Collagen Membranes: A Review

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Collagen materials have been utilized in medicine and dentistry because of their proven biocompatability and capability of promoting wound healing. For guided tissue regeneration (GTR) procedures, collagen membranes have been shown to be comparable to non-absorbable membranes with regard to probing depth reduction, clinical attachment gain, and percent of bone fill. Although these membranes are absorbable, collagen membranes have been demonstrated to prevent epithelial downgrowth along the root surfaces during the early phase of wound healing. The use of grafting material in combination with collagen membranes seems to improve clinical outcomes for furcation, but not intrabony, defects when compared to the use of membranes alone. Recently, collagen materials have also been applied in guided bone regeneration (GBR) and root coverage procedures with comparable success rates to non-absorbable expanded polytetrafluoroethylene (ePTFE) membranes and conventional subepithelial connective tissue grafts, respectively. Long-term clinical trials are still needed to further evaluate the benefits of collagen membranes in periodontal and peri-implant defects. This article will review the rationale for each indication and its related literature, both in vitro and in vivo studies. The properties that make collagen membranes attractive for use in regenerative therapy will be addressed. In addition, varieties of cross-linking techniques utilized to retard the degradation rate of collagen membranes will be discussed. *J Periodontol 2001; 72:215-229.*

KEY WORDS

Collagen/therapeutic use; guided tissue regeneration; guided bone regeneration; membranes, barrier; membranes, bioabsorbable; tooth root; wound healing.

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Traditional periodontal surgeries are often performed to gain access to diseased root surfaces and remove local factors, i.e., plaque, calculus, and endotoxin. Periodontal surgical techniques include gingivectomy, open flap debridement, modified Widman flap, and osseous surgery, which may offer probing depth reduction and gain of clinical attachment.[1-3] However, new attachment achieved by these procedures is usually a result of the formation of long junctional epithelium with slight or no new connective tissue attachment and negligible new cementum formation.[4-7] In contrast, guided tissue regeneration (GTR), the procedure in which a barrier is utilized to exclude epithelium from the root surfaces,[8] has been shown to partially regenerate lost periodontal tissue with new bone, periodontal ligament, and cementum.[9] Several materials, both non-absorbable and bioabsorbable barriers, have been developed and modified in an attempt to achieve complete periodontal regeneration.[9] More recently, the membrane barriers have also been applied for regenerating bone surrounding peri-implant defects, in the so-called guided bone regeneration (GBR) procedure.[10] The purpose of this article is to address the uses of collagen membranes for their roles in GTR, GBR, and other applications. The properties that make collagen membranes attractive for use in regenerative therapy will be addressed. The related literature, both in vitro and in vivo studies, as well as clinical trials will be discussed. Moreover, a description of how collagen membranes have been used clinically and the future role of this material in dental treatment will be explored.

Collagen Membranes and Their Properties

Due to the need for a second surgery to retrieve non-absorbable membranes, a demand for bioabsorbable membranes with comparable, if not better, clinical outcomes became apparent. Type I collagen is a predominant component found in periodontal connective tissue. Several commercially available collagen membranes have been developed using type I collagen as their major component (Table 1). In addition, collagen materials also possess additional advantages including hemostasis, chemotaxis for periodontal ligament (PDL) fibroblasts[11,12] and gingival fibroblasts,[13] weak immunogenicity,[14-16] easy manipulation, and ability to augment tissue thickness.[17] Hence, collagen material appears to be an ideal choice for an absorbable GTR barrier. It should be noted that although the main component of collagen membranes discussed in this paper is type I collagen, these membranes are, in fact, not identical depending on sources of material (bovine or porcine) and additional components. However, for the purposes of this review, these membranes are all considered as collagen membranes, while details of the components and cross-linking techniques are provided in the tables.

Quteish et al.[18] evaluated the biocompatability and resorption pattern of a human collagen graft material both in vitro and in vivo. Human collagen, extracted from placenta, was implanted subcutaneously in 10 Sprague Dawley rats. The graft was encapsulated by day 7 and was slowly resorbed over 56 days with minimal inflammatory response. Locci et al.[13] compared the degree of biocompatability between collagen and polytetrafluoroethylene (PTFE). Using ^{3}H-thymidine, it was found that PTFE membrane inhibited gingival fibroblast DNA synthesis, while the matrix membrane, consisting of 95% collagen and 5% chondroitin-4-sulfate, enhanced cell proliferation. In addition, the PTFE membrane caused a marked decrease in synthesis of extracellular collagen and glycosaminoglycan (GAG), the major components of extracellular matrix (ECM). Collagen matrix resulted in a significantly increased production of these components. Since ECM plays a major role in wound healing, the authors proposed that collagen may be more suitable than PTFE barrier to achieve periodontal regeneration. Schlegel et al.[16] evaluated the immune responses to the collagen membrane both in vivo and in a pilot study in

Table 1.

Collagen Membranes Currently Available or Under Investigation*

Name	Company	Sources	Methods of Cross-Link	Main Components	Resorption Rate†
BioMend	Sulzer Calcitek, Carlsbad, CA	Bovine tendon	Formaldehyde	100% type I collagen	6-8 weeks
BioMend-Extend	Sulzer Calcitek, Carlsbad, CA	Bovine tendon	Formaldehyde	100% type I collagen	18 weeks
Periogen	Collagen Inc., Palo Alto, CA	Bovine dermis	Glutaraldehyde	Types I and III collagen	4-8 weeks
Paroguide	Coletica, Lyon, France	Calfskin	DPPA‡	96% type I collagen and 4% chondroitin-4-sulfate	4-8 weeks
Biostite	Coletica, Lyon, France	Calfskin	DPPA	88% hydroxyapatite, 9.5% type I collagen, and 2.5% chondroitin-4-sulfate	4-8 weeks
BioGide	Geistlich, Wolhusen, Switzerland	Porcine dermis	None	Types I and III collagen	24 weeks
Tissue Guide	Koken Co., Tokyo, Japan	Bovine dermis + tendon	HMDIC§	Atelocollagen (1°) + tendon collagen	4-8 weeks
BioBar	Colbar Research & Dev. Ltd., Ramat-Hasharon, Israel	Bovine tendon	N/A	100% type I collagen	6-8 months

