Original Article

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Dentistry Section

Comparison of Physiochemical Properties and Biocompatiblity of Two Commercially Available Natural Xenogeneic Collagen Membranes: In-vitro Study

A GNANAMANI[1], VAMSI LAVU[2], RESHMA ACHU JOSEPH[3], R THILAGAM[4], SK BALAJI[5]

ABSTRACT

Introduction: Physical factors like stiffness and surface features are among the characteristics that affect the performance of barrier membranes and determine the results of regenerative processes. A perfect equilibrium between the membrane's rigidity and mechanical stability guarantees effective periodontal regeneration. The study's novelty lies in comparing the physical characteristics, namely morphology, tensile strength, wettability, and biological characteristics, namely biocompatibility and enzyme resistance properties, of the Fix-GideTM membrane against the gold standard membrane, Bio-Gide®.

Aim: To explore the physical and biological properties of two commercially available barrier membranes in oral tissue regeneration.

Materials and Methods: The present in-vitro study compared two commercially available membranes, namely Bio-Gide® and Fix-GideTM. Both membranes are bilayered resorbable membranes, with Bio-Gide composed of porcine dermis Type-I and III collagen and Fix-GideTM of bovine origin. The study was conducted at the Central Leather Research Institute, and the membranes were procured from Sri Ramachandra Institute of Higher Education and Research. Morphological characterisation was done using Scanning Electron Microscopy (SEM). Physical properties were evaluated using a tensile strength test, enzyme resistance test, and wettability measurement. Biocompatibility assessment was also performed. The Statistical Package for Social Sciences (SPSS) software was used to run the Mann-Whitney U test to analyse the statistical data obtained in the enzyme resistance test.

Results: Biocompatibility assessment showed no cytotoxic profile of both membranes, portraying their biocompatible nature. Morphological analysis using SEM showed the surface of the Bio-Gide® membrane to be considerably smoother than the Fix-GideTM membrane. Both membranes, however, have fibrous and porous features on their inner surfaces. Tensile strength assessment found that the percentage of elongation was better with Bio-Gide (1.7±0.4 and 4.8±0.4) when compared to Fix-Gide (15.8±0.2 and 2.2±0.2) in both wet and dry states, respectively. The enzyme resistance test evaluated in dry and wet settings showed that the membranes, namely, Bio-Gide® membrane exhibited around 29±2% of degradation, whereas the Fix-GideTM exhibited only 18±2%. These mechanical profiles exhibited that the membranes has appreciable differences, although there wasn't a statistically significant difference between them (p=0.68). According to wettability studies, Bio-Gide is hydrophilic, but Fix-GideTM is hydrophobic.

Conclusion: The observations of the present study showed that Fix-Gide had comparable physio-biological properties to that of the Bio-Gide membrane. This supports the suitability of the use of both membranes for various oral tissue regeneration procedures.

Keywords: Guided tissue regeneration, Scanning electron microscopy, Tissue scaffolds, Tissue engineering

INTRODUCTION

Periodontal disease is a multi-factorial condition that causes soft tissue damage and the progressive destruction of the periodontium, resulting in complete tooth loss, pocket formation, and concomitant alveolar bone resorption [1,2]. Regeneration of the periodontal attachment apparatus remains the holy grail for clinicians worldwide. For years, several therapeutic modalities have been involved in periodontal repair and regeneration, and as evidenced by the literature, these approaches hold limitations without the use of adjunctive materials [3]. Recently, the field of tissue engineering has made considerable progress using multiple biomaterials in the form of barrier membranes, osseous grafts, growth factors, and 3D scaffolds [4,5]. Guided Tissue Regeneration (GTR) and Guided Bone Regeneration (GBR) are common surgical procedures that majorly rely on barrier membranes to improve the prognosis in the regeneration of periodontal tissue, including the bifurcation area and bone augmentation in implant therapy [6,7]. Barrier membranes implanted over the tissue defect allow periodontal ligament cells to proliferate while concurrently eliminating the growth of epithelium and connective tissue in the bone compartment [8,9].

Barrier membranes are classified into two major types: resorbable and non-resorbable. Resorbable membranes are natural materials like collagen, acellular dermal matrix, and oxidised cellulose mesh, whereas non-resorbable membranes are manufactured from synthetic polymers, metals, or composites, including cellulose acetate, polytetrafluoroethylene, expanded polytetrafluoroethylene, titanium mesh, etc. [8]. Resorbable membranes remain superior to non-resorbable ones in terms of biodegradability and are regarded as the first choice of material [10,11].

