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Regeneration of periodontal tissues: combinations of barrier membranes and grafting materials – biological foundation and preclinical evidence

A systematic review

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Abstract

Background: Regenerative periodontal therapy aims to predictably restore the tooth's supporting periodontal tissues and should result in formation of a new connective tissue attachment (i.e. new cementum with inserting periodontal ligament fibres) and new alveolar bone. Histologic evidence from preclinical models has demonstrated periodontal regeneration following treatment with barrier membranes, various types of grafting materials or a combination thereof. However, it is still not clear to what extent a combination of barrier membranes and grafting materials may additionally enhance the regeneration process compared with barrier membranes alone, grafting materials alone or open flap debridement.

Objectives: To review with a systematic approach all preclinical (i.e. animal) studies presenting histologic support for periodontal regeneration using the combination of barrier membranes and grafting materials.

Material and Methods: Based on a focused question, an electronic and manual search was conducted for animal studies presenting histological data for the effect of the combined use of barrier membranes and grafting materials on the treatment of periodontal defects. A systematic approach was followed by two independent reviewers including eligibility criteria for study inclusion, outcome measures determination, screening method, data extraction, data synthesis and drawing of conclusions.

Results: Ten papers completely fulfilling the inclusion criteria were selected. All relevant data from the selected papers were extracted and recorded in separate tables according to the types of periodontal defects treated (i.e. supra-alveolar defects, intrabony defects, furcation defects and fenestration defects) with the combination of barrier membranes and grafting materials. Most studies have demonstrated periodontal regeneration following the combination approach. Most studies demonstrated superior histologic healing following the combination of barrier membranes and grafting materials than following open flap debridement. Histologically superior healing following the combination of barrier membranes and grafting materials when compared with barrier membranes alone or grafting materials alone were only obtained in non-contained two wall intrabony and supraalveolar defects.

Conclusion: Within its limits the present analysis indicates that:

(a) The combination of barrier membranes and grafting materials may result in histological evidence of periodontal regeneration, predominantly bone repair.

(b) No additional benefits of combination treatments were detected in models of three wall intrabony, Class II furcation or fenestration defects.
(c) In supra-alveolar and two wall intrabony (missing buccal wall) defect models of periodontal regeneration, the additional use of a grafting material gave superior histological results of bone repair to barrier membranes alone.
(d) In one study using a supra-alveolar model, combined graft and barrier membrane gave a superior result to graft alone.

Key words: animal models; barrier membranes; grafting materials; histology; preclinical models; regenerative periodontal therapy

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Conflict of interest and source of funding statement

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Regenerative periodontal therapy aims to predictably restore the tooth's supporting periodontal tissues (i.e. new periodontal ligament, new cementum with inserting periodontal ligament fibres and new bone) that have been lost due to periodontal disease or dental trauma (Polimeni et al. 2006). Nonsurgical and conventional surgical periodontal therapy may usually result in successful clinical outcomes such as probing depth reduction and gain of clinical attachment. Histologically, however, the healing following these treatment approaches was characterized by a long junctional epithelium along the treated root surfaces and no formation of cementum with an associated periodontal ligament (Caton & Greenstein 1993). Although in some cases a conventional periodontal therapy may result in bone regrowth, histological studies have demonstrated that an epithelial lining was often interposed between the root surface and the newly formed bone (Caton & Greenstein 1993).

One widely used technique in reconstructive periodontal surgery is the use of various types of grafting materials to fill the periodontal defects (Brunsvold & Mellonig 1993). Data from systematic reviews have suggested that the implantation of grafting materials may indeed result in superior clinical outcomes in terms of probing depth reduction and clinical attachment gain compared with open flap debridement (Trombelli et al. 2002, Reynolds et al. 2003).

The biologic rationale for using grafting materials is based on the assumption that these materials may facilitate formation of alveolar bone, periodontal ligament and root cementum through one of the following mechanisms:

1. Contain bone-forming cells (osteoneogenesis).
2. Serve as scaffold for bone formation (osteoconduction).
3. Contain bone-inducing substances (osteoinduction) (Brunsvold & Mellonig 1993).

On the other hand, recent evidence from preclinical studies using the critical-size supraalveolar defect has suggested that the main mechanism by which certain grafting materials appear to support periodontal and bone regeneration when used in combination with guided tissue regeneration (GTR) is rather by space provision than by the osteoconductive properties of the grafting material (Polimeni et al. 2004).