* Modified from reference 89.
† Company data.
‡ Diphenylphosphorylazide.
§ Hexamethylenediisocyanate.

humans. Using enzyme-linked immunosorbent assay (ELISA), the authors found no specific immunoreaction against collagen during an 8- to 65-day observation period. Thus, this confirmed the lack of immunogenicity of collagen membrane previously reported by Johns et al.[19] and Wang et al.[20]

The ideal properties of a barrier for GTR procedures include the ability to exclude unwanted epithelial cells and maintain a space for appropriate cells (e.g., PDL cells, bone cells, and/or cementoblasts) to repopulate the wounded area. Thus, a membrane must maintain its structural integrity during early wound healing. The degradation rate of collagen membranes varies depending on the source. Unsal et al.[21] compared different collagen materials including fascia lata, fascia temporalis, dura mater, and bovine origin. By placing the collagen materials subcutaneously in rats for up to 42 days, the authors observed that type I bovine collagen and fascia lata were resorbed faster and that the majority of materials were replaced by normal connective tissue after 42 days. In contrast, the resorption rates of dura mater and fascia temporalis were much slower. However, whether this information from an in vivo study performed subcutaneously could be applied to the human oral cavity requires further investigation.

Paul et al.[22] examined the degradation rate of non–cross-linked bovine collagen in an attempt to treat Class II furcation defects. No residual collagen material protruding from the gingival margin was noted at one week. However, Pitaru et al.[23] reported a half-life of 21 to 28 days for non–cross-linked collagen when implanted intramuscularly or subcutaneously. These differences in degradation times between these 2 studies may be attributed to different study designs with regard to placement site; i.e., intramuscularly or subcutaneous versus intraoral, as well as a closed versus an open environment. Several periodontal pathogens (e.g., *Porphyromonas gingivalis, Bacteroides melaninogenicus*) have been reported to be capable of producing collagenase[24,25] that may degrade membranes prematurely. In addition, it should be noted that Paul et al.[22] intentionally exposed the coronal part of the collagen membrane. This may allow bacterial colonization, resulting in early degradation and ultimately compromising the final outcomes.[26-28]

To prolong the absorption time of collagen membranes, various cross-linking techniques have been developed. These include ultraviolet light,[23,29] hexamethylenediisocyanate (HMDIC),[29,30] glutaraldehyde plus irradiation,[31] and diphenylphosphorylazide.[32,33] Pitaru et al.[23] treated surgically created defects in dogs with cross-linked collagen membranes obtained from rat-tail tendons. They reported partial regeneration consisting of new bone, PDL, and cementum in the apical half, with long junctional epithelium and connective tissue attachment in the coronal half of defects.

However, only long junctional epithelium with negligible new bone was observed in sites treated with flap debridement only. The authors attributed partial regeneration to early membrane exposure, resulting in quick degradation of the membrane. Using 250 rats, Minabe et al.[29] studied differences in wound healing between atelocollagen (purified from bovine dermis) and insoluble collagen (purified from bovine tendon) cross-linked with ultraviolet rays or HMDIC. Two layers of membrane were placed under palatal flaps in the treatment groups, while flaps were closed without any membrane in the control group. The greatest inhibitory effect on epithelial downgrowth was observed in the HMDIC weak cross-linked atelocollagen subgroup (31.2%). In addition, it was noted that the non–cross-linked collagen membrane disappeared in 5 to 7 days, while residues of the cross-linked collagen membrane were still observed at day 14. The authors concluded that the higher the degree of cross-linking, the slower the rate of degradation of the collagen membrane.

Since a non-absorbable (ePTFE) membrane is required to stay in place for 4 to 6 weeks before being surgically removed, one may question the effectiveness of the collagen membrane in enhancing regeneration due to its relatively fast degradation rate. Minabe et al.[29] demonstrated that rapid epithelial proliferation occurred on days 3 to 5, and then progressed up to 14 days in the non–cross-linked collagen group. In contrast, the rate of epithelial proliferation increased slightly up to day 5 with no subsequent downgrowth in the cross-linked atelocollagen group. Similar results were also obtained by Kodama et al.[30] in a 28-day study in 120 Wistar rats. In this study, the effect of collagen concentration in HMDIC cross-linked atelocollagen was evaluated. Regardless of the concentration, collagen-treated sites demonstrated a significantly slower rate of apical migration and more perpendicular fiber bundles compared to untreated controls. Thus, from these studies it may be postulated that the period of time in which a collagen membrane remains intact is sufficient for preventing apical migration of epithelium during early periodontal wound healing, since the critical time for epithelial proliferation occurs within the first 14 days. Furthermore, Numabe et al.,[34] using ^{3}H-thymidine autoradiography, observed epithelial cell kinetics during periodontal wound healing with and without atelocollagen membrane in a rat model. The authors reported that in the collagen membrane group, ^{3}H-thymidine labeled cells were noted in both basal and prickle cell layers of newly formed sulcular epithelium, compared to only a single layer of ^{3}H-thymidine-incorporated basal cells along the root surface in non-membrane treated controls. In addition, there was no apical extension of the epithelium beyond the cemento-enamel junction (CEJ) in the collagen group even though the membrane was completely resorbed at day 14. These

findings implied that the collagen membrane decreases the mitotic activity of basal cells resulting in piling up of dividing cells adjacent to the membrane, thus inhibiting apical migration of the epithelium. Although these reports showed an adequate barrier function of the collagen materials, it is noteworthy that most studies were conducted in a rat model in a short period of time. Further studies in this area are still needed.

