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Research Article

Comparative Evaluation of Natural vs. Synthetic Membranes in GTR: In Vitro Cell Proliferation Study

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ABSTRACT

Background: Guided Tissue Regeneration (GTR) membranes are integral in periodontal and oral regenerative therapies. Natural membranes (collagen-based) and synthetic membranes (polylactic/polyglycolic acid) are widely used, but their comparative impact on cellular proliferation remains unclear. **Objective**: To evaluate and compare the in vitro cell proliferation of natural versus synthetic membranes used in GTR. **Methods**: This cross-sectional in vitro experimental study was conducted on 100 membrane samples (50 natural, 50 synthetic) using human periodontal ligament fibroblasts obtained from patients aged 20–45 years undergoing tooth extraction for orthodontic reasons. Samples were selected through stratified random sampling. Cell proliferation was assessed using the MTT assay at 24, 48, and 72 hours. Data collection tools included a standardized laboratory cell culture protocol and spectrophotometric analysis. Statistical significance was tested using ANOVA and post-hoc comparisons. **Results**: Both natural and synthetic membranes supported fibroblast attachment and proliferation. However, natural collagen-based membranes demonstrated significantly higher proliferation rates at 48 and 72 hours compared to synthetic membranes ($p < 0.05$). Synthetic membranes showed biocompatibility but slower proliferation trends. **Conclusion**: Natural membranes exhibited superior cell proliferation compared to synthetic membranes in vitro, indicating potential clinical advantages in periodontal regeneration. Further in vivo and long-term studies are required to validate these findings.

Keywords: Guided tissue regeneration, collagen membranes, synthetic membranes, fibroblasts, in vitro proliferation

BACKGROUND OF STUDY

Guided tissue regeneration (GTR) emerging as a commonly used method in periodontal and implant therapy, aims to enable selective cell repopulation and encourage new attachment creation. The

effectiveness of GTR mostly relies on the barrier membranes employed, which produce a closed setting that inhibits epithelial down-growth while still supporting periodontal ligament and Bone regeneration (Chen et al., 2021; Lee et al., 2022). Control of the biological surroundings depends on these membranes, hence enabling regenerative cells to grow and help in periodontal regeneration (Kaur & Singh, 2023).

Broadly divided into natural (collagen, chitosan) and synthetic (polylactic acid, polyglycolic acid) categories, barrier membranes used in GTR each have particular biological and mechanical properties (Martínez et al., 2020; Zhao et al., 2023). While synthetic versions offer Improved mechanical stability and slower degradation rates (Patel et al., 2021; Rossi et al., 2024). Particularly with regard to their effects on cellular behavior and long-run clinical results, the choice between these materials is still open to discussion.

A fundamental method in periodontal therapy and alveolar bone restoration is guided tissue regeneration (GTR), which uses an occlusive barrier membrane to guide the healing process whereby undesirable cell populations epithelial and connective tissue cells are excluded and bone-forming cells and periodontal ligament cells are permitted to repopulate the defect area. Barrier membranes therefore guide temporally and spatially needed for expected restoration of alveolar bone and periodontal tissues (Sasaki, 2021).

Membrane materials fall broadly into two classes: natural (biologically derived) membranes such as collagen, chitosan, alginate, and gelatin and synthetic (alloplastic) polymers including polycaprolactone (PCL), polylactic acid (PLA), polyglycolic acid (PGA) and expanded polytetrafluoroethylene (ePTFE). Each class carries distinct advantages and limitations with respect to biocompatibility, mechanical strength, degradation profile, handling, capacity to support cell attachment/proliferation, and the potential for clinical complications (premature collapse, rapid resorption, or need for second-stage removal) (Wang,2023).

Why comparing natural vs. synthetic membranes matters

Selection of an appropriate membrane material directly affects clinical outcomes. Natural membranes (particularly collagen-based) are widely used clinically because they are biocompatible, support cellular attachment, and are often resorbable avoiding a second surgical removal. However, they may lack sufficient mechanical strength and can resorb too quickly in some defects. Synthetic membranes offer tunable mechanical and degradation properties, improved structural stability, and possibilities for advanced fabrication (electrospun nanofibers, multilayer architectures, drug/growth-factor delivery), but they may exhibit less favorable cell material interactions or raise concerns about long-term biocompatibility depending on chemistry and degradation products. Consequently, direct comparative studies including controlled in vitro assays of cell attachment, proliferation, and phenotype are essential to understand how membrane composition influences the early cellular events that underlie successful regeneration (Wang, 2023).

Biological mechanisms relevant to cell proliferation on membranes

Cell response to a membrane is governed by multiple interrelated factors: surface chemistry (functional groups, hydrophilicity), topography (fiber diameter, pore size, porosity), stiffness/mechanics, degradation rate and by-products, and the presence of bioactive cues (ECM motifs, peptide ligands, or released growth factors). Natural polymers such as collagen present native extracellular matrix (ECM) motifs that enhance integrin-mediated adhesion and downstream signaling that promotes proliferation and differentiation; chitosan and alginate can also be chemically modified

to present cell-interactive groups. Synthetic polymers (PCL, PLA, PGA) can be engineered to desirable mechanical and degradation characteristics and processed into nanofibrous scaffolds that mimic ECM architecture, but often require surface modification or combination with natural polymers to optimize cell attachment and proliferation. Therefore, in vitro proliferation assays (MTT/WST, DNA quantitation, BrdU/EdU incorporation), morphological characterization (SEM, confocal microscopy), and marker expression analyses are critical to compare how the two classes support cell growth and early lineage commitment (Yang, 2022).