Histological findings from a series of animal experiments have demonstrated the important role of periodontal ligament cells in creating a new connective tissue attachment (i.e. new periodontal ligament and new cementum with inserting periodontal fibres) (Löe & Waerhaug 1961, Melcher 1976, Karring et al. 1980, 1984, 1985, Nyman et al. 1980, Lindhe et al. 1984, Isidor et al. 1985). These studies have also indicated that the potential of periodontal ligament cells for forming a new connective tissue attachment could only develop if the epithelium and the gingival connective tissue were prevented from occupying the wound area adjacent to the root. Other observations from preclinical studies evaluating treatment of experimentally-created intrabony defects via implantation of different types of grafting materials or flap surgery have shown a healing characterized by formation of a long junctional epithelium until the most apical part of the instrumented root surface and no periodontal regeneration (Caton et al. 1980). The placement of a barrier membrane (i.e. GTR) over the denuded root surfaces and the debrided periodontal defect has been shown to exclude epithelial downgrowth and allow periodontal ligament and alveolar bone cells to repopulate the isolated space selectively (Nyman et al. 1982, Gottlow et al. 1984). On the other hand, the amount of regenerated tissues is limited by the available space under the membrane, thus suggesting that the space-provision and wound-stabilizing effects of the barrier membrane may substantially influence the healing process (Wikesjö & Nilvéus 1990, Haney et al. 1993). One way in which clinicians attempt to overcome the problems related to a collapse of the barrier membrane is the use of a combination of barrier membranes and grafting materials. However, the data from controlled clinical studies do not seem to clearly indicate improved clinical outcomes in terms of probing depth reduction, clinical attachment level (CAL) gain and defect fill when the combination of grafting materials and GTR is compared with GTR alone or grafting materials alone (Blumenthal & Steinberg1990, Chen et al. 1995, Mellado et al. 1995, Gouldin et al. 1996, Trejo et al. 2000, Paolantonio 2002, Murphy & Gunsolley 2003, Nygaard-Østby et al. 2008).

From a biological point of view, the use of any type of regeneration method needs to be supported by histologic evidence from preclinical (i.e. animal) experiments, thus supporting its rationale (Polimeni et al. 2006).

The aim of this study is to review with a systematic approach all preclinical (i.e. animal) studies presenting histologic support for periodontal regeneration using combination therapy of barrier membranes and grafting materials.

Material and Methods

Development of a protocol

A protocol was developed before commencing the review and covered all aspects of the systematic review methodology (Needleman 2002). These aspects were: definition of a focused question, search strategy, eligibility criteria for study inclusion, outcome measures determination, screening methods and data extraction, data synthesis and drawing of conclusions.

Definition of a focused question

The question addressed was the following: what is the effect of the combination of barrier membranes and grafting materials on the healing of periodontal defects in animal studies?

Search strategy

Literature search, for articles published up to and including November 2007, was performed using MEDLINE database. Combinations of searching terms were used to identify the proper studies (Table 1).

Also the reference lists of review articles were scanned. In addition, the reference lists of articles selected for inclusion in the present review were screened. Finally, a hand searching including the *Journal of Dental Research, Journal of Clinical Periodontology, Journal of Periodontology, Journal of Periodontal Research* and *The International Journal of Periodontics and Restorative Dentistry* was performed.

Table 1. Searching terms were used to identify the proper studies

Searching 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 ''furcation defect'' OR ''furcation invasion'' OR ''furcation involvement'')
AND
(''guided tissue regeneration'' OR ''GTR'' OR ''membrane'' OR ''barrier'' OR ''periodontal regeneration'')
AND
(''bone graft'' OR ''bone replacement graft'' OR ''bone substitute'' OR ''osseous graft*''OR ''bone transplantation'' OR ''bone regeneration'' OR ''bone matrix''OR ''autologous bone graft*'' 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 ''synthetic graft'' OR ''polymer'' OR ''ceramic graft*'' OR ''bioactive ceramic graft*'' OR ''bioglass*'' OR ''bioglass graft*'' OR ''bioceramic*'' OR ''hydroxyapatites'' 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'')
AND
(''animal model'' OR ''animal study'' OR ''preclinical study'' OR ''dog study'' OR ''monkey study'' OR ''rabbit study'' OR ''rat study'')

Criteria selection for study inclusion

The selection was limited to animal studies evaluating the combined use of barrier membranes and grafting materials for the treatment of periodontal defects. Any type of barrier membrane and grafting material was considered. Studies involving biomimetic substances/growth factors were excluded. A time limitation of 4 weeks regarding postoperative evaluation period was applied. All the studies with histological, radiographic or clinical outcome parameters evaluating soft tissue and/or bone healing were included. Only studies published in English were analyzed.

Outcome measures determination

Primary outcomes of interest were:

- Formation of new periodontal tissues based on histologic evaluation: periodontal ligament, cementum and bone formation as a linear measure (mm) or as a percentage of the instrumented root length (%).
- Change in defect size based on measurement after radiographic examination or re-entry surgical procedure.
- Change in CAL or change in probing pocket depth in millimetres after clinical evaluation.

When specific data were reported, defect resolution and defect fill were calculated.

Also, secondary outcomes were evaluated as:

- adverse effects related to the additional use of barrier membrane/grafting materials
- post-operative complications

Screening methods and data extraction

The titles and abstracts were independently screened by two reviewers (A. S. and D. N.). Titles and abstract screening was based on the following questions.

- Was the study conducted on animals presenting periodontal defects requiring combined use of barrier membranes and grafting materials?
- Was treatment outcome evaluated histologically, radiographically or clinically?
- Was the post-surgery evaluation period of at least 4 weeks?

The full text of an article was obtained whether the response was ''yes'' or ''uncertain'' to the screening questions. Disagreement regarding inclusion was resolved by discussion. The level of agreement between reviewers was determined by κ scores. Authors of the trials were contacted to provide missing data where possible.