It should be noted that a prolonged resorption rate of the collagen membrane does not always result in greater periodontal/bone regeneration. Using rat calvarial defects, Brunel et al.[32] evaluated the effect of degrees of cross-linking on guided bone regeneration. Their results confirmed previous reports that the higher degree of cross-linking, the longer the resorption rate, and more defect fills were noted on membrane-treated sites. However, no statistically significant differences in bone fills were observed among sites treated with collagen membranes with different cross-linking degrees. Interestingly, highly cross-linked, slow-resorbing collagen membranes appeared to have negative effects on final clinical outcomes as reported by Crigger et al.[35] in a dog model. The authors found the greatest attachment gain when using weak cross-linked, rapid-resorbing collagen membranes, followed by ePTFE membranes and high cross-linked collagen membranes. Membrane exposure, one of the primary contributing factors for GTR/GBR failure, was commonly found in ePTFE membranes and highly cross-linked collagen membranes. At 6 months, the highly cross-linked membrane group demonstrated extensive recession and loss of attachment. In contrast, the weak cross-linked collagen showed 84% new connective tissue attachment while ePTFE membranes had only 53%.

Regarding cross-linking techniques, the glutaraldehyde technique, the most widely used chemical cross-linking technique, was reported to leave cytotoxic residue during the process.[36] To overcome the drawback of this technique, the diphenylphosphorylazide (DPPA) technique was developed. Collagen barriers manufactured through this process showed comparable results when compared to other collagen membranes.[33]

In addition to cross-linking, heparan sulfate and fibronectin have also been added in an attempt to enhance the inhibitory effect on epithelial migration. In a dog model, Pitaru et al.[37] compared the length of the root surfaces repopulated by connective tissue between sites treated with collagen membrane alone, and those treated with a fibronectin and heparan sulfate-enriched collagen membrane. Approximately 30% more root surfaces were repopulated with connective tissue in the fibronectin and heparan sulfate-treated group compared to the collagen alone group.

Overall, studies have shown that collagen materials have an ability to inhibit apical migration of epithe-lium regardless of the fast degradation rate. It should be noted that once the epithelium becomes mature, membrane integrity is no longer essential for tissue regeneration. Hence, the crucial period for the membrane to remain intact is during the early wound healing period when the epithelium actively migrates along the root surface.[38] After that, the presence of a barrier may cause a delay in connective tissue maturation during the late phase of wound healing.

Animal Studies

Several animal studies have been conducted to evaluate the safety and efficacy of collagen barriers (Table 2). Most earlier studies focused on collagen biocompatibility and its resorption rate,[12,13,16,29,30,34] and the majority of them used surgically created defects. In addition, most studies ended within 4 to 12 weeks, with few studies lasting for 6 months. These factors should be considered when applying results obtained from these in vivo reports. Nevertheless, the conclusions drawn from these animal studies can be used as a reference for the material. They are: 1) collagen membranes are weak immunogen and biocompatible; 2) collagen membranes possess the chemotactic ability to attract gingival and/or PDL fibroblasts, thus promoting proliferation and extracellular matrix maturation, and facilitating wound healing;[13,16] 3) collagen membranes have been shown to be an effective barrier in preventing apical migration of epithelium;[29,30,34] 4) the higher degree of cross-linking, the slower the resorption rate;[29-33,35] and 5) collagen membranes provide better clinical results when compared to open flap debridement alone.[23,35,37,39,40]

Guided Tissue Regeneration

Intrabony defects. Since the late 1980s, collagen membranes have been tested for their ability to promote regeneration in periodontal intrabony defects (Table 3).[31,41-46] Most studies have shown significantly greater probing depth (PD) reduction, clinical attachment gain, and bone fill in collagen-treated groups than open debridement controls.[31,42-45] The mean PD reduction from these studies ranged from 3.1 to 5.5 mm in collagen-treated groups, while only 2.2 to 2.8 mm reductions were noted in controls. However, Tanner et al.[41] showed no difference in clinical attachment gain when comparing collagen membranes to flap debridement alone. It should be noted that this study was done in only 4 patients with hopeless teeth, and results were obtained only at 3 months. Other studies had more participants (9 to 52) with longer follow-up (6 months). Factors contributing to these variable results include different cross-linking techniques, initial probing depths, width of defects, measuring techniques, and different analytical methods.[47] For example, Chung et al.[42] used mean PD and attachment level from each tooth for data analysis instead

Table 2.

