Morphological Analysis of Resorbable Collagen Membranes by Scanning Electron Microscopy

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Abstract

Objective*:* The composition and morphology of the internal and external surfaces of membranes are important for preventing migration of epithelial and connective cells, and allow the installation of osteogenic cells for bone growth. The objective of this study was to analyze the morphology and composition of three types of commercially available resorbable collagen membranes.

Methods*:* Three types of collagen membranes, with different compositions and coming from different animals, were used: 1) Dental Surgidry F (bovine collagen type I); 2) Bio-Gide® (porcine collagen type I and III); and 3) OsseoGuard™ (bovine collagen type I). These membranes were analyzed using scanning electron microscopy and energy dispersive spectrometry.

Results*:* The membranes showed distinct superficial architectures, porosities and chemical compositions. The membranes exhibited different surfaces and thicknesses, ranging from 0.32 mm to 0.75 mm. The chemical compositions exhibited a high percentage of niobium (Nb) in the Surgidry and OsseoGuard™ membranes; the Bio-Gide® membrane showed a greater proportion of calcium and aluminum relative to other elements.

Conclusion: Different types of resorbable collagen membranes exhibit different morphologies and chemical composition, which could lead to differences in the mode and time of resorption of the membranes used for guided tissue regeneration procedures.

Key words: Membranes, collagen, biodegradation, bone regeneration

Introduction

Collagen membranes are used in guided tissue regeneration to prevent the migration of epithelial and connective cells, and to allow the proliferation of slower-growing osteogenic and periodontal connective tissues under the membrane (Dahlin *et al.*, 1988; Crump *et al.*, 1996). A review of membranes suggested five essential criteria for their successful design: 1) tissue integration; 2) cell-occlusivity; 3) clinical manageability; 4) spacemaking; and 5) biocompatibility (Scantlebury, 1993).

Collagen membranes have shown the same efficacy as non-resorbable membranes in guided tissue regeneration (GTR) procedures (Bunyaratevej and Wang, 2001). They eliminate the need for a second surgical procedure to remove non-resorbable membranes (Sandberg *et al.*, 1993).

Several indications for their use in regenerative applications in the oral cavity have been described. These include immediate or delayed extraction of socket defects, bone defects or fenestration, lateral or vertical ridge augmentation and sinus floor elevation (Buser *et al.*, 1999).

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The membrane must be rigid enough to maintain an area for tissue formation for sufficient time (Zellin *et al.*, 1995). Inflammatory reactions caused by decomposition and resorption of the membrane should not interfere with tissue healing (Jansen *et al.*, 1995; Piattelli *et al.*, 1996).

Changes in cell proliferation can be explained by differences in surface topography, surface characteristics and pore size (Kasaj *et al.*, 2008).

In this context, the aim of this study was to analyze and compare the morphology and composition of three commercially available resorbable collagen membranes: Bio-Gide®, OsseoGuard™ and Surgidry Dental F, using scanning electron microscopy (SEM), a spectrometer and energy dispersive X-ray software EDX.

Materials and methods

Membranes

Three types of commercially available collagen membranes, with different composition and coming from different animals, were used: (1) Dental Surgidry F (bovine type I collagen; Technodry Lyophilized Medical Ltd., Belo Horizonte, Brazil), (2) Bio-Gide® (porcine collagen type I and III; Geistlich Pharma, Wolhusen, Switzerland) and (3) OsseoGuard™ (bovine collagen type I; Collagen Matrix, Inc., Franklin Lakes, USA).

Scanning electron microscopy (SEM)

The membranes were cut into 8 mm x 8 mm pieces and mounted on an aluminum support with the aid of adhesive tape. The membrane specimens were subjected to a metallization process in a vacuum chamber (Denton Vacuum Desk V, Denton Vacuum, Moorestown, USA) and covered with gold microparticles. One blinded, experienced and trained examiner analyzed the specimens, using a scanning electron microscope (SEM, Jeol JSM - 6510LV). The evaluation protocol was based on the exam of the structure of the collagen surface and morphology of the top, bottom, and side surfaces of the membranes. Digital images were obtained by detecting signals of secondary electrons emitted by the samples when they were exposed to an electron beam.

Analysis of the atomic composition of the membranes was performed using a spectrometer and energy dispersive X-ray software EDX-720 Shimadzu associated with the images of SEM (Jeol JSM - 6510LV). The interaction between the electron beam and the samples produced a variety of emissions, X-rays being one of them. The detector of the dispersive energy absorbed and separated the characteristic X-rays of each element. The semi-quantitative analysis of each atom in the analyzed membrane samples, and the results, were expressed as histograms.

