving passive flap adaptation and wound closure for primary intention healing. For example, in one clinical study it was observed that sites with a tissue thickness buccally of ≤1 mm presented statistically significant greater recession, 6 months after regenerative treatment, than sites with tissues thicker than 1 mm (1). Indeed, the need for full soft-tissue coverage of a wound to a level above the cemento–enamel junction following regenerative surgery is often overlooked, largely because of failure to regenerate those soft tissues at the same time as the hard-tissue regenerative surgery.

Taking into consideration the above limitations, when evaluating the outcomes of regenerative procedures in human biopsies, one has to bear in mind the fact that large amounts of regeneration may not necessarily arise as a result of the strong biologic potential of a technique or material but rather may reflect biases arising from the method of biopsy harvesting or histological evaluation. For example, sections representing areas close to the original bone walls of an intrabony defect would probably demonstrate a better regenerative response than more peripheral (i.e. furthest from the bone wall) sections (Fig. 9). This is exemplified by the observations of Paolantonio et al. (61), who took a core biopsy from the interdental area of a site treated 8 months earlier with a deproteinized bovine bone substitute and a collagen membrane and found that some periodontal regeneration had occurred in the vicinity of the pre-existing lingual alveolar plate only, and that the majority of the defect space was occupied by deproteinized bovine bone particles embedded in connective tissue.

In contrast, a lack of periodontal regeneration may similarly not necessarily indicate absence of the biological potential of a technique or material, but may

Table 16. Histologic results of human periodontal defects treated using combination techniques (20 studies)

Study: authors (year) (ref. no.)	No. of patients No. of defects	Defect type Defect depth	Healing time Biomaterials	Healing type	Histological results
Moskow & Lubarr (1983) (54)	1 1	Extensive periodontal defects	9 weeks Autograft + hydroxyapatite	Eventful healing	No notches were placed. Encapsulated hydroxyapatite particles in connective tissue and active osteogenesis only on autograft particles were observed
Bowers et al. (1985) (7)	2 6	1-3 osseous walls 5–10 mm	6 months Decalcified freeze-dried bone allograft+barrier	Long junctional epithelium Regeneration	Notches at the most apical extent of the defect and at the level of the alveolar crest were placed. Four defects showed signs of regeneration and two defects showed long junctional epithelium. Graft particles were incorporated in new bone or surrounded by connective tissue
Stahl & Froum (1991) (90)	2 4	1-2 osseous walls Deep intrabony defects	5–6 weeks Decalcified freeze-dried bone allograft + barrier	Long junctional epithelium Regeneration	Notches at the gingival margin and the most apical extent of calculus were placed. Three defects showed limited signs of cementogenesis, osteogenesis and functionally inserted fibers. One defect exhibited long junctional epithelium
Bowers et al. (1991) (4)	6 14	1-3 osseous walls 7 mm	6 months Decalcified freeze-dried bone allograft+osteogenin	Long junctional epithelium Regeneration	Two notches at the alveolar crest and at the most apical extent of calculus were placed. New cellular cementum, new periodontal ligament fibers and osteogenesis in 78% of the cases
Camello et al. (1998) (11)	2 2	2-3 osseous walls 7 mm	7–9 months Bovine derived xenograft +barrier	Regeneration	No notches were placed. Regeneration of periodontal tissues was reported. Graft particles were incorporated in new bone or were surrounded by connective tissue coronally. Collagen membrane was partially resorbed
Mellonig (2000) (50)	4 4	1-3 osseous walls 6–10 mm	4–6 months Bovine-derived xenograft +barrier	Long junctional epithelium Regeneration	Notch at the apical extent of calculus was placed. In three cases, new cellular cementum, new periodontal ligament fibers perpendicular or parallel to the tooth surface and osteogenesis with graft particles incorporated in new bone were reported. In one case, long junctional epithelium with encapsulated biomaterial particles was seen
Camello et al. (2001) (10)	3 3	3 osseous walls 6–7 mm	9 months Autograft+ bovine-derived xenograft + barrier	Regeneration	Notch at the base of the defect was placed. Root surface conditioning (tetracycline) was used. New cellular cementum, new periodontal ligament fibers and osteogenesis were reported. Graft particles were incorporated in new bone
Paolantonio et al. (2001) (61)	1 4	1 osseous wall Intrabony defects	8 months Bovine-derived xenograft +barrier	Regeneration	No notch was placed. New cementum, new periodontal ligament fibers and osteogenesis with graft particles surrounded by dense connective tissue or new bone were observed