Collagen Membrane in Treating Periodontal Defects in Animal Studies

Reference	Animal (n)	Defect Type	Collagen Type	Study Length	Treatment Groups	Results
Pitaru et al. 1988[23]	Beagle dogs (3) – 3 teeth/side	Surgically created buccal defects (4 mm)	Rat-tail tendon (type I collagen)	30 days	Split-mouth 1) Flap debridement 2) Collagen membrane	Length of JE (mm): 2.1 / 1.0 / $P<0.0005$ — CAL (mm): 0.8 / 1.6 / $P<0.025$ — New bone (mm): 0.4 / 1.9 / $P<0.005$ — New Cementum (mm): 0 / 1.6
Blumenthal 1988[39]	Mongrel dogs (4) –15 defects/dog	Surgically and ligature-induced defects (5 mm)	Purified bovine collagen (cross-linked with glutaraldehyde)	84 days	Split-mouth 1) No treatment 2) Flap debridement 3) Collagen membrane	Healing 1) Inflamed tissue new CT 2) Long junctional epithelium (new CT = 0.2 mm) 3) No apical migration of JE, new CT attachment with new cementum (new CT = 1.6 mm)
Minabe et al. 1989[29]	Wistar rats (250)	Surgically created palatal dehiscence defects on maxillary 1st molar	Atelocollagen (purified from bovine dermis) & insoluble collagen (purified bovine tendon)	14 days	1) No membrane 2) Non x-linked 3) UV x-linked 4) HMDIC 0.5 5) HMDIC 5	Regardless of the type of cross-link, all collagen significantly decreased the rate of epithelial downgrowth when compared with the control group (64.6% vs. 31.2%) at day 14.
Kodama et al. 1989[30]	Wistar rats (120)	Surgically created palatal dehiscence defects on maxillary 1st molar	Atelocollagen (purified from bovine dermis cross-linked with HMDIC)	28 days	1) 0.5% atelocollagen membrane 2) 1.0% atelocollagen membrane 3) 1.3% atelocollagen membrane	The concentration of collagen has no significant influence on the apical migration rate of the epithelium.
Pitaru et al. 1991[37]	Mongrel dogs (8)	Surgically created dehiscence defects (6 mm) on 12 maxillary canines	Rat-tail tendon (type I collagen)	20 days	1) 2-layer collagen membranes (inner layer- heparan sulfate and fibronectin-enriched membrane) 2) 2-layer collagen (non-enriched) 3) Only outer layer of collagen	CT attachment: 5.3 ± 0.5 / 3.5 ± 0.5 / 3.6 ± 0.7 — JE: 0.2 ± 0.1 / 1.2 ± 0.3 / 1.4 ± 0.3
Choi et al. 1993[90]	Beagle dogs (7)	Maxillary canines (fenestration defects 6 × 4 mm)	Collagen matrix	28 days	1) No treatment 2) Collagen membrane	No significant differences between groups with regard to new cementum and bone formation.
Crigger et al. 1996[35]	Beagle dogs (6)	P2, P3, and P4 Surgically created Class III furcation defects	Bovine collagen cross-linked with diphenylphosphoryl-azide	180 days	3 groups (4 sites each) 1) ePTFE membrane 2) low cross-linked collagen 3) high cross-linked collagen	Defect height (mm): 3.9 ± 0.3 / 3.9 ± 0.4 / N/A — AL gain (mm): 2.1 ± 0.5 / 3.2 ± 1.1 / N/A — New cementum (mm): 1.2 ± 0.5 / 1.4 ± 0.9 / N/A — New bone (mm): 0.5 ± 0.8 / 1.2 ± 1.1 / N/A
Cirelli et al. 1997[40]	Mongrel dogs (4)	Class II surgically created furcation defects in 4 mandibular premolars	Bovine tendon cross-linked with glutaraldehyde	90 days	2 groups (8 sites each) 1) Open debridement 2) Collagen membrane	% epithelium: 37.8 ± 29.7 / 1.9 ± 3.5 / $P = 0.005$ — % new cementum: 59.3 ± 29.4 / 92.4 ± 15.7 / $P = 0.019$ — % new bone: 48.6 ± 26.9 / 56.3 ± 11.4 / NS

Table 3.

Collagen Membrane in Treating Intrabony Defects in Humans

Reference	N (mean age)	Collagen Type	Study Length	Treatment Groups	Mean Initial PD (mm)	Mean PD Reduction (mm)	Mean CAL Gain (mm)	Defect Fill (%)
Chung et al. 1990[42]	15 patients (21–39 years) with 20 paired defects	Cross-linked bovine type I collagen	12 months (reentry)	Split-mouth design 1) Flap debridement	5.4 ± 1.9	NA	–0.7 ± 0.9	N/A
				2) Collagen membrane	6.0 ± 2.2 NS	N/A	0.6 ± 0.6 $P < 0.01$	N/A
Quteish and Dolby 1992[31]	19 patients (N/A) with 52 matched defects	Irradiated glutaraldehyde human type I collagen extracted from placenta	6 months	Split-mouth design 1) Flap debridement	5.8 ± 1.4	2.2	2.3	N/A
				2) Collagen membrane	6.4 ± 1.9 NS	4.2 $P < 0.0001$	4.0 $P < 0.0001$	N/A
al-Arrayed et al. 1995[43]	14 patients (43.7 years)	Cross-linked human type I collagen	6 months	Split-mouth design 1) Open flap debridement	6.4	2.8	3.7	N/A
				2) Collagen	6.7 NS	4.3 $P < 0.05$	3.9 $P < 0.05$	N/A
Mattson et al. 1995[44]	9 patients (49.7 years)	Type I bovine collagen	12 months (reentry)	Split-mouth design, but not balanced for severity 1) Open flap debridement	6.6 ± 1.2	2.2 ± 1.8	0.4 ± 2.0	28
				2) Collagen	7.2 ± 0.9	3.1 ± 1.6	2.4 ± 2.1	77
Benquét et al. 1997[45]	52 AP patients and 16 RPP patients	DPPA cross-linked bovine type I collagen	6 months	One defect/patient 1) Adult periodontitis	9.6 ± 2.0	5.5 ± 2.4	3.6 ± 2.2	N/A
				2) RPP	8.3 ± 1.6	4.1 ± 2.6	2.6 ± 3.0	N/A
Mattson et al. 1999[46]	19 patients (50.4 years) with at least one 2- to 3- walled interproximal defect	Cross-linked bovine tendon (type I collagen)	6 months (reentry)	1) Collagen membrane (n = 11)	6.18 ± 0.9	3.27 ± 1.91	2.58 ± 1.9	2.15 ± 2.0
				2) PGA/PLA copolymer membrane (n = 12)	6.14 ± 2.02 NS	3.55 ± 2.47 NS	2.77 ± 2.13 NS	1.9 ± 1.9 NS

RPP—rapidly progressive periodontitis.

of those of the actual intrabony defects. With this analytical approach, little or no improvement from shallow PD sites may mask the actual improvement observed on the treated defect sites. Therefore, it is not surprising that the results from this study showed less attachment level gain than other studies. Nevertheless, the majority of these studies showed significantly greater clinical improvements when collagen membranes were used.

Parodi et al.[48] reported a human histological biopsy of a mesial circumferential defect on a maxillary molar treated with DPPA cross-linked collagen membrane containing 4% chondroitin-4-sulfate. At 5 months, a clinical attachment gain of 4 mm was observed. Histological observation demonstrated highly vascularized periodontal ligament, and new cementum with an active cementoblast front and new bone formation up to the crestal level. Moreover, no sign of inflammation was noted. It was concluded that collagen membranes can enhance partial periodontal regeneration in humans. Evans et al.[49] used meta-analysis to analyze GTR articles published between 1994 to early 1996. The mean PD reduction and CAL gain with a GTR procedure utilizing collagen membranes were 4.1 mm and 4.0 mm, respectively. These results were comparable to those of studies using ePTFE mem-

Table 4.