Data were extracted based on the general characteristics (authors and year of publication), study characteristics (type and number of animals; tooth type; defect characteristics; intervention strategies; evaluation period; outcome measures; complications), methodological characteristics (study design and methodological quality) and conclusions.

Data analysis

There was a substantial heterogeneity in the data collected regarding animal type, study design, used materials, evaluation methods, outcome measures and observation periods. Additionally, most of the studies had either a split-mouth or a mixed design without providing data about intra-individual variance. Weighted mean differences could not be calculated, and consequently, it was impossible to conduct a quantitative data synthesis leading to a meta-analysis. Therefore, the mean and SD, the 95% confidence intervals (CI) and the statistical significance were found and extracted from the reviewed articles. These data were summarized in separate tables based on the different type of periodontal defects treated with the combined application of barrier membranes and grafting materials. The defects were classified as follows:

supra-alveolar defects, intrabony defects, furcation defects and fenestration defects (Tables 4–7). Furthermore, the results of the studies that used the same methods of evaluation and similar outcome measurements were combined and the data are presented in a statistical graph (Fig. 2).

Table 2. Paper excluded for clearly not fulfilling inclusion criteria after full-text screening

Study	Reason for exclusion
Artzi et al. (2003a)	No periodontal defects (bony defects)
Artzi et al. (2003b)	No periodontal defects (bony defects)
Kohles et al. (2000)	No periodontal defects (bony defects)
Moon et al. (1996)	No outcome measurements (descriptive histology)
Ellender et al. (1992)	No outcome measurements (descriptive histology)

Results

Data extraction after literature searching

The MEDLINE literature search resulted in 632 hits (Fig. 1). After the first selection step, based on the title of the collected studies, 70 articles were included for further analysis (inter-reader agreement $k = 0.96$). The hand searching revealed 45 articles, which were added to this step. The second step, based on abstract screening, resulted in 15 studies (inter-reader agreement $k = 0.87$). From these studies, 10 papers completely fulfilling the inclusion criteria were selected (inter-reader agreement $k = 1$). Five studies were excluded at the last step and the reasons for exclusion are presented in Table 2. The papers that remained after the third selection ($n = 10$) are presented in Table 3.

Effect of the combined use of GTR and bone grafts on supra-alveolar defects

Three studies related to the periodontal regeneration of a supra-alveolar defect model were identified (Wikesjö et al. 2003, Polimeni et al. 2004, Koo et al. 2005). The results of the studies are presented in Table 4.

In all three studies, the supra-alveolar defects were created by removing surrounding bone extending approximately 6 mm apical to the cemento-enamel junction of premolar teeth in a canine model (i.e. Beagle dogs). Cementum was fully removed by hand and rotating instruments from the exposed root surfaces. Non-resorbable ePTFE membrane (GTR) and coral-derived calcium carbonate graft were evaluated as treatment approaches for periodontal regeneration. Two studies have tested the combination (GTR+CI) *versus* GTR alone (Wikesjö et al. 2003, Polimeni et al. 2004) whereas the other study tested the combination (GTR+CI) *versus* CI alone (Koo et al. 2005). After a 4-week healing period, clinical observation at sacrifice revealed uneventful healing in all cases. Membrane exposure was not observed. Only one animal, which received the combined approach, exhibited an extensive inflammatory reaction during histological observation and minimal bone regeneration (Wikesjö et al. 2003). Histomorphometrical parameters such as extension of long junctional epithelium and connective tissue repair, cementum and bone formation, presence of ankylosis and root resorption were assessed. Limited cementum regeneration was observed in all three studies. Root resorption and ankylosis appeared to be insignificant. A combination of woven and lamellar new bone was observed. Bone formation was more extensive in the groups receiving the combined approach. Particles of the used biomaterial were surrounded by bone and soft tissue and appeared with scalloped borders, suggesting active resorption. In all groups, connective tissue intervened between new bone and instrumented root surface. Additionally, in the groups using either the combination approach or the barrier alone, long junctional epithelium was absent on the instrumented roots, whereas in the graft group long junctional epithelium (approximately 10% of the root length) was also observed.

Effect of the combined use of GTR and bone grafts on furcation defects

The combination barrier membranes and grafting materials for the treatment of furcation defects was evaluated in three canine studies (Caffesse et al. 1993, Lekovic & Kenney 1993, Deliberador et al. 2006). The results of these studies are presented in Table 5.

Class II furcation defects were either created surgically (Deliberador et al. 2006) or they developed naturally due to periodontal disease (Caffesse et al. 1993, Lekovic & Kenney 1993).

Deliberador et al. (2006) evaluated in mongrel dogs the following treatments: control, autogenous bone (AB) and combination AB+calcium sulphate paste as barrier (CS). The healing period was 3 months. Most specimens failed to show complete bone fill of the furcation. New bone formation was moderate and restricted from the notch area to the mid portion of the defect. Fibrous connective tissue occupied the remaining portion of the defect, whereas epithelial migration extended to the coronal portion of the furcation. An inflammatory infiltrate was also present in all groups. The amount of periodontal regeneration (periodontal ligament fibres inserting into new cementum and new bone) in the three groups was approximately 50% of the root length without differences between the groups. The root surfaces were partially covered with new cementum of varying thickness. Newly formed periodontal ligament was present and it was highly vascularized. The fibres were oriented perpendicular or oblique to the root surface and were inserting into the new cementum and new bone. Areas of dentoalveolar ankylosis were also present in some sections, but no active root resorption was observed.