Table 16. (*Continued*)

Study: authors (year) (ref. no.)	No. of patients No. of defects	Defect type Defect depth	Healing time Biomaterials	Healing type	Histological results
Nevins et al. (2003) (56)	2 2	1-3 osseous walls 4–7 mm	9 months Bovine-derived xenograft +barrier	Long junctional epithelium Regeneration	Notch at the most apical extent of calculus was placed. Root surface conditioning (tetracycline) was applied. New cellular cementum, new attachment apparatus and bone formation were obvious. Biomaterial particles were surrounded by dense connective tissue or new bone
Sculean et al. (2003) (84)	2 2	1-2 osseous walls	6 months Bovine-derived xenograft + enamel matrix derivative	Regeneration	Notches at the most apical extent of defect and at the level of alveolar crest were placed. New cellular-acellular cementum with inserting collagen fibers and bone formation around biomaterial particles were noticed
Yunka et al. (2002) (107)	1 1	1-2 osseous walls 4 mm	6 months Bovine-derived xenograft + PepGen-P-15	Regeneration	Notch at the most apical extent of calculus was placed. Root surface conditioning (citric acid) was applied. New cellular cementum, new periodontal ligament fibers and graft particles surrounded by new bone were observed
Nevins et al. (2003) (57)	6 6	Interproximal intrabony defects	9 months Decalcified freeze-dried bone allograft+ platelet-derived growth factor-BB	Long junctional epithelium Regeneration	Notch at the most apical extent of calculus was placed. Root surface conditioning (tetracycline) was applied. Four defects exhibited regeneration
Hartman et al. (2004) (30)	1 2	1 osseous wall 5–6 mm	6 months Bovine-derived xenograft +barrier	Long junctional epithelium	Notch at the apical extent of calculus was placed. Root surface conditioning (tetracycline) was used. Encapsulated biomaterial particles and long junctional epithelium were observed
Sculean et al. (2004) (83)	8 8	1-2 osseous walls 3–6 mm	6 months Bovine-derived xenograft +barrier	Long junctional epithelium Regeneration	Notch at the bottom of the defect was placed. New cellular cementum, new periodontal ligament fibers and graft particles surrounded by new bone were seen. No membrane remnants were reported. Long junctional epithelium was observed in one case
Sculean et al. (2005) (86)	3 3	1-2 osseous walls 4–6 mm	6 months Enamel matrix derivative+bioglass	Regeneration	Notch at the most apical extent of calculus was placed. Regeneration of the periodontal tissues was noticed. Graft particles were present surrounded by bone-like tissue, indicating ongoing mineralization
Ridgway et al. (2008) (68)	8 8	Deep intrabony defects	6 months Beta-tricalcium phosphate + platelet-derived growth factor	Long junctional epithelium Connective tissue attachment Regeneration	Notch at the most apical extent of calculus was placed. Regeneration of the periodontal tissues was noticed. Graft particles were encapsulated in connective tissue. Minimal osseogenesis in contact with graft was seen

Table 16. (*Continued*)

Study: authors (year) (ref. no.)	**No. of patients No. of defects**	**Defect type Defect depth**	**Healing time Biomaterials**	**Healing type**	**Histological results**
Sculean et al. (2008) (88)	1 1	1-2 osseous walls Deep intrabony defects	5 years Enamel matrix derivative + bovine-derived xenograft	Regeneration	No notch was placed. Graft particles were present incorporated into bone. New cementum and periodontal ligament were opposite to xenograft. Partial defect resolution was reported
Sculean et al. (2008) (77)	9 9	1-2 osseous walls 3–7 mm	9 months Enamel matrix derivative + bicalcium phosphate	Long junctional epithelium Connective tissue attachment Regeneration	Notch at the most apical extent of calculus or the defect bottom were placed. New acellular/cellular cementum, new inserting fibers and encapsulated graft particles were seen. Minimal new bone formation was reported
Stavropoulos et al. (2011) (99)	5 5	1-2 osseous walls ≥3 mm	6 months Bovine-derived xenograft +barrier (bovine pericardium)	Long junctional epithelium Connective tissue attachment	Two notches were placed: one at the postsurgical level of the gingival margin and one at the apical extent of root planing or calculus. Graft particles mostly embedded in connective tissue. An amorphous, mineralized, cellular tissue in contact with bone graft. No new bone formation. No resorption or ankylosis
Stavropoulos et al. (2011) (96)	10 10	1-2 osseous walls 6.7 mm	6 months Recombinant human growth/differentiation factor 5 + beta-tricalcium phosphate	Long junctional epithelium Regeneration	Two notches were placed: one at the postsurgical level of the gingival margin and one at the apical extent of root planing. Cementum, bone and periodontal ligament regeneration. No ankylosis, no root resorption. Beta-tricalcium phosphate particles were occasionally observed surrounded by connective tissue with signs of inflammation or foreign body reaction