Combined Use of Collagen Membrane and Other Materials in Humans

Reference	N (mean age)	Collagen Type	Study Length	Treatment Groups	Mean Initial PD (mm)	Mean PD Reduction (mm)	Mean CAL Gain (mm)	Defect Fill (%)
Blumenthal and Steinberg 1990[50]	10 patients (34–57 years) with 71 defects	Purified bovine collagen, allogenic freeze-dried bone & microfibrillar collagen gel	12 months (reentry)	1) Open debridement	N/A	1.5 ± 0.2	0.8 ± 0.2	5.9 ± 1.5
				2) Membrane alone	N/A	2.0 ± 0.1	1.2 ± 0.1	30.6 ± 2.8
				3) Bone graft alone	N/A	2.0 ± 0.1	1.4 ± 0.1	47.7 ± 2.7
				4) Bone and collagen gel	N/A	2.6 ± 0.1	1.9 ± 0.2	48.5 ± 3.5
				5) Bone, collagen gel, and membrane	N/A	2.7 ± 0.1	2.0 ± 0.1	62.7 ± 2.5
Dowell et al. 1995[51]	16 patients (43.7 ± 4 years) with 78 bilateral defects	Cross-linked human type I collagen with or without metronidazole (8 mg/cm^2)	6 months	Split-mouth design 1) Collagen membrane	6.8	4.3 ± 0.9	NS	N/A
				2) Metronidazole-impregnated collagen	6.7	4.3 ± 0.9		
					NS	NS		
Chen et al. 1995[52]	10 patients (26–62 years) with bilateral defects	Cross-linked bovine tendon with or without DFDBA	6 to 12 months (reentry)	Split-mouth design 1) Collagen membrane	7.4 ± 0.4	3.2 ± 0.4	2.0 ± 0.4	39.6
				2) Collagen membrane with DFDBA	7.6 ± 0.4	3.4 ± 0.4	2.3 ± 0.5	35.4
					NS	NS	NS	NS
Benqué et al. 1997[53]	43 AP patients (49.2 ± 9.9 years) and 14 RPP patients (38 ± 4.7 years) with deep 2-wall bony defects	DPPA cross-linked bovine type I collagen with or without hydroxyapatite/ collagen/ chondroitin sulfate grafting material	6 months	One defect/patient 1) AP patients 2) RPP patients	9.5 ± 2.0 8.2 ± 1.8	6.1 ± 2.1 4.7 ± 1.2 *P* <0.05	4.2 ± 2.1 3.0 ± 1.6 *P* <0.05	N/A N/A

AP—adult periodontitis.
RPP—rapidly progressive periodontitis.

branes (5.1 mm and 3.6 mm, respectively). The authors also reported that there were no statistically significant differences in clinical outcomes among various GTR membranes (ePTFE, collagen, collagen plus bone graft, polylactic acid/polyglycolic acid copolymer [PLA/PGA], and rubber dam). However, it should be remembered that even with the best results ever reported, none of these studies demonstrated a complete fill of periodontal defects.

Combined Use of Collagen Membrane and Other Materials

Several attempts have been made to enhance regeneration outcomes by combining/adding other materials. These include a combination of collagen membranes and bone grafting materials, metronidazole-impregnated collagen, fibronectin and heparan sulfate-

enriched collagen, and a combination of collagen membrane and collagen gel (Table 4).[37,50-53] These techniques mainly aim to provide space maintenance and to recruit cells with regenerative potential. It is believed that bone grafting materials not only maintain the space, but also provide an osteoinductive and/or osteoconductive capacity. In 1990, Blumenthal and Steinberg[50] reported statistically significant improvement in defect fill with the use of freeze-dried bone and microfibrillar collagen gel‡ in addition to collagen barrier. However, it should be noted that this study was conducted in only 10 patients (71 defects) with 5 different treatment groups. Also, the original PD or description of defect morphology is lacking. Chen et

‡ Zyderm, Collagen Corp., Palo Alto, CA.

al.,[52] in a randomized split-mouth design clinical trial, showed that demineralized freeze-dried bone allograft (DFDBA) had no additional benefit to the GTR therapy with collagen membrane alone. The use of grafting material containing hydroxyapatite, collagen, and chondroitin-sulfate[§] in addition to the collagen membrane was also studied by Benqué et al.[53] The authors attempted to treat 2-wall intrabony defects in both chronic adult periodontitis and rapidly progressive periodontitis patients. The results indicated that this combined technique significantly improved PD and CAL in both patient groups. In addition, there were statistically significant greater PD reduction and CAL gain in chronic adult periodontitis patients than rapidly progressive periodontitis patients. However, this study lacked a negative control (open debridement), positive control (membrane alone), and placebo control (membrane plus gel carrier) groups; therefore, it is inconclusive whether the spacer or its components accounted for the improvement.

In addition to space maintaining materials, antibiotic-impregnated collagen membranes have also been utilized to reduce the risk of bacterial contamination. Dowell et al.,[51] in a double-blinded, randomized, split-mouth design clinical trial, failed to demonstrate the extra benefit of metronidazole-impregnated collagen over the collagen membrane alone.

Class II furcation defects. It is generally agreed that treatment of furcation defects is more complicated and challenging than that of non-furcated

§ Biostite, Coletica Corp., Lyon, France.

Table 5.