Lekovic & Kenney (1993) evaluated in mongrel dogs four different barriers in conjunction with porous tricalcium phosphate (β-TCP) granules. Histomorphometrical measurements were obtained 6 months postoperatively. All animals had an uneventful healing. The histometric analysis assessed the presence of root resorption and ankylosis, formation of new cementum and bone in the furcation area, extent of epithelial migration, direction of periodontal fibres and degree of tissue inflammation. The four combined approaches demonstrated similar histomorphometric measurements regarding formation of new cementum, new bone and epithelial downgrowth. On the other hand, the control group (no material) revealed significantly less cementum and bone formation. The inflammatory reaction was significantly increased in two groups (polycarbonate barrier+β-TCP, polycaprolactone barrier+β-TCP) compared with the other groups (ePTFE barrier+β-TCP, silicone barrier+β-TCP or control), questioning the biocompatibility of polycarbonate and polycaprolactone materials. The β-TCP particles were partially resorbed and

Table 3. Data extraction from the included studies ($n = 10$)

No.	Study	Year	Model	Design	Animals	Defect type	Tooth	Treatment	n	Healing	Evaluation
1	Koo et al.	2005	Dog	Parallel	12	Supra-alveolar	Premolar	CaC	5	4 weeks	histomorphometry
								CaC+ePTFE	7		LJE, CT, NC, NB (distance in mm)
2	Polimeni et al.	2004	Dog	Split-mouth	4	Supra-alveolar	Premolar	ePTFE	4	4 weeks	histomorphometry
								CaC+ePTFE	4		NB (distance in mm)
3	Wikesjö et al.	2003	Dog	Split-mouth	5	Supra-alveolar	Premolar	ePTFE	5	4 weeks	histomorphometry
								CaC+ePTFE	5		LJE, CT, NC, NB (distance in mm)
4	Deliberador et al.	2006	Dog	Split-mouth	6	Furcation	Premolar	Control	6	3 months	histomorphometry
						Class II		AB	6		LJE, CT, NC, NB (% of root length)
								AB+CaSB	6		
5	Caffesse et al.	1993	Dog	Split-mouth	4	Furcation	Premolar	ePTFE	16	4 months	histomorphometry
						Class II	Molar	ePTFE+DFDB	16		LJE, CT, NC, NB (distance in mm)
6	Lekovic & Kenney	1993	Dog	Split-mouth	6	Furcation	Molar	Control	6	6 months	histomorphometry
						Class II		ePTFE+CaP	6		NC, NB (distance in mm)
								Silrub+CaP	6		
								PC+CaP	6		
								PCL+CaP	6		
7	Blumenthal et al.	2003	Baboons	Split-mouth	9	Intrabony		CollB	9	6 months	clinical parameters
						Three walls		DFDB	9		histomorphometry
						Two walls		DFDB-glyc	9		Periodontal regeneration (distance in mm)
								CollB+DFDB	9		
8	Kim et al.	1998	Dog	Split-mouth	4	Angular	Premolar	Control	4	8 weeks	histomorphometry
						Three walls		CaSB	4		LJE, CT, NC, NB (distance in mm)
								DFBM	4		
								CaSB+DFBM	4		
9	Tal et al.	2005	Dog	Mixed	12	Fenestration	Canine	Control	3	3 months	histomorphometry
								CollB	3		CT, NC, NB (% of defect height)
								BBM	3		
								BBMC	4		
								CollB+BBM	3		
								CollB+BBMC	4		
10	Caplanis et al.	1998	Dog	Split-mouth	6	Fenestration	Canine	ePTFE	6	4 weeks	histomorphometry
								ePTFE+DFDB	6		NC, NB (distance in mm)

Biomaterials: CaC, coral-derived calcium carbonate graft; ePTFE, expanded polytetrafluoroethylene barrier; AB, autogenous bone graft; CaSB, calcium sulfate barrier; DFDB, demineralized freeze-dried bone graft; CaP, calcium phosphate graft; Silrub, silicone rubber barrier; PC, polycarbonate barrier; PCL, polycaprolactone barrier; CollB, collagen barrier; DFDB-glyc, demineralized freeze-dried bone graft with glycoprotein; BBM, bovine bone mineral graft; BBMC, bovine bone mineral graft with collagen.
Histological parameters: LJE, long junctional epithelium; CT, connective tissue; NC, new cementum, NB, new bone.