Table 17. Histomorphometric results of human periodontal defects treated using combination techniques

Study*	Tooth notation	Defect depth (mm)	Osseous walls	Collagen fibers on tooth surface (mm)	New cementum (mm)	New bone (mm)	Junctional epithelium (mm)	Healing type
Bowers et al. (1985) (7)	13 mesial	8	1-2	0.0	0.0	0.0	–	Long junctional epithelium
	25 mesial	5	3	0.2	1.7	2.3	–	Regeneration
	35 mesial	9	2	1.5	0.2	1.2	–	Regeneration
	35 distal	7	2	0.0	0.0	2.7	–	Long junctional epithelium
	33 distal	10	1	0.8	1.0	2.3	–	Regeneration
	34 mesial	7	2	1.5	1.2	3.3	–	Regeneration
Bowers et al. (1991) (4)	*14x*	6.6	1-3	0.3	2.4	2.7	–	Regeneration
Camello et al. (1998) (11)	43 distal	7	3	–	7.0	5.3	–	Regeneration
	13 distal	7	2	–	7.6	4.5	–	Regeneration
Camello et al. (2001) (10)	44 distal	7	3	–	5.3	4.7	–	Regeneration
	44 distal	6	3	–	3.9	3.8	–	Regeneration
	44 distal	6	3	–	4.5	4.8	–	Regeneration
Nevins et al. (2003) (56)	23 mesial	7	1-2-3	–	2.2	3.0	–	Long junctional epithelium, regeneration
	25 mesial	4	1-2-3	–	1.9	3.1	–	Long junctional epithelium, regeneration
Sculean et al. (2003) (84)	15	–	1-2	–	2.2	0.5	–	Regeneration
	16	–	1-2	–	1.9	1.8	–	Regeneration
Hartman et al. (2004) (30)	12 mesial	6	1	0	0	0	1.9	Long junctional epithelium
	22 mesial	5	1	0	0	0	3.5	Long junctional epithelium

Table 17. (*Continued*)

Study*	Tooth notation	Defect depth (mm)	Osseous walls	Collagen fibers on tooth surface (mm)	New cementum (mm)	New bone (mm)	Junctional epithelium (mm)	Healing type
Sculean et al. (2004) (83)	26 mesial	6	1	–	2.5	2.8	–	Regeneration
	27 distal	5	1-2	–	2.9	3.0	–	Regeneration
	26 distal	4	1-2	–	3.8	3.8	–	Regeneration
	37 distal	6	2	–	2.8	3.4	–	Regeneration
	16 distal	4	1-2	–	0	0	–	Long junctional epithelium
	26 distal	4	2	–	2.4	1.6	–	Regeneration
	16 mesial	3	1-2	–	1.5	0.5	–	Regeneration
	16 distal	5	1-2	–	2.2	1.0	–	Regeneration
Sculean et al. (2005) (86)	36	4	1-2	–	1.6	1.4	–	Regeneration
	47	5	1-2	–	1.5	1.4	–	Regeneration
	46	6	1-2	–	2.9	2.8	–	Regeneration
Ridgway et al. (2008) (68)	12 mesial	–	–	0	0	0	3.5	Long junctional epithelium
	33 distal	–	–	1.3	1	4.9	2.6	Regeneration
	13 distal	–	–	1.6	1.8	0.8	2.1	Regeneration
	13 mesial	–	–	1.1	1.6	5.4	2.3	Regeneration
	36 distal	–	–	1	1.9	1.4	0.6	Regeneration
	35 mesial	–	–	0	0.9	1.3	1.2	Regeneration
	35 distal	–	–	0.7	0.9	1.6	1.3	Regeneration
	25 distal	–	–	0.3	0.7	0.4	-0.3	Regeneration
Sculean et al. (2008) (88)	11	7	–	–	0	0	–	Long junctional epithelium
	12	4	–	–	0.4	0	–	Connective tissue attachment
	32	3	–	–	1.3	0.2	–	Regeneration
	11	6	–	–	1.1	0.5	–	Regeneration
	26	5	–	–	0	0	–	Long junctional epithelium
	41	5	–	–	0.6	0	–	Connective tissue attachment
	21	6	–	–	0	0	–	Long junctional epithelium
	41	5	–	–	0.7	0	–	Connective tissue attachment
	25	5	–	–	2.1	0.7	–	Regeneration