Results

SEM

SEM was performed to analyze the external (facing the soft tissue), internal (facing the bone or tooth root) and lateral surfaces of the collagen membranes. The magnifications were chosen according to the best images provided by the microscope.

The results showed that the morphology of the surfaces varied considerably among the collagen membranes analyzed.

The Bio-Gide® membrane had an irregular side surface with several circular depressions arranged homogeneously throughout its extension (*Figure 1A*). The membrane thickness was uniform and measured approximately 0.73 mm. The outer surface was smoother (*Figure 1B*), with no visible pores even at large magnification (650X), and occasional focal areas showing collagen fibers with low thicknesses (*Figure 1C*). The inner surface appeared to be very heterogeneous and irregular, with smooth and fibrous areas (*Figures 1D and 1E*). The smooth areas showed scale formations without visible pores under the SEM (*Figure 1F*), whereas the fibrous areas exhibited a wide mesh of intertwined fibers of varying thicknesses, forming retentive regions (*Figure 1G*).

The OsseoGuard™ membrane presented an irregular lateral surface with two distinct layers (*Figure 2A*). The outermost layer was thicker and more compact while the internal layer, formed by interconnecting septa, was extremely porous (*Figure 2B*). The thickness varied from 0.54 mm to 0.75 mm under a microscope (*Figure 2A*). The outer surface appeared to be very smooth and homogeneous (*Figure 2C* and *2D*) with no visible pores, even at large magnification (650X; *Figure 2E*). Small and dispersed polyhedral structures adsorbed on the membrane surface (*Figure 2E*) were also noted. The inner surface was composed of numerous rectangular cavities arranged symmetrically (*Figure 2F*), measuring approximately 420 μm × 150 μm. The centers of the cavities had smooth surfaces and fibers with varying thicknesses that formed a mesh toward the bottom of the holes (*Figure 2G*).

Analysis of the Surgidry Dental F membrane revealed lateral surfaces composed of several superimposed layers, with depressed ovals, and of varying thicknesses averaging approximately 0.32 mm (*Figures 3A* and *3B*). The external surface was rough and filled with structures of varying forms, but had a smooth bottom (*Figure 3C* and *3D*). Pores were not observed, even at larger magnification (650X; *Figure 3E*). The inner surface showed numerous fibers of varying thicknesses, arranged in several directions, forming an interconnected mesh (*Figure 3F* and *3G*).