Collagen Membrane in Treating Class II Furcation Defects in Humans

Reference	N (mean age)	Collagen Type & Additional Materials	Study Length	Treatment Groups	Results Initial V-Depth	Initial H-Depth	V-Defect Fill	H-Defect Fill	% Cl II → Cl I Defects
Paul et al. 1992[22]	7 patients (51.7 years) with bilateral defects	Type I bovine collagen	6 months (reentry)	Split-mouth design 1) Flap debridement 2) Collagen membrane	6.1 ± 1.8 6.4 ± 1.9 NS	3.9 ± 2.1 4.7 ± 2.0 NS	0.4 ± 0.8 0.7 ± 0.9 NS	0.0 ± 0.9 0.9 ± 0.9 P <0.05	N/A
Van Swol et al. 1993[60]	38 patients (31–71 years)	Chemical cross-linked purified bovine dermal collagen	3 months (reentry)	One defect/patient 1) Flap debridement (10) 2) Collagen membrane (28)	7.1 ± 1.6 6.9 ± 1.6 NS	5.7 ± 2.5 5.1 ± 1.4 NS	0.7 1.7 P <0.05	0.7 2.3 P <0.0001	>50% H-Fill 0/10 5/28
Blumenthal 1993[62]	12 patients (31–80 years) with paired buccal Class II mandibular molar furcation defects	Chemical cross-linked purified bovine dermal collagen	12 months (reentry)	Split-mouth design 1) ePTFE membrane 2) Collagen membrane	6.2 ± 2.2 7.00 ± 2.2 NS	4.9 ± 0.7 5.3 ± 1.3 NS	1.0 ± 1.0 1.6 ± 1.2 NS	1.7 ± 0.5 2.5 ± 0.7 P <0.05	N/A
Black et al. 1994[63]	13 patients (43.2 years) with a pair of comparable Class II molar defects	Cross-linked bovine tendon type I collagen	6 months	Split-mouth design 1) ePTFE membrane 2) Collagen membrane	5.3 ± 1.6 5.00 ± 1.8 NS	4.3 ± 2.0 4.4 ± 1.5 NS	1.1 ± 0.8 1.4 ± 1.7 NS	0.8 ± 2.2 1.5 ± 2.0	N/A
Wang et al. 1994[20]	12 patients (46.4 years) with bilateral mandibular furcation defects	Cross-linked type I bovine collagen	12 months (reentry)	Split-mouth design 1) Flap debridement 2) Collagen membrane	4.2 ± 2.2 4.2 ± 1.7 NS	5.6 ± 2.7 6.0 ± 2.7 NS	1.5 ± 0.3 2.8 ± 0.4 P <0.05	1.1 ± 0.6 2.0 ± 0.4 NS	>50% H-Fill 4/12 3/12
Yukna and Yukna 1996[64]	59 patients (46.8 years) from 7 centers	Type I bovine tendon collagen	6 to 12 months (mean 11.1) (reentry)	Split-mouth design 1) Flap debridement (27) Collagen membrane (27) 2) ePTFE membrane (32) Collagen membrane (32)	3.2 ± 1.6 3.6 ± 1.7 1.9 ± 2.5 1.3 ± 1.0	5.3 ± 2.0 5.0 ± 2.0 4.3 ± 1.7 4.7 ± 1.6	0.8 ± 1.5 1.7 ± 1.6 N/A N/A	1.1 ± 2.0 2.0 ± 1.7 1.7 ± 2.2 1.7 ± 1.9	% improved defects 7 44 53

defects because of the complexities of tooth anatomy including root concavities,[54] cervical enamel projections,[55] bifurcation ridges,[56] accessory canals,[57] and small entrance access.[58] Nonetheless, attempts have been made to regenerate lost periodontium in furcation defects through varieties of approaches. Table 5 lists studies that have utilized collagen membranes to promote regeneration in furcated molars. In 1988, Garrett et al.[59] used a collagenous membrane, freeze-dried dura mater, for treating furcation-involved teeth. The results, however, showed less favorable outcomes than coronally positioned flaps with allograft-treated sites. Paul et al.[22] showed minor improvement in horizontal defect fill, with no difference in vertical bone fill when comparing a collagen membrane-treated group to an open debridement control group. In contrast, Van Swol et al.[60] and Wang et al.[20] reported significantly greater defect fills in a collagen-treated group than an open debridement control group. This may be explained by the differences in the protocol; i.e., collagen membranes were intentionally left supragingivally in the former study while primary coverage was attempted in the latter studies. Since primary coverage is a key factor in periodontal regen-eration and premature membrane exposure often leads to bacterial colonization and compromised clinical outcomes,[26,28,61] it is possible that the unfavorable outcomes from Garrett et al.[59] and Paul et al.[22] were partly the consequences of exposed membranes. When comparing the use of collagen material to non-absorbable ePTFE membrane, comparable clinical results were noted in treating Class II furcation defects.[62-64] Nevertheless, it should be noted that only 14% of these treated sites showed complete furcation fill.[64] Hence, further studies or attempts are still needed to identify the best methods/materials for furcation defect regeneration/repair.

Root Coverage Procedures

Different techniques have been used to replace lost, damaged, or diseased gingival tissues. Traditionally, multiple procedures are often needed to achieve optimal esthetic results. These include lateral pedicle graft, free gingival graft, and subepithelial connective tissue graft. However, these procedures often require a second surgical site for harvesting grafts, and may cause postsurgical bleeding and patient discomfort. Recently, attempts have been made to achieve root coverage

Table 6.