Table 17. (*Continued*)

Study*	Tooth notation	Defect depth (mm)	Osseous walls	Collagen fibers on tooth surface (mm)	New cementum (mm)	New bone (mm)	Junctional epithelium (mm)	Healing type
Stavropoulos et al. (2011) (99)	–	≥3	1-2	–	0.1	0	–	Connective tissue attachment
	–	≥3	1-2	–	2.1	0	–	Connective tissue attachment
	–	≥3	1-2	–	0	0	–	Long junctional epithelium
	–	≥3	1-2	–	1.0	0	–	Connective tissue attachment
	–	≥3	1-2	–	0.9	0	–	Connective tissue attachment
Stavropoulos et al. (2011) (96)	35	7	1-2	–	2.98	3.79	2.84	Regeneration
	36	5	1-2	–	1.45	2.77	2.60	Regeneration
	11	4	1-2	–	2.91	2.53	3.48	Regeneration
	34	5	1-2	–	0.95	2.17	2.78	Regeneration
	12	4	1-2	–	4.99	5.02	2.05	Regeneration
	23	11	1-2	–	1.81	1.88	0	Regeneration
	21	7	1-2	–	1.80	1.03	3.94	Regeneration
	23	11	1-2	–	0	0	3.35	Long junctional epithelium
	21	4	1-2	Excluded				
	45	9	1-2	–	2.52	0.48	1.42	Regeneration
Mean (in mm)		6.1 ± 1.7	1-3	0.5 ± 0.5	1.9 ± 1.5	1.9 ± 1.6	2.1 ± 1.2	75% regeneration

14x is given in italics because there were 14 defects and the author of the study gave mean values and no single values per defect. Thus, the mean value contributed in a 14-fold manner to the final result.
*Thirteen of the 20 studies given in Table 16 provided appropriate histomorphometric data, which are presented here.

Table 18. Histomorphometric results of human periodontal defects treated following use of several biomaterials

	Defect depth (mm)	Osseous walls (*n*)	Collagen fibers on tooth surface (mm)	New cementum (mm)	New bone (mm)	Long junctional epithelium (mm)	Healing type	% Regeneration
Autografts	3.0 ± 2.1	1-3	2.1 ± 1.5	1.9 ± 1.5	1.9 ± 1.0	2.3 ± 1.0	Regeneration, long junctional epithelium	80
Allografts	6.0 ± 1.1	1-3	0.5 ± 0.9	1.3 ± 0.7	1.8 ± 0.9	1.4 ± 0.9	Regeneration, connective tissue attachment, long junctional epithelium, osseous repair	70
Xenografts	6.6 ± 0.9	1-3	1.4 ± 1.2	2.4 ± 1.9	2.3 ± 1.8	1.2 ± 0.6	Regeneration, connective tissue attachment, long junctional epithelium	70
Alloplastic	>4	1-3	0.1 ± 0.1	0.6 ± 0.8	0.4 ± 0.6	2.9 ± 1.4	Regeneration, connective tissue attachment, long junctional epithelium	34
Barriers	5.1 ± 2.5	1-3	3.1 ± 2.1	2.6 ± 1.6	1.7 ± 1.3	–	Regeneration, connective tissue attachment, long junctional epithelium	75
Biologic active factors	4.8 ± 2.4	1-3	2.5 ± 1.6	1.3 ± 1.5	0.6 ± 1.0	–	Regeneration, connective tissue attachment, long junctional epithelium	45
Combinations	6.1 ± 1.7	1-3	0.5 ± 0.5	1.9 ± 1.5	1.9 ± 1.6	2.1 ± 1.2	Regeneration, connective tissue attachment, long junctional epithelium	75

Fig. 2. Healing of an intrabony defect following grafting with an alloplastic material (e.g. Bioglass). The healing is characterized by formation of a long junctional epithelium (between the two red arrowheads). Formation of a new cementum is limited to the most apical part of the defect (the green arrow indicates the most coronal extent of new cementum).

Fig. 3. Periodontal regeneration following treatment with guided tissue regeneration. Formation of new cementum (green arrowheads) and new bone (blue stars) are clearly visible coronally to the most apical part of the defect indicated by the apical notch.