The basic properties required for barrier membranes include high biocompatibility, low permeability to cells, tight adhesion to host tissues, moderate mechanical strength, stable storage stability, and good handling properties for clinical use [12,13]. To date, several clinical outcomes are available on various types of barrier membranes [14,15]. However, most practitioners prefer resorbable membranes, especially collagen membranes, because of their biocompatibility, easy handling, and gradual degradability at the defect site without the need for additional surgery to remove them [16].

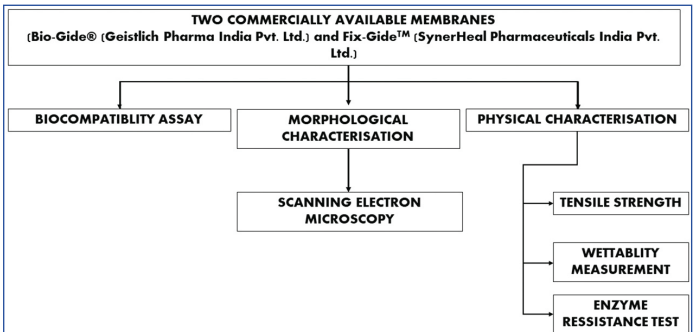
Among the properties that influence the success of barrier membranes, physical properties such as stiffness and surface

characteristics decide the regenerative outcomes. Though the available reports describe the general properties of the membranes [9,12], biological characterisation inputs on barrier membranes are missing in the public domain. In GBR procedures, several clinical reports prove that the use of barrier membranes with insufficient stability and inadequate space maintenance leads to the displacement of grafts, resulting in reduced new bone growth [17,18]. A recent consensus report conducted by Elgali I et al., showed that a balance between the mechanical stability and the stiffness of the membrane ensures proper regeneration [6]. Among the properties that influence the success of barrier membrane elasticity, surface characteristics influence the regenerative outcomes. Therefore, the ideal membrane should be sufficiently rigid to withstand the compression generated by peripheral soft tissues and possess the required degree of plasticity to be easily contoured and moulded into the desired shape to conform to the defect. A balance between the mechanical stability and the stiffness of the membrane ensures proper periodontal regeneration [6].

Currently, there are still insufficient guidelines for the types of barrier membranes in GTR and GBR procedures. Although the properties such as composition, morphology, and physiochemical profile of the Bio-Gide® membrane have already been explored [19], there is a lack of literature on the comparison of the physical and biological properties of Fix-GideTM with the gold standard membrane Bio-Gide®, which remains the novelty of the study. Therefore, understanding the biological and mechanical properties of the available membranes becomes important for dentistry. Hence, the present study is focused on evaluating and comparing the physio-biological properties and biocompatibility of two commercially available collagen membranes which facilitate their suitability for oral tissue regeneration.

MATERIALS AND METHODS

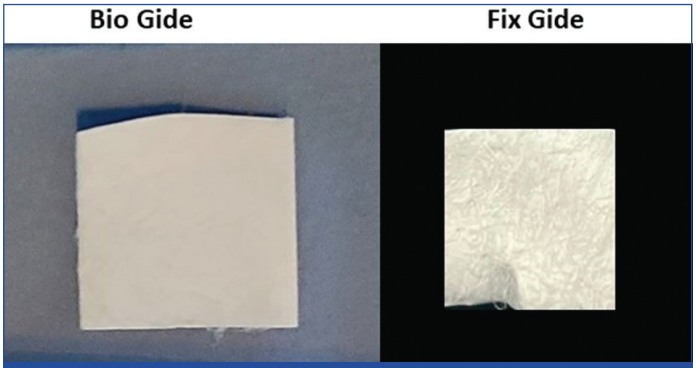
The in-vitro study was conducted over a period of one month at the Central Leather and Research Institute, and the membranes were procured from Sri Ramachandra Institute of Higher Education and Research. Two commercially available resorbable collagen membranes, namely Bio-Gide® (Geistlich Pharma India Pvt., Ltd.) and Fix-GideTM (SynerHeal Pharmaceuticals India Pvt., Ltd.), which are used in GTR/GBR procedures, were chosen for the study. Bio-Gide is made of porcine dermis Type-I and III collagen, while Fix-GideTM is made from bovine collagen. Both membranes have a bilayered structure, with the first dense layer serves as a tissue barrier against soft tissue, and the second layer being spongy and porous, allowing for the integration of bone tissue. The biocompatibility of both membranes was assessed. Following which the physical and morphological characterisation [Table/Fig-1].