Table 4. Data extraction in the supra-alveolar defect model

Study	Design	Parameters	Distance on the root surface (mm) treatment groups control m (95% CI)	graft m (95% CI)	barrier m (95% CI)	combination m (95% CI)	p value
Koo et al. (2005)	Graft *versus* combination	Junctional epithelium		0.4 (0.1–0.8)		0.0	0.01
		Connective tissue		4.3 (3.6–5.1)		4.6 (4.1–5.2)	0.44
		Cementum		< 0.1		<0.1	0.45
		Bone		1.2 (0.1–2.3)		2.3 (1.8–2.9)	0.04
Polimeni et al. (2004)	Barrier *versus* combination	Bone			1.3 (0.6–2.1)	2.1 (1.4–2.9)	<0.01
Wikesjö et al. (2003)	Barrier *versus* combination	Connective tissue			4.6 (4.0–5.2)	4.5 (3.9–5.1)	0.78
		Cementum			0	0	–
		Bone			1.2 (0.5–2.0)	1.9 (1.2–2.6)	0.04

m (95% CI) = mean and 95% confidence interval.

Table 5. Data extraction in the furcation defect model

Study	Design	Parameters	Treatment groups control m (95% CI)	graft m (95% CI)	barrier m (95% CI)	combination m (95% CI)	p value
			Percentage of root length (%)				
Deliberador et al. (2006)	Graft *versus* combination Control included	Junctional epithelium	5 (− 4–14)	6 (− 3–14)		10 (0–20)	0.29
		Connective tissue	35 (16–54)	33 (15–51)		27 (17–37)	0.59
		Cementum	57 (33–81)	59 (40–78)		62 (45–79)	0.85
		Bone	62 (53–71)	65 (48- 82)		60 (38–82)	0.69
			Distance on the root surface (mm)				
Caffesse et al. (1993)	Barrier *versus* combination	Junctional epithelium			1.6 (0.7–2.5)	1.9 (0.8–3.0)	>0.05
		Connective tissue			1.5 (0.7–2.3)	1.4 (0.4–2.4)	>0.05
		Cementum			1.4 (0.6–2.2)	1.0 (0.1–2.0)	>0.05
		Bone			1.0 (0.3–1.7)	1.1 (0.0–2.3)	>0.05
			Distance on the root surface (mm)				
Lekovic & Kenney (1993)	4 different combinations Control included	Cementum	0.3 (0.0–0.5)			2.3 (0.7–3.9)	<0.05
						2.5 (0.9–4.1)	<0.01
						2.6 (1.0–4.2)	<0.01
						2.4 (0.7–4.0)	<0.05
		Bone	0.3 (0.0–0.6)			1.7 (0.6–2.9)	<0.01
						2.0 (1.0–3.0)	<0.01
						2.0 (1.0–3.0)	<0.01
						1.8 (0.9–2.8)	<0.01

m (95% CI) = mean and 95% confidence interval.

surrounded by new bone. There was no evidence of root resorption or ankylosis.

Caffesse et al. (1993) compared in Beagle dogs the use of ePTFE (GTR) barrier *versus* the combination of GTR+demineralized freeze-dried cortical bone grafts (DFDBA) (GTR+DFDBA). The animals were sacrificed at 4 months following surgery. The healing pattern was identical for both treatment groups (barrier *versus* combination). New periodontal fibres were evident including cementum deposition and bone regeneration coronal to the notch. These fibres were oriented perpendicular to the root surface and inserted into the new bone and new cellular cementum. Approximately 70% of the defect area was occupied by bone and 30% by connective tissue. Epithelial migration was minimal. No signs of inflammatory reaction or root resorption were observed. However, some sections in the graft group demonstrated signs of ankylosis. Both treatments resulted in periodontal regeneration but adjunctive bone grafting did not appear to enhance the regeneration process.

Table 6. Data extraction in the intrabony angular defect model

Study	Design	Parameters	Distance on the root surface (mm) treatment groups control m (95% CI)	graft m (95% CI)	barrier m (95% CI)	combination m (95% CI)	p value
Blumenthal et al. (2003)	All treatment modalities except control	Periodontal regeneration	Non-contained defects:	1.7 (0.9–2.5)	1.3 (0.8–1.8)	1.9 (1.6–2.2)	Not mentioned
			Contained defects:	1.9 (1.4–2.3)	2.0 (1.3–2.6)	2.2 (1.7–2.7)	
Kim et al. (1998)	All treatment Modalities	Junctional epithelium	0.9 (0.1–1.7)	0.5 (0.2–0.8)	1.0 (0.2–1.8)	0.6 (0.1–1.1)	>0.05
		Connective tissue	1.6 (0.8–2.4)*	0.4 (0.0–0.9)	0.5 (0.2–0.8)	0.4 (0.0–0.9)	*<0.05
		Cementum	1.6 (1.1–2.1)*	3.1 (2.5–3.7)	2.5 (1.9–3.1)	3.0 (2.5–3.5)	*<0.05
		Bone	0.7 (0.5–0.9)*	2.7 (2.2–3.2)	1.8 (1.0–2.6)	2.7 (2.1–3.3)	*<0.05

m (95%CI) = mean and 95% confidence interval.