Collagen Membrane in Root Coverage Procedures

Reference	N (mean age)	Collage Type	Study Length	Defect Type	Results				
					Mean Initial Recession Depth (mm)	Gain in CAL (mm)	Mean Root Coverage (mm)	Mean % Root Coverage	% Sites With Complete Coverage
Shieh et al. 1997[74]	10 patients (40.7 years)	Cross-linked type I bovine collagen	6 months	Miller Class I or II buccal marginal tissue recession ≥ 2.5 mm	3.2 ± 0.3	1.3 ± 0.5 $P <0.05$	1.7 ± 0.2 $P <0.05$	51.6 ± 6.8 $P <0.05$	10
Zahedi et al. 1998[75]	15 patients (46.7 years)	DPPA cross-linked bovine 96% type I collagen with 4% chondroitin-4-sulfate	24 months	Miller Class I	3.7 ± 1.4	3.5 ± 1.3 $P <0.0001$	2.9 ± 1.1	82.2 ± 23.2 $P <0.0001$	53
Özcan et al. 1997[76]	10 patients (38.5 years) with 28 Miller's Class I or II (≥3 mm recession depth) defects	Tutoplast fascia temporalis	6 months	1) Coronally positioned flap 2) Collagen membrane, fibrin/fibronectin and tetracycline root conditioning	4.1 4.3	2.9 4.2 $P <0.001$	2.8 3.1 NS	68.6 71.7 NS	N/A N/A
Wang et al. 1999[77]	16 patients (40.6 years) with paired Miller's Class I or II (≥3 mm recession depth) defects	Newly designed double-thickness membrane (bovine tendon)	6 months	Split-mouth design 1) Subepithelial connective tissue graft 2) Newly designed collagen membrane	3.4 ± 1.0 3.7 ± 1.1 NS	2.3 ± 1.0 2.8 ± 0.7 NS	2.8 ± 0.8 2.5 ± 0.7 NS	84 ± 25 73 ± 26 NS	43.7 43.7 NS

using surgical techniques based on the principles of GTR.[65-68] Similar results were reported when GTR-based techniques were compared to traditional root coverage procedures.[69-72] However, GTR therapy offers an additional benefit through its potential of achieving new attachment formation along the previously denuded root surface, while it rarely occurred when a subepithelial connective tissue graft was utilized.[73]

Collagen membranes have recently been used in root coverage procedures (Table 6). While Shieh et al.[74] gained 51.6% root coverage after 6 months, Zahedi et al.[75] reported mean root coverage of 82.2% after 2 years. Since neither of these studies included a control group, it is inconclusive if the GTR-based procedure is comparable to the conventional procedures. In 1997, Özcan et al.[76] compared the use of collagen membranes containing fibrin/fibronectin plus tetracycline root conditioning to coronally positioned flaps. They reported no significant differences between groups with regard to mean root coverage. However, significantly more gain in clinical attachment was found in the collagen membrane group compared to the coronally positioned flap (4.21 mm versus 2.86 mm). Wang et al.[77] compared a conventional subepithelial connective tissue graft to a GTR-based procedure using a newly designed double-thickness collagen membrane (prepared from cross-linked bovine tendon and supplied in individual $20 \times 15 \times 0.4$-0.5 mm pieces) in 16 patients with bilateral Miller's Class I or II (gingival recession ≥3.0 mm) recession defects. Data from this study indicated that the GTR-based procedure resulted in statistically significant ($P < 0.05$) improvements in recession depth, CAL, and width of keratinized and attached gingiva compared to baseline. Moreover, the results were comparable to those achieved from subepithelial connective tissue grafts, with mean root coverage of 73% and 84% for the newly designed collagen membrane and conventional connective tissue graft, respectively. Recently, Kimble et al.[78] studied the effect of adjunctive bone graft placement during GTR-based root coverage procedures. Twenty patients with a Miller Class I or II buccal recession defect ≥2.5 mm were randomly treated with either type I collagen membrane alone or type I collagen membrane plus DFDBA. Preliminary results from this study indicated both forms of treatment resulted in statistically significant ($P < 0.05$) reduction of recession depths (69% for membrane alone and 79% for membrane plus DFDBA). It was concluded that there is a tendency for an additional benefit of bone graft for percent root coverage; however, no statistically significant difference was found between groups.

In summary, data from available resources indicate that GTR-based procedures and subepithelial connective tissue grafts are clinically equivalent. In addition, using a barrier may also enhance more clinical attachment gain.[76] It should also be noted that with the GTR-based procedure, adequate flap thickness (≥0.5 mm in the defect area) seems to have a great influence in improving the percent root coverage as reported by Harris[70] (26.7% versus 95.9% root coverage in thin and thick tissue, respectively). Therefore, careful case selection is crucial for the success of this procedure.

Guided Bone Regeneration (GBR)

Implants have slowly emerged as an alternative procedure for replacing missing teeth. Ridge deficiency or inadequate bone height/width often compromises the overall treatment plan. Bone augmentation using GTR principles has become a common means to resolve these concerns. Studies have reported success in using barriers to augment bone prior to or after implant placement.[79] When selecting an ideal material for GBR, the following requirements must be considered: wound stabilization, space creation and maintenance, protection of the underlying blood clot, and the ability to exclude unwanted tissues or cells (connective tissue and epithelium). Since collagen membranes fulfill all these criteria, it has become one of the materials used in GBR procedures. However, the fast resorption rate of collagen material remains a concern to most clinicians. So far, no study has defined the actual time needed for GBR barriers. Iglhaut et al.[80] reported that during GTR procedures, bone and/or PDL cell migration reach their peaks in 2 to 7 days after surgery, with a decrease in mitotic activity to almost normal levels by the end of the third week. It could be implied that cells needed for regeneration arrive at the wound sites in around 3 to 4 weeks; therefore, the length of time that the collagen membrane retains its integrity should be sufficient to allow selective cell repopulation.

GBR procedures utilizing collagen membranes have been widely reported (Table 7).[81-85] In a pilot study, Colangelo et al.[81] created through and through defects on the lateral aspect of rabbit mandibles and then treated these defects with either a collagen membrane or no-treatment control. The histologic evaluation at 30 days demonstrated a nearly complete continuous layer of lamellar bone with osteoblastic activity in the collagen membrane-treated group compared to only fibrous connective tissue in the control group. Sevor et al.[82] created buccal dehiscence defects after placement of a total of 48 implants (24 hydroxyapatite [HA]-coated and 24 grit-blasted) in 6 dogs. At 8 weeks, the mean defect fill was 80.29% in the collagen membrane-treated group compared to 38.62% in the control group.