[Table/Fig-1]: Methodology.

Biocompatibility assesment: The NIH 3T3 cells received from Child's Trust Hospital, Chennai, were passaged and grown in Dulbecco's modified eagle's medium (Lonza India Pvt., Ltd.) supplemented with 10% foetal bovine serum (Himedia India Pvt., Ltd.) and 100 U/mL penicillin-streptomycin (Lonza India Pvt., Ltd.). The cells were propagated in a 75-cm^2 T-flask in a humidified atmosphere containing 5% CO_2 at 37°C. Before experiments, the membranes (10×10 mm) were exposed to 70% ethanol, followed by a sterile Phosphate Buffer Solution (PBS) wash. Cells were seeded at a concentration of 0.5×105 cells/well in a 24-well plate and incubated under standard parameters. Fresh medium was replaced at 48-hour intervals. After five days of incubation, a live-cell fluorescence staining procedure was followed to ensure the adhesion to the membranes. In brief, membranes were washed with PBS to remove the media traces and also to avoid artifacts during staining, then incubated in a live-cell tracker stain with 1 µM calcein-AM for 30 minutes. Excess stain was washed with PBS, and images were acquired using an inverted fluorescence microscope (Euromex) using a blue filter. The experiments were run in duplicate, and the images were captured.

Morphological Characterisation: Scanning Electron Microscope (SEM): Three dry samples of each membrane (Bio-Gide and Fix Gide) were measured and sliced to a precise size using scissors to assess their morphological features under SEM [Table/Fig-2]. The cross morphology of the Bio-Gide® and Fix GideTM membranes was evaluated using a SEM (Phenom 1817 by Thermo Fisher Scientific Pvt. Ltd., the United States). The scaffolds were sputtered with gold using a Hitachi S-3400 before analysis. The coated samples were imaged, and micrographs were taken at different magnifications.



[Table/Fig-2]: Images of dry samples of each membrane.

Physical Characterisation

Tensile strength: The mechanical profile of the Bio-Gide® and Fix GideTM membranes were assessed using a tensile tester (Instron India Pvt. Ltd.) equipped with a load cell with a maximum range of 100N. The barrier membranes were cut into standard-sized pieces (10×25 mm) and placed on a custom-made mounting plate. The samples were tested in both dry and wet conditions. To achieve wet samples, the membranes were soaked in a saline solution for 30 minutes according to the manufacturer's instructions. An equal number of samples from each group (n=3) from different lots were tested in dry and wet conditions at a crosshead speed of 1 mm/min until the membrane tore. The tensile strength of the membranes was plotted as a strain vs. stress graph.

Wettability Measurement: To analyse surface energy and wettability, the static contact angle of the rough/smooth surface of the barrier membranes was assessed using the sessile drop method and analysed with a contact angle meter. Three microliters of Milli-Q water were dropped on the surface of the membranes, and the contact angle was measured at 4 and 30 seconds after applying the Laplace-Young fitting of the drop profile with software. The test was performed in triplicates for each group using membranes from different lots. The materials were cut, and a drop of water was placed over the material to assess hydrophilicity.

Enzyme Resistance Test: The in-vitro degradation profile was assessed by exposing the membrane samples to 0.025% Trypsin-Ethylene Diamine Tetra-Acetic Acid(EDTA) (Lonza India Pvt. Ltd.) and incubated at 37°C for 24 hours. The reduction in mass was observed by withdrawing the samples at regular time intervals of 4, 10, 18, and 24 hours and drying them at 37°C. The rate of

degradation was calculated from the observed mass. The remaining mass or percentage of degradation was calculated according to the following equation: Percentage Remaining mass=Final weight / Initial weight ×100.