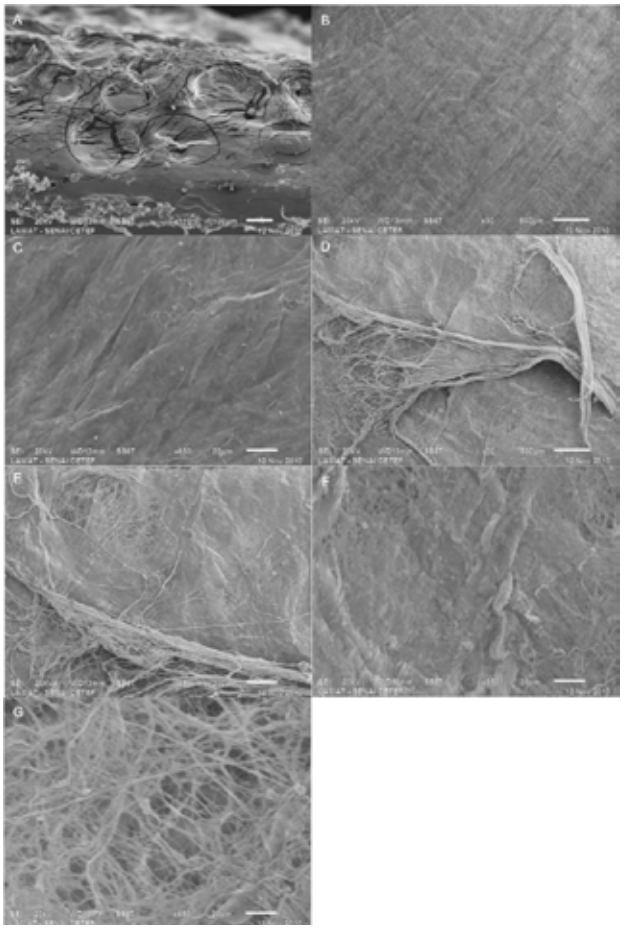

Figure 1. A) The Bio-Gide® membrane showed an irregular lateral surface with several circular depressions arranged homogeneously throughout its length. The thickness of the membrane was uniform and measured approximately 0.73 mm. B) The outer surface of Bio-Gide® was smoother than the lateral surface. (C) The outer surface of Bio-Gide® showed no visible pores, even at a large magnification (650X); there were occasional focal areas exhibiting collagen fibers of small thickness. D and E) The inner surface of Bio-Gide® appeared heterogeneous and irregular, with smooth as well as extremely fibrous areas. F) The smooth areas of the internal surface of Bio-Gide® presented scale formations without visible pores under the scanning electron microscope. G) The fibrous areas of the inner surface of Bio-Gide® exhibited wide crosslinked fibers of various thicknesses, forming very retentive areas.

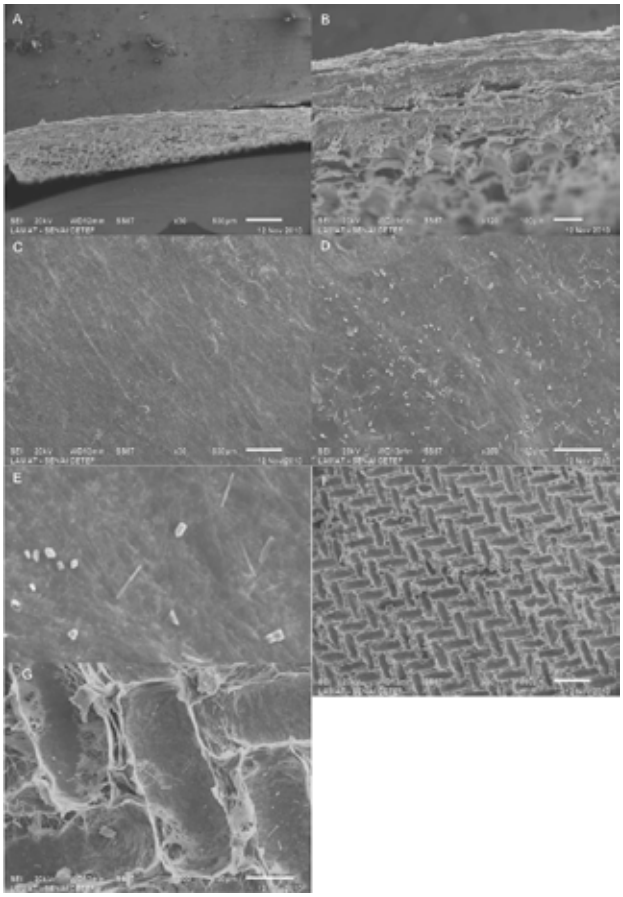

Figure 2. A) The OsseoGuard™ membrane presented an irregular lateral surface with two distinct layers. Its thickness varied from 0.54 mm to 0.75 mm. B) The outermost layer of OsseoGuard™ appeared to be denser and more compact than the innermost layer, formed by interconnecting septa, and was extremely porous. C and D) The outer surface of OsseoGuard™ appeared smooth and homogeneous. E) Pores were not visualized in the external surface of OsseoGuard™ even at a large magnification (650X). Small dispersed polyhedral structures were adsorbed on the surface. F) The internal surface of OsseoGuard™ consisted mainly of numerous, symmetric, rectangular perforations, measuring approximately 420 µm × 150 µm. G) The centers of the perforations in OsseoGuard™ had smooth surfaces, and the septa had fibers of varied thicknesses, forming crosslinks toward the bottom of the perforations.

Energy dispersive spectrometry

Through energy dispersive spectrometry (EDS), it was possible to partly identify the chemical composition of each membrane. Data were expressed as percentages relative to the weight. Carbon and nitrogen atoms were not considered because their low atomic numbers were not precisely quantified. For each membrane, two areas were marked on the inner surface to analyze the chemical composition. Differences in the chemical compositions of the membranes were observed. In particular, the presence of a high percentage of niobium (Nb), a rare element, was observed in the weight of the Surgidry and OsseoGuard™ membranes, relative to other elements. The Bio-Gide® membrane showed a greater proportion of calcium (Ca) and aluminum (Al).

Discussion

Optimized membrane design and composition could prevent or minimize inflammatory reactions, fill and maintain blood clots, and avoid invasion by undesirable cells (Hardwick *et al*., 1995). Furthermore, the degradation time of collagen membranes must be sufficient to permit periodontal tissues or bone regeneration (Schlegel *et al*., 1997).