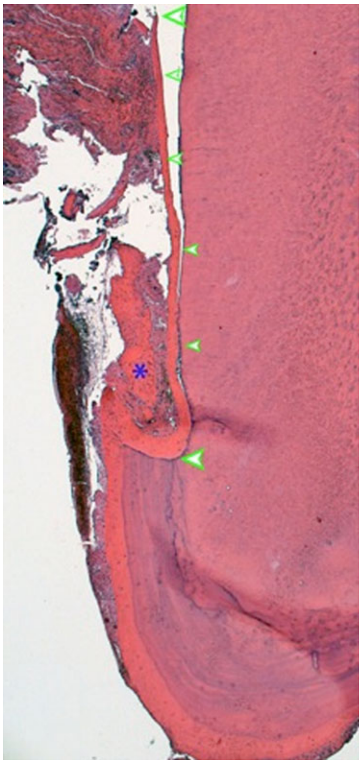

Fig. 4. **Healing following treatment with enamel matrix derivative, demonstrating periodontal regeneration. New cementum (green arrowheads) and new bone (blue star) have formed coronally to the most apical part of the defect indicated by the notch.**

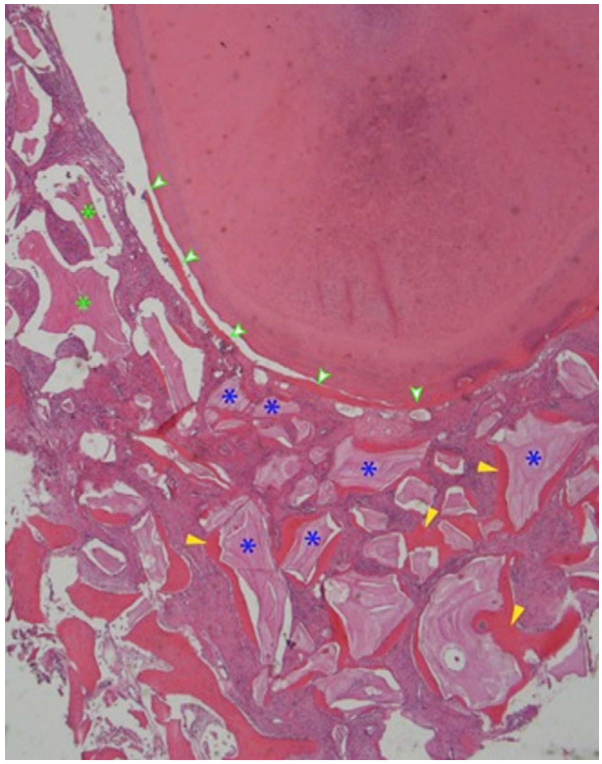

Fig. 5. **Healing following treatment with a bovine-derived xenogeneic bone grafts and guided tissue regeneration. New cementum (green arrowheads) and remnants of the graft material (blue and green stars) can be distinguished. New bone (yellow arrowheads) has formed around some of the graft particles, whereas some other particles (green stars) are surrounded by connective tissue.**

derive from technical problems arising during harvesting and/or processing of the biopsy. For example, because of the anatomic nuances discussed earlier, or perhaps for accidental reasons, the biopsy may not comprise a complete or representative portion (i.e. root, periodontal ligament or bone) of the original defect or the newly regenerated tissues. Moreover, the teeth included in such analyses are usually deemed to have a hopeless prognosis, amongst other reasons, because of advanced periodontitis, and thus the regenerative potential at such sites may be extremely limited and/or exhausted. Moreover, teeth showing an especially favorable outcome following periodontal regenerative procedures are frequently not harvested for obvious ethical reasons. Thus, limited, or lack of, periodontal regeneration may simply be a result of the evaluation of 'hopeless' cases. Nevertheless, in several of the studies discussed in the present review, the teeth