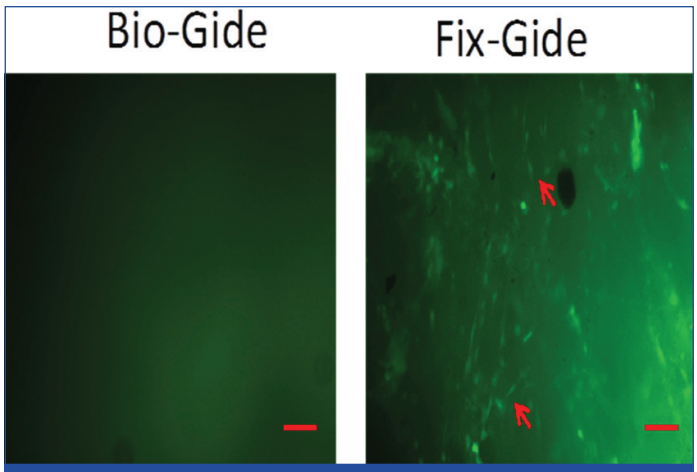
STATISTICAL ANALYSIS

Statistical analysis for the data obtained from the enzyme resistance test comparing both membranes in wet and dry states was done through Mann-Whitney U test. The statistical analysis was performed using Statistical Package of Social Sciences (SPSS) version 20.0 (SPSS for Windows, Version 20.0, Chicago, IL).

RESULTS

Biological Properties

Biocompatibility assessment: A biocompatibility assessment was made between two commercially available membranes. [Table/Fig-3] illustrates cell adhesion in the Bio-Gide® and Fix-GideTM membranes. Between the two membranes, the Fix-GideTM membrane promoted cell adhesion with the presence of spindle-shaped cells. However, the nil cytotoxic profile implies the biocompatible nature of both membranes. The biocompatibility appears to be similar. In regards to cell exclusion, the Bio-Gide membrane showed lower cell adhesion than the Fix-Gide membrane.

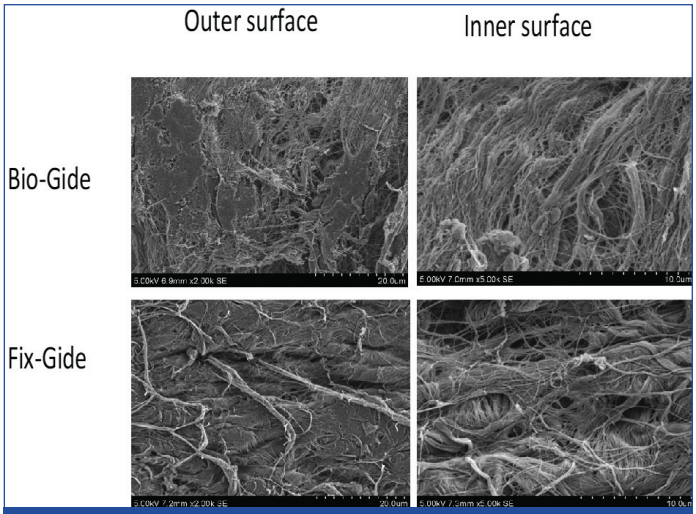


[Table/Fig-3]: NIH 3T3 Cell adhesion in Bio-Gide® and Fix-GideTM membranes.

Physical Properties

Scanning Electron Microscopy (SEM): The Bio-Gide® membrane displayed a relatively smoother outer surface with no visible pores even at higher magnifications [Table/Fig-4]. Occasional focal areas showed interconnected collagen fibres. The inner surface appeared irregular and very heterogeneous, mainly possessing high fibrous networks. The fibrous areas were tightly interwoven and exhibited a wide mesh of varying thickness [Table/Fig-4]. The Fix-GideTM membrane showed a rough outer surface with irregular and tightly packed structures than the Bio-Gide® membrane. It also possessed large-sized fibers that run all over the surface. The inner surface was similar to Bio-Gide®, which is highly fibrous interwoven networks heterogeneously distributed and no pores. Thus, none of the two evaluated membranes showed pores on their surface, which would allow cell migration, since cells have an average diameter of 15 µm to 20 µm [6]. Exceptionally, the Fix-GideTM membrane allowed cell adhesion, which may be due to the presence of an uneven grooved surface, whereas, the homogenous flat surface architecture of Bio-Gide® failed to promote cell adhesion.

Tensile strength test: Following testing in a dry state, the Fix-Gide sample exhibited a tensile strength of 15.83 MPa, showing higher stability in terms of strength compared to Bio-Gide (1.69 MPa) in the dry state. For experiments in wet conditions, the samples were



[Table/Fig-4]: Scanning Electron Microscopy (SEM) of the membranes: Both the outer (scale 10 µm) and inner surface (scale 20 µm) of Bio-Gide® and Fix-GideTM.