Table 1. Chemical composition of Bio-Gide®, OsseoGuard® and Surgidry Dental F® membranes, using energy dispersive spectrometry

	Al	*Ca*	*Rb*	*Tc*	*Hg*
Bio-Gide (a1)	34.900	65.100			0.000
Bio-Gide (a2)		47.826	52.174	0.000	0.000

	N	*O*	*Al*	*K*	*Rb*	*Nb*	*Tc*	*Hf*	*Ta*
OsseoGuard (a1)	0.000	52.960	1.357	6.543		31.559	5.979	0.118	1.484
OsseoGuard (a2)	0.000	42.656		5.513	3.644	39.803	8.385		

	Na	*Al*	*P*	*Cl*	*Ca*	*Rb*	*Nb*	*Pd*
Surgidry (a1)	13.205			10.586	3.179	6.916	66.114	0.000
Surgidry (a2)	8.790	0.153	17.716	30.967	7.312		35.062	

Values expressed as a percentage of the total weight, excluding carbon and nitrogen atoms.
(a1, a2) – area 1 and area 2

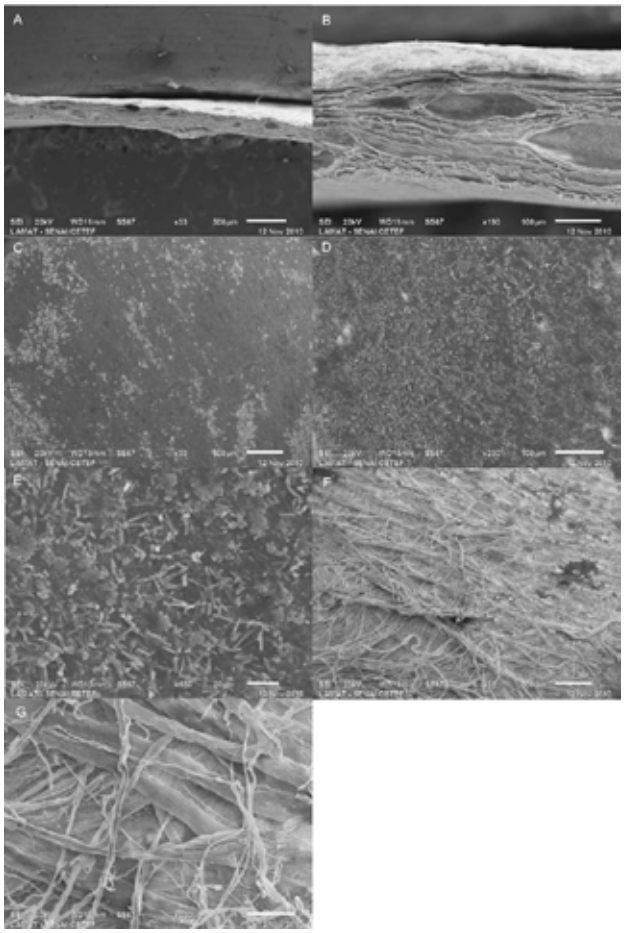

Figure 3. A and B) Analysis of the membrane showed that the lateral surface was composed of several superimposed layers, exhibiting oval depressions and variable thicknesses with an average of approximately 0.32 mm. C and D) The outer surface of Surgidry Dental F was rough with a flat bottom, and filled with structures of various shapes, adsorbed on the plain background. E) Pores on the outer surface of Surgidry Dental F were not visualized even at large magnification (650X). F and G) The inner surface of Surgidry Dental F presented numerous fibers with various thicknesses, arranged in several directions and forming highly interconnected crosslinks.

Moreover, the physical characteristics of membranes such as surface topography, porosity, stiffness and chemical composition of the membrane barrier can influence guided tissue or guided bone regeneration (de Santana *et al.*, 2010). In this context, the objectives of this study were to evaluate the surface morphology of different resorbable collagen membranes in an attempt to correlate the surface architecture and chemical composition of a membrane as a biocompatible barrier.

Osteopromotive efficacy is different among biodegradable membranes that are chemically similar (Zellin *et al.*, 1995). SEM revealed considerable differences in the architecture and chemical composition of the membranes used in this study. The outer surfaces of the Bio-Gide® (*Figure 1B*) and OsseoGuard™ (*Figure* 2C) membranes showed a more homogeneous and flat architecture than that of the Dental Surgidry F membrane (*Figure 3E*). The latter presented numerous polyhedral structures of different shapes adsorbed to its surface, forming a relatively rough area. Although the OsseoGuard™ membrane presented a smooth outer surface, it also showed sparsely adsorbed polyhedral structures (*Figure 2E*).