Table 7. Data extraction in the angular fenestration defect model

Study	Design	Parameters	Treatment groups control m (95% CI)	graft m (95% CI)	barrier m (95% CI)	combination m (95% CI)	p value
			Percentage of defect height (%)				
Tal et al. (2005)	All treatment modalities	Connective tissue	83	69/72	26	33/37	SD, SE or p values are not provided
		Cementum	17	28/32	84	63/67	
		Bone	13	39/44	78	81/87	
			Distance on the root surface (mm)				
Caplanis et al. (1998)	Barrier *versus* combination	Cementum			1.6 (0.0–3.4)	2.0 (0.6–3.4)	0.39
		Bone			1.5 (0.7–2.3)	0.8 (0.2–1.4)	0.14

m (95% CI) = mean and 95% confidence interval.

Effect of the combined use of GTR and bone grafts on intrabony defects

Two studies providing data on the effect of the use of GTR and graft material in intrabony defects were identified (Kim et al. 1998, Blumenthal et al. 2003). The results of these two studies are summarized in Table 6.

In the first study, the authors created surgically three walled contained and two walled non-contained intrabony defects in nine adult baboons (Blumenthal et al. 2003). Four treatment modalities were tested: (a) GTR alone (i.e. collagen membrane), (b) DFDBA alone, (c) DFDBA/glycoprotein sponge and (d) combination of DFDBA+ collagen membrane. Six months post-operatively, clinical and histological parameters were evaluated. For the contained defects, no significant clinical or histological differences were found among the groups. In non-contained defects, the combined therapy (i.e. graft+GTR) resulted in clinically and histologically superior results compared with the single therapies. Unfortunately, no p values were provided in the study. Periodontal regeneration ranged from 37% to 48% of the total defect height among the groups. Connective tissue adhesion was also observed. Epithelial migration was limited and encapsulated graft particles were evident. No evidence of inflammation was noticed.

The second study has evaluated in four mongrel dogs the following treatment modalities: (a) control (flap alone, (b) CS, (c) graft alone (DFDBA) and (d) DFDBA+CS (Kim et al. 1998). Three-wall intrabony defects were surgically created and subsequently treated with one of the four treatment options. Clinical healing was uneventful. Block sections of the defects were collected at sacrifice 8 weeks post-operatively and processed for histometric analysis. Bone graft particles were observed clearly without evidence of residual barrier particles. Ankylosis was found in three animals. Connective tissue adhesion (connective tissue contact to the root without apparent cementum formation), cementum regeneration and new bone formation were calculated. None of the sections including DFDBA provided evidence of bone metabolic activity. The use of DFDBA and CS barrier, alone or in combination, resulted in significantly improved regeneration of alveolar bone and cellular cementum in this preclinical model compared with the control group. However, the barrier alone resulted in less bone formation compared with the groups treated with grafts alone or in the combination groups. The authors speculated that space-providing properties of the materials supported the observed regeneration.

Effect of the combined use of GTR and bone grafts on fenestration defects

Two studies have evaluated in fenestration defects the effect of a combination barrier membranes and grafting materials (Caplanis et al. 1998, Tal et al. 2005). In both studies, circular periodontal defects were created by removing alveolar bone, PDL and cementum at the mid-buccal aspect of canines.

Caplanis et al. (1998) evaluated the effect of barrier (ePTFE) against the combination of demineralized bone matrix (DBM)+ePTFE on fenestration defects in a dog model. The six dogs were sacrificed 4 weeks post-surgically in order to assess formation of new cementum and new bone. Clinical healing was uneventful and membrane collapse was not observed. DBM particles were surrounded by connective tissue. No evidence of metabolic activity associated with the graft particles was observed. Connective tissue was in contact with the instrumented root surfaces in the absence of cementum formation. Areas of perpendicular fibre insertion to the defected root surface were confirmed. Ankylosis was not observed. Limited and similar amounts of bone and regeneration were observed for both groups.

Tal et al. (2005) used 12 mongrel dogs and six treatment modalities were applied including collagen barrier alone, bovine bone mineral (BBM) alone, bovine bone mineral collagen (BBMC), collagen barrier+BBM, collagen barrier plus BBMC and control (i.e. flap alone). Three months post-operatively, histological sections were prepared and new bone, new cementum and connective tissue adhesion were calculated as percentage of the defect height. The authors did not provide any data about SD, SE or *p* values in each group; thus, further analysis is impossible. However, the use of barrier alone achieved higher mean values regarding new bone and new cementum compared with all the other groups. The control group demonstrated the lowest values. The authors concluded that the presence of the collagen barrier was the predominant factor influencing bone and cementum regeneration.