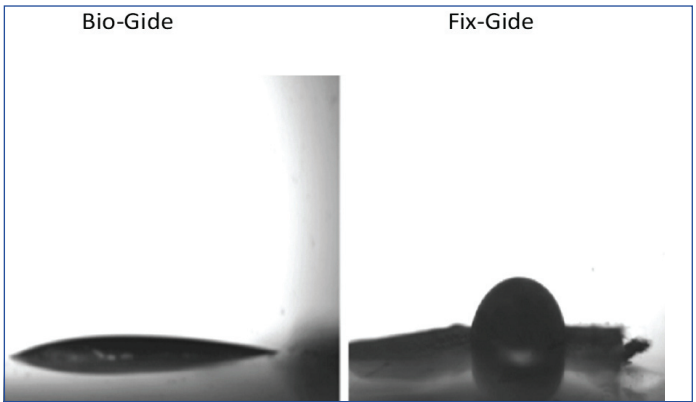
immersed in PBS for 24 hours. The strength of the membranes became weak and exhibited values of 4.8 MPa and 2.2 MPa for Fix-GideTM and Bio-Gide®, respectively. The percentage of elongation was better with Bio-Gide (46%, 96.79%) compared to Fix-Gide (33.91%, 24.17%) in both wet and dry states, respectively [Table/Fig-5].

Materials	Dry condition		Wet condition	
	Tensile strength (MPa)	Break elongation (%)	Tensile strength (MPa)	Break elongation (%)
Bio-Gide®	1.7±0.4	96.8±2.4	4.8±0.4	33.9±2.4
Fix Gide™	15.8±0.2	24.2±1.2	2.2±0.2	46±2

[Table/Fig-5]: Mechanical properties of Bio-Gide® and Fix-GideTM at two different conditions.

Enzyme resistance test: In the initial four hours of enzyme exposure, the Bio-Gide® membrane exhibited around 29±2% degradation, whereas the Fix-GideTM exhibited only 18±2% degradation. Extending the experimental period to 24 hours, >80% reduction in mass was observed with the Bio-Gide® membrane and ~70% mass reduction with the Fix-GideTM membrane.

Wettability capacity of membranes: At the experiments, the Bio-Gide® membrane absorbed water at once and became infeasible for measurement, concluding that the membrane was extremely hydrophilic, whereas the Fix-GideTM membrane exhibited 88.32±1.2% hydrophobicity [Table/Fig-6], mainly attributed to the excellent strength observed in the tensile strength measurement.



[Table/Fig-6]: Wettability measurements on (i) Bio-Gide®, (ii) Fix-Gide™.

DISCUSSION

Biological membranes are used as a barriers to enable cell exclusion for regenerative procedures such as GBR or GTR. The proper selection of these membranes is aided by an understanding of the physical and chemical characteristics of the membrane. The two variants of collagen membranes considered in the

present study display distinctive features in their respective clinical applications. Each membrane has a distinct biological effects on cellular proliferation, adhesion, and tissue healing. Porcine-derived membranes promote cellular proliferation by providing a scaffold for cell attachment and growth. The porous structure of porcine-derived membranes facilitates nutrient exchange and cell migration, supporting tissue regeneration. Additionally, the biochemical composition of these membranes can stimulate cellular responses, enhancing tissue healing processes such as angiogenesis and extracellular matrix deposition. On the other hand, bovine-derived membranes possess biocompatible properties conducive to tissue regeneration. These membranes promote cellular adhesion by offering a surface for cell attachment and spreading. The mechanical strength and stability of bovine-derived membranes provide structural support during tissue healing, particularly in GBR applications where bone regeneration is critical. Additionally, the biochemical cues present in bovine-derived membranes can modulate cellular behaviour, influencing processes such as osteogenesis and collagen synthesis.

The choice between these membranes often depends on factors such as their source, mechanical properties, and intended clinical application. The porcine-derived membrane possesses the ability to resist long-term degradation, which helps achieve a significant amount of bone growth effect in clinical applications such as GBR procedures by virtue of its excellent histocompatibility and reliable biosafety [19]. On the other hand, bovine-derived membranes have the capacity to promote tissue healing, making them suitable choices for procedures such as GTR [20-22]. Therefore, the membrane's surface characteristics and chemical composition determine the potential electrostatic interactions, which in turn orchestrate the various biological processes that occur in clinical settings.