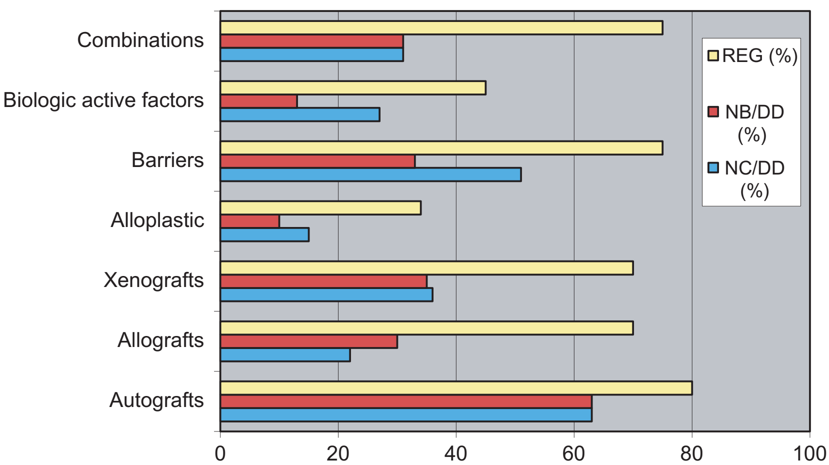

Fig. 6. **Diagram illustrating percentages of treated defects with histological evidence of regeneration (REG %), and amount of cementum (NC/DD%) and bone (NB/DD%) formation presented as percentage of defect depth.**

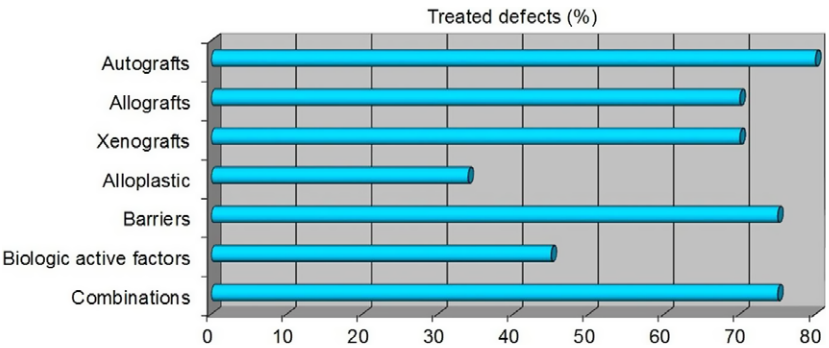

Fig. 7. Diagram showing the percentage of treated defects (%) with histologic evidence of regeneration among the biomaterials group.

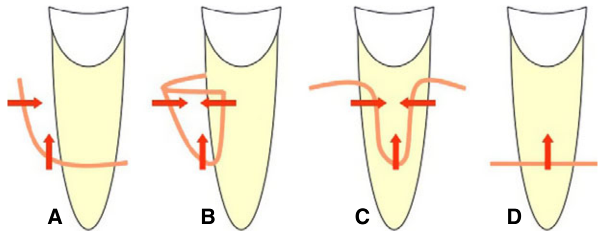

Fig. 8. Distribution and contribution of tissue resources (red arrows) for regeneration is dramatically different depending upon the morphology of the remaining bone walls (pink line). For example, three-walled defects (B) and narrow dehiscence defects (C) possess greater potential for regeneration than one-walled defects (A) or horizontal defects (D).

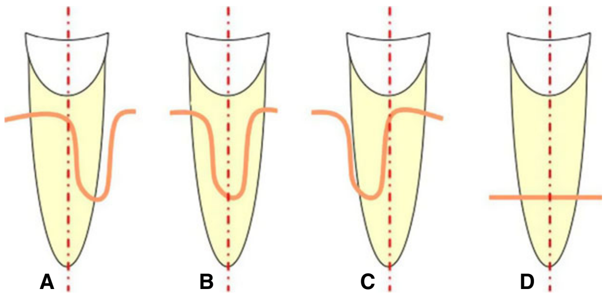

Fig. 9. Sections (dotted red line) representing areas close to the original bone walls (pink line) of the inrabony defect (A and C), would probably demonstrate a better regenerative response than those representing the mid-portion of the defect (B). Such a problem does not exist with horizontal defects (D).

included, despite advanced disease, were described as having a reasonable potential for regeneration owing to the presence of a deep intrabony component and the one- or two-bone wall (or combination thereof) configuration.

In summary, it is important to recognize that, despite the limitations and challenges in both technical and ethical histological studies of periodontal regeneration in humans, there is strong proof-of-principle evidence for partially successful regenerative outcomes, although these remain inconsistent and somewhat unpredictable.

Conclusion

The histological evaluation of human specimens in the study of periodontal regeneration is fraught with challenges of a technical nature, as are those associated with data interpretation. Nevertheless, human histology provides important information on the biological potential of various regenerative protocols and biomaterials, which is vital to advancing this field of research and clinical practice. As the outcomes of such studies can be influenced by various factors outlined in this review, the information obtained from such studies needs to be carefully interpreted in the light of the evidence available from pre-clinical and clinical studies, designed to serve as 'proof-of-principle' studies.

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