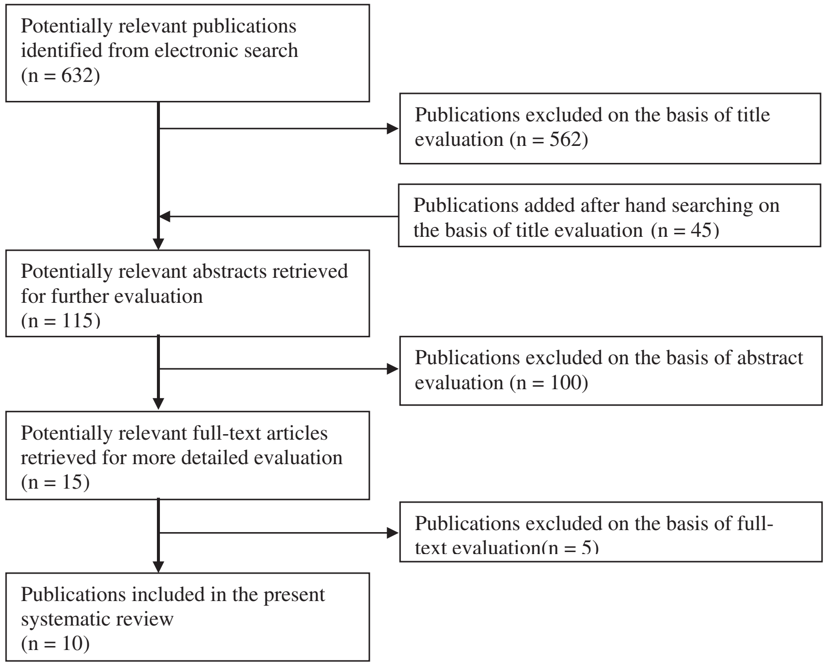

Fig. 1. Flow-chart of the screening of the relevant publications

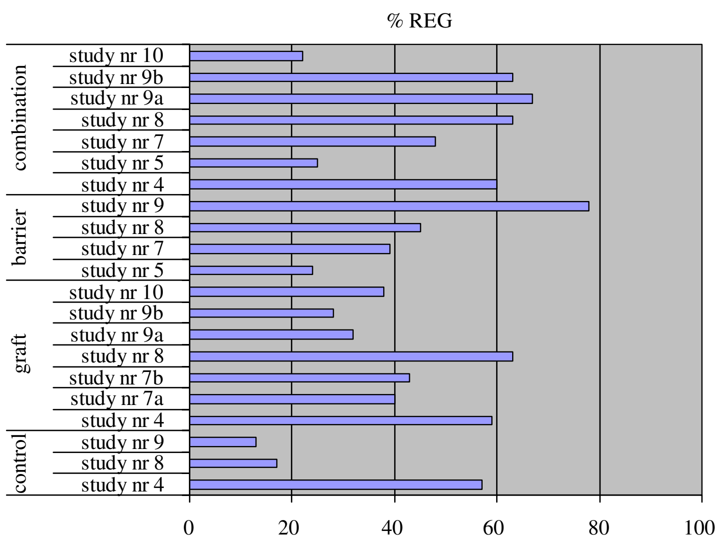

Fig. 2. Mean values of periodontal regeneration (new cementum with inserting periodontal ligament fibres and new bone) were calculated as percentage of the instrumented root length. Study numeration is described in Table 3.

Data synthesis

Studies using the same methods of evaluation and similar outcome measurements were combined and the data are presented in statistical graph (Fig. 2). The mean values of periodontal regeneration (new cementum with inserting periodontal ligament fibres and new bone) were calculated as percentage of the instrumented root length based on relevant data (distance of new periodontal tissues divided by the root length). The combined approach demonstrated periodontal regeneration ranging from 22% to 67% of the instrumented root length whereas the groups graft and membrane alone reported 28–63% and 24–78%, respectively.

Discussion

The present systematic review has attempted to provide a biological foundation for the use of combinations of barrier membranes and grafting materials and to address a specific question, i.e. whether the preclinical evidence (i.e. data from animal studies) supports the use of such combinations in regenerative periodontal therapy. The review has evaluated the available data from four different types of experimental models (i.e. supraalveolar, Class II furcation, intrabony and fenestration defects) regarding the effects of various types of barrier membranes and grafting materials on the histologic healing. The results indicate that, independent of defect type and animal model, regenerative periodontal surgery using combinations of barrier membranes and grafting materials may result in periodontal regeneration to a varying extent (Caffesse et al. 1993, Lekovic & Kenney 1993, Caplanis et al. 1998, Kim et al. 1998, Blumenthal et al. 2003, Wikesjö et al. 2003, Polimeni et al. 2004, Koo et al. 2005, Tal et al. 2005, Deliberador et al. 2006). All evaluated grafting materials (i.e. AB, DBM, DFDBA, BBM, BBMC, β-TCP and CI) appeared to be biocompatible and there were no adverse effects such as allergies or other immunologic reactions, abscess formation or rejection of the grafting materials reported. The bone metabolic activity of DBM and DFDBA appeared to be limited and was observed in two (i.e. Caffesse et al. 1993, Blumenthal et al. 2003) out of the four studies using these materials in combination with barrier membranes (Caffesse et al. 1993, Caplanis et al. 1998, Kim et al. 1998, Blumenthal et al. 2003). Signs of ankylosis were observed in one study evaluating treatment of Class II furcation defects with membrane barriers and DFDBA while no ankylosis was observed following treatment with membrane barriers alone (Caffesse et al. 1993). In another study, some of the DFDBA graft particles exhibited mineralization-osteoid formation, indicating possible osteoinduction (Blumenthal et al. 2003). However, other DFDBA graft particles were encapsulated either in connective tissue or incorporated into new alveolar bone (Blumenthal et al. 2003) while in two studies (Caplanis et al. 1998 and Kim et al. 1998) DBM particles were found to be embedded in dense connective tissue without evidence of bone metabolic activity. Thus, it appears that the osteoinductive capacity of DFDBA or DBM is highly variable probably due to differences in methods of harvesting, processing and sterilizing (Schwartz et al. 1996).