The methodology used in this investigation to assess the physical characteristics of the two membranes was standard and had previously been used in a prior study by Caballé-Serrano J et al., [22]. In-vitro cultures of primary Human Gingival Fibroblasts (HGF), Periodontal Ligament Fibroblasts (PDLF), and Human Osteoblast-like (HOB) cells were used by Kasaj A et al., [23] to examine the biological effects of various bioabsorbable and non-resorbable membranes. The authors noticed that, compared to non-resorbable PTFE membranes, bioabsorbable membranes were more effective at promoting cellular proliferation.

The present study was found to be in agreement to the study done by Kasaj A et al., as the biocompatibility between the Fix-Gide membrane and Bio-Gide membrane was similar. Upon analysing the mechanical properties in the present study, the Fix-GideTM membrane exhibited a greater tensile strength both in a dry and wet state when compared to Bio-Gide. In wettability measurements, the Fix-GideTM membrane showed higher hydrophobicity, which mainly attributes to the excellent strength observed in the tensile strength measurement. This study findings were in line with the studies done by Kasaj A et al., and Raz P et al., [23,24].

In terms of analysing wettability measurements, the Fix-GideTM membrane showed higher hydrophobicity, which mainly attributes to the excellent strength observed in the tensile strength measurement. The results of the present study was in agreement the studies conducted by Shi X et al., and An YZ et al., [19,25]. Exceptionally, the Fix-GideTM membrane allows cell adhesion, which may be due to the presence of an uneven grooved surface, whereas the homogeneous flat surface architecture of Bio-Gide fails to promote cell adhesion. It is also notable that the composition of collagen composition and the manufacturing process greatly influence the resorption rate of the membrane. As observed from the SEM images [Table/Fig-4], the Bio-Gide membrane had a smaller fibre area and was thinner compared to Fix-GideTM; thus, Bio-Gide showed a higher degradation rate compared to Fix-GideTM.

The majority of current tissue engineering efforts have been centered on identifying and modifying the biochemical variables that control tissue regeneration at a specific place. However, this biochemical paradigm does not address the importance of mechanical forces in controlling tissue regeneration in in-vitro and in-vivo conditions. Mechanical signals have been discovered to function as important controllers of tissue regeneration in-vivo and as indicators of the type and amount of tissue that can be produced at a specific site [25]. These signals primarily arise from the physical properties of the membrane, such as its stiffness, porosity, and surface topography. The mechanical stiffness of the membrane affects cellular behaviour by providing resistance to cell traction forces.

Membranes with specific pore sizes and permeability characteristics regulate the exchange of nutrients, oxygen, and growth factors within the regenerative microenvironment. Mechanical signals derived from the flow of fluids through the membrane can influence cell migration, proliferation, and matrix deposition. Mechanical cues from surface characteristics can trigger intracellular signaling pathways involved in cytoskeletal organisation and cell-matrix interactions. According to Hood L et al.'s area code hypothesis, there is a recognition system that directs cell location [26]. The results obtained agree with a few previous studies shown in [Table/Fig-7] [19,22,24,27].

Therefore, knowing their physical qualities give insight into the mechanical microenvironment that cells typically

Author and year	Type of membrane	Commercially available membrane	Characteristics	Inference
Caballé-Serrano J et al., 2019 [22]	Biologic Origin Allogenic Collagen Xenogenic collagen Synthetic origin: Resorbable Non resorbable	Alloderm Biogide Collprotect Collagen P Evolution Imploflex Cytoplast Ossix Osteoguide Tisseos	Surface morphology and roughness	Porcine pericardium: Thinner collagen fibres. Equine Pericardium membranes: More dense collagen fibers. Bovine Tendon membrane: Thicker but less compact fibres. Smoother surface.
			Tensile strength	Dry state: Dried Porcine Bone Lamina, exhibited the highest tension values. Wet State: Allogenic Collagen membrane that withstood high values of tension despite being wet.
			Stiffness	Alloplastic membranes are less stiff.
			Wettability	Alloplastic membranes had a high contact angle.
Raz P et al., 2019 [24]	Xenogenic Collagen membranes	Bio-Gide® Remaix™ Ossix Plus®	Maximal load (N)	Remaix produced maximal loading.
			Tensile strength	Remaix™ was significantly more resistant to tensile force
			Maximal extension (mm)	Remaix produced the longest extension under both dry and wet conditions
			Energy applied during loading (J)	The energy needed for tearing the Remaix membrane was the highest