These structures were fewer than those observed on Surgidry Dental F, and did not form an area for retentive cells. The outer surface constitutes a physical barrier to the migration of epithelial cells or tissues, but is permeable to the passage of macromolecules necessary for providing nutrition for tissue repair in the underlying membrane (de Santana *et al.*, 2010). In this context, none of the three evaluated collagen membranes showed presence of pores on their surfaces, which would allow cell migration, since tissues and cells have an average diameter of approximately 15 μm to 20 μm.

Another important characteristic of a membrane barrier is sufficient stiffness to avoid sagging and deformation in the repair area, allowing maintenance of the blood clot volume underlying bone formation.

Furthermore, the thickness and composition of the membrane should allow maintenance of the surgical area for several weeks until a bone structure can be formed in the covered area, avoiding invasion by cells of the soft tissues, which grow faster than mineralized bone tissues (Bunyaratavej and Wang, 2001; de Santana *et al*., 2010; Stavropoulos *et al*., 2002).

The results of this study revealed massive thickness of the lateral surface of the Bio-Gide® membrane, with narrow circular depressions and cracks (*Figure 1A*). The OsseoGuard™ membrane showed two distinct layers: a superficial and very compact layer, occupying most of the membrane thickness, and another layer at the underside, presenting a porous aspect (*Figure 2B*). The Surgidry Dental F membrane showed the presence of thin, overlapping layers with spaces between them, with a less massive structure than the other membranes (*Figure 3B*).

It was also observed that the thicknesses of the evaluated membranes ranged from approximately 0.3 mm to 0.7 mm. The Bio-Gide® membrane was thickest, followed by the OsseoGuard™ and Surgidry Dental F membranes, with the latter showing a thickness approximately half that of the former two. The thickness of the Surgidry Dental F membrane was less than that of the two other membranes, suggesting a shorter period of stability in the tissues. However, it is notable that the composition of collagen (collagen type) and the manufacturing process can influence the resorption rate of the membrane. Several cross-linking techniques of collagen fibers such as the use of ultraviolet light, hexamethylenediisocyanate, glutaraldehyde plus radiation, and diphenylphosphorylazide have been developed with the goal of slowing down the degradation of membranes in tissue implantation sites (Bunyaratavej and Wang, 2001).

Large differences were observed between the inner surfaces of the three membranes. Bio-Gide® exhibited highly heterogeneous surfaces (*Figure 1D* and *1E*) with extensive flat areas (*Figure 1F*) alternating with porous ones, and consisting of many interconnected fibers of various sizes, arranged in various directions (*Figure 1G*). OsseoGuard™ exhibited uniformly porous surfaces formed by undercut rectangular structures (*Figure 2F*) along its length. Surgidry Dental F exhibited surfaces rich in fibers of various thicknesses, forming an extensive, three-dimensional mesh with spaces of various sizes (*Figure 3F* and *3G*).

Kasaj *et al.* (2008) compared the surfaces of resorbable collagen membranes and observed, using SEM, that the Bio-Gide® membrane had a smaller fiber area in the internal surface and that these fibers were thinner than those in the Tutodent® and Resodent® membranes. Results of the present study also showed that Bio-Gide® exhibited an internal surface with a smaller fiber area (*Figure 1D*), and these thin fibers formed structures with pore sizes that were much smaller (*Figure 1G*) than those in the OsseoGuard™ (*Figure 2G*) and Dental Surgidry F (*Figure 3G*) membranes.

In this study, the membranes showed differences in structure and chemical composition. Zellin *et al.* (1995) found differences in the surface architecture of several resorbable and non-resorbable membranes, analyzed using SEM, which showed apparent chemical similarity. The study by Kasaj *et al.* (2008) also examined the ability of three collagen membranes (Tutodent®, Resodont® and Bio-Gide®) to sustain the proliferation of osteoblastic cells, gingival fibroblasts and periodontal ligament *in vitro*. The results showed that, although all the membranes were composed of collagen, cell proliferation was significantly different among them. The proliferation of fibroblasts and osteogenic cells was lower on the Bio-Gide® membrane compared with the other two. According to these studies, differences in the surface topography and pore size of the membranes, observed using SEM, may contribute to differences in their effect on cell proliferation. In addition, the discrepancies observed between the collagen membranes could be explained by the difference in the dissolution of the membrane materials (Zhao *et al*., 2000). These authors performed histological analyses of different resorbable membranes implanted in the subcutaneous tissue of rats, and found that the Bio-Gide® membrane dissolved in the early phase of the experiment, and showed inflammatory giant cells (Zhao *et al*., 2000).