Generally, the use of barrier membranes, grafting materials or a combination of barrier membranes and grafting materials did not seem to induce ankylosis or root resorption. A common histologic feature was the limited inflammatory reaction following the use of different types of barrier membranes, grafting materials or combinations thereof. There was only one study reporting increased numbers of chronic inflammatory cells following implantation of resorbable polycarbonate and polycaprolactone barriers compared with controls (i.e. flap surgery alone) or with non-bioresorbable membranes made of polytetrafluoroethylene or silicone rubber (Lekovic et al. 1993).

There were only three studies also including open flap debridement as control (Lekovic et al. 1993, Kim et al. 1998, Deliberador et al. 2006). In all three studies, healing following flap surgery alone was characterized by incomplete defect fill and limited cementum and bone regeneration. This finding seems to indicate that flap surgery alone has only a limited effect in promoting periodontal regeneration.

Superior histologic outcomes, predominantly bone repair, following the use of a combination of grafting materials and barrier membranes compared with grafting materials alone or barrier membranes alone were only found in non contained periodontal defects (i.e. intrabony defect with missing buccal wall or supraalveolar defects). However, in contained defects (i.e. fenestration defects, three-wall intrabony defects or Class II furcation defects) no additional advantage of a combination of grafting materials and barrier membranes compared with grafting materials alone or barrier membranes alone was found. Taken together, these histologic findings seem to support the results of some clinical studies where treatment of intrabony defects with a complicated, non contained morphology with a combination of barrier membranes and grafting materials resulted in superior clinical outcomes compared with treatment with barrier membranes alone (Blumenthal & Steinberg 1990, Paolantonio 2002). Furthermore, the suggestion that the principal mechanism by which a grafting material may support regeneration appears rather to be related to its space-provision capacity than to the osteoconductive properties needs to be addressed in further preclinical and clinical studies (Kim et al. 1998, Polimeni et al. 2004, Koo et al. 2005).

It also needs to be pointed out that most of the studies did not report adequate method of randomization to avoid selection bias. It was also unclear how the selected studies included allocation concealment, examiner blinding and operator blinding to minimize the performance and measurements bias (Needleman 2002).

There was a substantial heterogeneity in the collected data regarding animal type (i.e. dog or baboon model), study design (i.e. parallel, split mouth or mixed), used materials (i.e. resorbable and non-resorbable membranes, several grafts such as autograft, allograft xenograft or alloplastic materials), defect and tooth type, outcome measures and observation periods (from 4 weeks to 6 months). Additionally, most of the studies had either a split-mouth or a mixed design without providing data about intra-individual variance. Therefore, the combination of the data for a meta-analysis approach was impossible.

Animal research and its value to human experience remains controversial (Weinberg & Bral 1999). It seems impossible to expect different species to respond identically or even similarly to the same challenge except within very narrow limits. However, animal data can provide adequate models of biologic trends before proceeding to human application. Features of periodontal diseases in humans and animals vary greatly depending on which form of the disease is present and the stage of the development (Weinberg & Bral 1999). Although periodontal defects can be experimentally or surgically induced in most mammalian species, it is important to choose a laboratory animal model that has similar characteristics of human anatomy and periodontal disease. Monkey and dog models seem to respond comparably to humans regarding the treatment of periodontal defects (Caton et al. 1994, Weinberg & Bral 1999). Furthermore, it appears that the use of discriminating animal models such as the critical-size supraalveolar periodontal defect model appears to provide important information on the biologic potential and safety of novel regenerative therapies. It was thus suggested that preclinical research should use such discriminating animal models before starting clinical evaluation (Polimeni et al. 2006).

In conclusion, within its limits, the present analysis indicates that:

(a) The combination of barrier membranes and grafting materials may result in histological evidence of periodontal regeneration, predominantly bone repair.

(b) No additional benefits of combination treatments were detected in models of three wall intrabony, Class II furcation or fenestration defects.

(c) In supra-alveolar and two wall intrabony (missing buccal wall) defect models of periodontal regeneration, the additional use of a grafting material gave superior histological results of bone repair to barrier membranes alone.

(d) In one study using a supra-alveolar model, combined graft and barrier membrane gave a superior result to graft alone.

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Clinical Relevance

Scientific rationale for the study: A systematic review of preclinical animal studies was conducted in order to evaluate the effectiveness of a combination of barrier membranes and grafting materials on periodontal regeneration.

Principal findings: Most studies have demonstrated periodontal regeneration following the combination of barrier membranes and grafting materials. Superior histologic healing following the combined technique when compared with barrier membranes alone or grafting materials alone was only obtained in non-contained two wall intrabony and supraalveolar defects.

Practical implications: A defect morphology-directed rationale may be of importance when a combination of barrier membranes and grafting materials is considered for regenerative periodontal therapy.