Contrary to the GTR procedures, additional bone grafting materials for space creation/maintenance tend to improve GBR outcomes. This was supported by

Table 7.

Collagen Membrane in Guided Bone Regeneration Procedures

Reference	Model (n)	Collagen Type	Study Length	Treatment Groups	Results Clinical and Histologic Observations		
Sevor et al. 1993[82]	Mongrel dogs with surgically created buccal dehiscence defects (6 sites/dog)	Highly cross-linked collagen	1 and 2 months	Randomly assigned HA-coated implants (24) 1) No treatment 2) Collagen membrane Grit-blasted implants (24) 1) No treatment 2) Collagen membrane	% Defect Fill at 4 Weeks 47.11 ± 23.13 83.13 ± 3.32 1.02 ± 75.12 55.18 ± 28.34	% Defect Fill at 8 Weeks 32.66 ± 17.61 81.80 ± 6.11 44.58 ± 28.73 78.79 ± 21.70 *P* <0.007	
Colangelo et al. 1993[81]	Rabbits with 3 x 9 mm defects on the lateral aspect of the mandible (15)	Cross-linked bovine tendon type I collagen	1 month	1) No treatment control (3) 2) Collagen membrane (12)	1) The defects were filled with fibrous CT. 2) Layer of lamellar bone was noted with osteoblastic activity.		
Zitzmann et al. 1997[86]	84 exposed implant surfaces (25)	Porcine collagen types I and III	24 months	Split-mouth design 1) ePTFE membrane and BioOss (41) 2) Collagen membrane and BioOss (43)	Mean % Bone Fill 78% 92% NS		
Parodi et al. 1998[88]	Patients (mean age 44.8 years) with insufficient ridge (width <5 mm) (16)	Calfskin collagen (96%) with 4% chondroitin-4-sulfate	7 to 12 months	Native collagen sponges were used to maintain the space buccally and lingually. The membranes were placed to cover the sponges.	75% of the defects demonstrated sufficient width for implant placement. Mean increase in the size of the crest was 2.5 mm (3 to 5.5 mm).		
Hürzeler et al. 1998[83]	Monkeys (*Macaca fascicularis*) with surgically created defects (4 x 4–5 x 20 mm) (5)	Calfskin collagen (96%) with 4% chondroitin-4-sulfate	6 months	1) Collagen membrane 2) Collagen membrane and BioOss 3) ePTFE membrane and BioOss 4) No membrane control	Bone/implant contact 2.2 ± 0.9 mm 3.3 ± 1.9 mm 3.9 ± 1.4 mm 1.4 ± 0.7 mm *P* <0.05 between 1, 4 and 2, 3		
Dongieux et al. 1998[84]	Mongrel dogs received 6 monocortical onlay grafts on the mandibles (4)	Cross-linked bovine type I collagen	2.5 months	Split-mouth design (2 for each group in each animal) 1) Onlay graft alone 2) Onlay graft with ePTFE membrane 3) Onlay graft with collagen membrane	Initial Graft Volume (ml) 0.15 ± 0.02 0.18 ± 0.03 0.16 ± 0.03 NS	Final Graft Volume (ml) NS	Final: Initial Volume Ratio 0.06 ± 0.04 0.40 0.06 ± 0.06 0.32 0.07 ± 0.06 0.46 NS

Hürzeler et al.[83] The authors reported collagen membrane combined with bovine HA significantly enhanced bone-to-implant contact when compared to collagen membrane alone, or non-membrane treated control groups. In addition, the results were comparable to those in the ePTFE membrane plus bovine HA group. This was further confirmed by Zitzmann et al.[86]

Collagen membranes have been reported to be superior to non-absorbable membranes with regard to healing. Also, a higher incidence of dehiscence, membrane exposure, and/or premature membrane removal was noted in non-absorbable membranes.[81,82,86] Similar to GTR procedures, membrane exposure has a negative impact on the amount of bone regenerated in GBR. Simion et al.[87] reported significantly less bone gain when the membranes were exposed compared to non-exposed membrane treated sites (41.6% versus 96.6%).

Collagen sponges have also been placed beneath collagen membranes to provide space needed for new bone formation. Parodi et al.[88] used collagen membranes combined with a collagen sponge to correct 16 cases with insufficient ridges (<5 mm in width). After 7 to 12 months, 75% of the sites demonstrated sufficient bone volume for implant placement with the mean increase in crestal bone of 2.49 mm. Recently, monocortical onlay grafts were introduced to augment bone prior to implant placement. Dongieux et al.[84] evaluated the necessity of barrier membranes in addition to onlay grafts in a dog model. This 10-week split-mouth design demonstrated that neither ePTFE membranes nor collagen membranes have an extra benefit to the onlay graft alone.

To date, the factors that influence bone regeneration are complex and not yet fully understood. Bone regeneration utilizing collagen membranes seems promising. It should also be noted that the available data are from studies within the 1990s. Long-term studies are needed to confirm the success rate of implants placed in regenerated bone.

CONCLUSION

In conclusion, collagen membranes have been used in both medical and dental (especially periodontal) fields for decades. The benefits of utilizing this absorbable material include promoting wound healing through clot stabilization, wound stability, and hemostasis; enhancing primary wound coverage via its chemotactic ability to attract fibroblasts; and augmenting flap thickness by providing a collagenous scaffold. Cross-linked membranes allow a slower degradation rate, therefore providing more time for selective cells to repopulate the wound areas. Generally, collagen membranes have been proven to significantly enhance periodontal regeneration in various animal studies and human clinical trials; however, none of these studies has shown a complete regeneration. In addition, collagen membranes have been applied to guided bone regeneration and root coverage procedures with comparable success rates to non-absorbable ePTFE membranes and conventional subepithelial connective tissue grafts, respectively. More research is necessary to improve clinical outcomes and achieve true periodontal regeneration. A better understanding of the factors orchestrated in the regeneration process is required to achieve 100% predictable outcomes in treating osseous defects around both teeth and implants.

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