In the present study, the results of the chemical analysis using EDS showed the presence of chemical elements and different proportions of these elements among the membranes. Elements such as technetium (Tc), tantalum (Ta), rubidium (Rb), and Nb were detected. These findings should be interpreted with caution, as they express the percentage of each element within the total sample weight but do not take into account carbon and nitrogen atoms. The latter two are major components of collagen molecules; the actual percentage of other elements can be significantly lower. Nevertheless, the presence of rare and unexpected elements such as Nb and Al at proportions higher than other common elements, such as chloride and potassium, is an interesting finding and may represent contaminants derived from the membrane manufacture process. However, a limitation of this technique is that the EDS device cannot distinguish and quantify atoms with atomic numbers lower than or equal to 6 (carbon).

In conclusion, this study shows that different types of resorbable collagen membranes exhibit different morphology and chemical composition. This could lead to differences in the mode and time of resorption of the membranes used for guided tissue regeneration procedures.

References

Bunyaratavej P and Wang HL. Collagen membranes: A review. *Journal of Periodontology* 2001; **72**:215-229.

Buser D, Dula K, Hess D, Hirt HP and Belser UC. Localized ridge augmentation with autografts and barrier membranes. *Periodontology 2000* 1999; **19**:151-163.

Crump TB, Rivera-Hidalgo F, Harrison JW, Williams FE and Guo IY. Influence of three membrane types on healing of bone defects. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* 1996; **82**:365-374.

Dahlin C, Linde A, Gottlow J and Nyman S. Healing of bone defects by guided tissue regeneration. *Plastic and Reconstructive Surgery* 1988; **81**:672-676.

de Santana RB, de Mattos CM, Francischone CE and Van Dyke T. Superficial topography and porosity of an absorbable barrier membrane impacts soft tissue response in guided bone regeneration. *Journal of Periodontology* 2010; **81**:926-933.

Hardwick R, Hayes BK and Flynn C. Devices for dentoalveolar regeneration: An up-to-date literature review. *Journal of Periodontology* 1995; **66**:495-505.

Jansen JA, de Ruijter JE, Jassen PTM and Paquay YGCJ. Histological evaluation of a biodegradable polyactive®/hydroxyapatite membrane. *Biomaterials* 1995; **16**:819-827.

Kasaj A, Reichert C, Götz H, Röhrig B, Smeets R and Willershausen B. *In vitro* evaluation of various bioabsorbable and nonresorbable barrier membranes for guided tissue regeneration. *Head & Face Medicine* 2008; **4**:22.

Piattelli A, Scarano A, Russo P and Matarasso S. Evaluation of guided bone regeneration in rabbit tibia using bioresorbable and non-resorbable membranes. *Biomaterials* 1996; **17**:791-796.

Sandberg E, Dahlin C and Linde A. Bone regeneration by the osteopromotion technique using bioabsorbable membranes: An experimental study in rats. *Journal of Oral and Maxillofacial Surgery* 1993; **51**:1106-1114.

Scantlebury TV. 1982-1992: A decade of technology development for guided tissue regeneration. *Journal of Periodontology* 1993; **64**:1129-1137.

Schlegel AK, Möhler H, Busch F and Mehl A. Preclinical and clinical studies of a collagen membrane (Bio-Gide®). *Biomaterials* 1997; **18**:535-538.

Stavropoulos F, Nale JC and Ruskin JD. Guided bone regeneration. *Oral and Maxillofacial Surgery Clinics of North America* 2002; **14**:15-27.

Tal H, Moses O, Kozlovsky A and Nemcovsky C. Bioresorbable collagen membranes for guided bone regeneration. *INTECH - Open Access Publisher*, 2012.

Zellin G, Gritli-Linde A and Linde A. Healing of mandibular defects with different biodegradable and non-biodegradable membranes: An experimental study in rats. *Biomaterials* 1995; **16**:601-609.

Zhao S, Pinholt EM, Madsen JE and Donath K. Histological evaluation of different biodegradable and non-biodegradable membranes implanted subcutaneously in rats. *Journal of Cranio-Maxillofacial Surgery* 2000; **28**:116-122.