Da Silva brum l et al., 2021 [27]	Non-crosslinked type 1 bovine-derived collagen membranes	Green Membrane®	Surface Morphology Analysis	The collagen fibers are structured in five layers.
			Surface roughness and wettability	Good wettability (60s= 55.89o).
			Tensile strength	The elasticity is adequate for complex procedure (Young modulus= 3.58 GPa).
Shi X et al., 2023 [19]	Membrane obtained from Porcine Dermis (PD), Bovine Dermis (BD), Bovine Pericardium (BP).	Bio-Gide Heal All Membrane Lyoplant membrane	Surface morphology	Collagen bundles of the PD and BP membranes were regular and arranged closely.
			Mechanical properties: Tensile strength, Elongation, Young's modulu	The tensile strength and Young's modulus of the BP membrane in both the dry and wet states were signifcantly higher
			Hydrophilic property	The contact angle on both the smooth and rough surfaces of the PD membrane was the lowest, while that of the BP membrane was the highest
			Degradation rate	The bovine-derived membrane degrades at the highest rate, while the PD membrane degrades at the lowest rate.
Present study	Membranes of porcine orgin and bovine orgin	Bio Gide membrane Fix Gide membrane	Surface morphology	The Bio Gide membrane allows cell adhesion which may be due to the presence of an uneven grooved surface, whereas, the homogenous flat surface architecture of the Fix gide membrane fails to promote cell adhesion.
			Mechanical properties: Tensile strength	The percentage of elongation was found to be better with Bio gide (46%, 96.79%) when compared to Fix gide (33.91%, 24.17%) in both wet and dry state respectively.
			Wettablity	Bio Gide membrane was extremely hydrophilic, whereas the Fix-GideTM membrane exhibited hydrophobicity
			Enzyme resistance test	Bio-Gide® membrane exhibited around 29±2% of degradation, whereas the Fix-GideTM exhibited only 18±2% of degradation.
			Biological properties: Biocompatibility assessment	Biocompatibility appears to be similar for both the membranes. In regards to cell exclusion Bio Gide membrane showed lesser cell adhesion than Fix Gide.

[Table/Fig-7]: Recent studies focusing physiochemical and biological properties of membrane [22,24,27,19,present study].

experience within a particular tissue, together with a full grasp of the native biomechanical environment of the tissue to be replaced. Knowing the physical characteristics of the tissue or engineered construct in which the cells are located can assist in understanding how changes in the mechanical microenvironment affect cell behaviour and whether desirable cell behaviours can be generated by particular manipulation of the physical properties of the tissue or designed construct. Hence, the fourth node needed for effective designed tissue growth might be stated as mechanical qualities. The goal of the current study was to gather information that would help clinicians choose collagen membranes that would produce the best therapeutic outcomes.

Limitation(s)

The limitations of the present study include a lack of experimental repeats and the small size of the membrane used (13x25 mm). The small size of the membrane could have impeded the precise interpretation and generalisation of the result. Due to the lack of experimental repeats, the consistency and reproducibility of the present findings were not reassessed. Therefore, further research is warranted to employ sufficiently large sample sizes and include experimental repeats to ensure even more precise interpretations and conclusions.

CONCLUSION(S)

In conclusion, having in-depth knowledge of the material and techniques relevant to specific barrier membranes pave the maximum way to the maximum success of procedures. The present study shows that the two collagen membranes chosen exhibit different morphology, physical, and biological characteristics. However, the choice of the appropriate membrane depends on the particular case, and the present findings could serve as one of the guiding lights.

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PARTICULARS OF CONTRIBUTORS:
1. Chief Scientist and Head, Department of Microbiology, CSIR-Central Leather Research Institute, Chennai, Tamil Nadu, India.
2. Professor, Department of Periodontology, Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu, India.
3. Senior Lecturer, Department of Periodontology, Ragas Dental College and Hospital, Chennai, Tamil Nadu, India.
4. Research Scholar, Department of Microbiology, CSIR-Central Leather Research Institute, Chennai, Tamil Nadu, India.
5. Professor and Head, Department of Periodontology, Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
A Gnanamani,
Chief Scientist and Head, Department of Microbiology, CSIR-Central Leather Research Institute, Adyar, Chennai-600020, Tamil Nadu, India.
E-mail: gnanamani3@gmail.com

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