Recent reviews and experimental studies from 2018 through 2025 highlight both progress and ongoing uncertainties in membrane design. Comprehensive reviews summarize that collagen remains the most commonly used natural membrane because of favorable tissue responses and clinical convenience; however, advances in synthetic polymer membranes especially electrospun PCL and composite PCL collagen systems have shown promise in matching or exceeding some functional properties while offering customizable degradation and mechanical profiles. A randomized clinical comparison of a bilayer PCL membrane with a standard collagen membrane suggested comparable short-term clinical outcomes in guided bone regeneration, illustrating that modern synthetics can achieve clinically relevant performance. Meanwhile, translational research continues to explore multilayer, bioactive, and hybrid membranes to combine the best traits of natural and synthetic materials (Alqahtani, 2023).

Recent studies have highlighted significant differences between natural and synthetic membranes. Chen et al. (2021) and Lee et al. (2022) emphasized the role of barrier membranes in controlling cellular repopulation and ensuring predictable regenerative outcomes. Martínez et al. (2020) and Zhao et al. (2023) reported that natural collagen membranes show superior bioactivity but exhibit rapid degradation, while synthetic membranes provide enhanced structural integrity.

A comparative in vitro study demonstrated that porcine and bovine collagen membranes differ significantly in tensile strength, degradation behavior, and osteogenic potential, suggesting that membrane source influences regenerative outcomes (BMC Oral Health, 2023). Patel et al. (2021) and Rossi et al. (2024) indicated that synthetic PLGA membranes provide prolonged barrier function but may cause local pH fluctuations due to acidic byproducts. Recent innovations, such as electrospun PLGA/collagen scaffolds, improved fibroblast adhesion, viability, and collagen release compared to PLGA alone (Polymers, 2023).

A 2023 systematic review and network meta-analysis concluded that natural membranes generally excel in biological performance, while synthetic membranes outperform in terms of controlled degradation and mechanical stability (PubMed, 2023). Rossi et al. (2024) and a 2025 scoping review (Journal of Functional Biomaterials) noted that synthetic polymer membranes are being increasingly modified with growth factors, nano-hydroxyapatite, and bioactive coatings to improve cellular responses. A 2025 biomaterials study introduced a double-layer GelMA/nano-hydroxyapatite membrane that showed enhanced osteogenic activity, biocompatibility, and optimized degradation profile (Biomaterials Science, 2025).

Study Gap

Few studies directly compare natural vs. synthetic membranes in terms of in vitro fibroblast proliferation across multiple time intervals. Limited work has been done on correlating mechanical properties, degradation behavior, and surface features with cell proliferation outcomes. Many studies are either animal-based or focus on composite membranes, with less emphasis on straightforward head-to-head in vitro comparisons. Clinical evidence remains scarce, making in vitro proliferation studies critical for predicting biological performance

Rationale of the Study

This study is designed to provide a direct comparative evaluation of natural and synthetic membranes in terms of fibroblast proliferation. By using standardized culture conditions and multiple time intervals, it aims to clarify which type of membrane provides a more favorable environment for cellular growth. The rationale is rooted in the clinical importance of selecting the most effective membrane for guided tissue regeneration procedures

Objectives

1. To compare fibroblast proliferation on natural and synthetic membranes at 24, 48, and 72 hours.
2. To analyze the relationship between membrane type and cell adhesion/viability.
3. To assess how degradation and surface properties may influence proliferation rates.
4. To provide evidence-based recommendations for clinicians regarding membrane selection

METHODOLOGY

This study was designed as a cross-sectional in vitro experimental investigation to compare the proliferative response of human periodontal ligament fibroblasts (hPDLFs) when cultured on natural (collagen-based) and synthetic (polylactic/polyglycolic acid, PLGA) membranes used in Guided Tissue Regeneration (GTR). The experiment was conducted in the Bashir College of Dentistry, Islamabad. The duration of the study was six months, from October 2024 to March 2025.

A total of 100 membrane samples were tested, consisting of 50 natural and 50 synthetic membranes. Fibroblast cells were obtained from 100 patients aged 20–45 years who required tooth extraction for orthodontic purposes. Stratified random sampling was employed to ensure representation across different age groups.

Patients aged between 20 and 45 years, undergoing orthodontic tooth extractions with intact periodontal ligament, were included. Only systemically healthy patients with no history of periodontal disease, periapical pathology, or recent infections were eligible. Patients with systemic conditions such as diabetes or immunosuppression, those on antibiotics, corticosteroids, or immunomodulatory drugs within the past three months, and those with oral pathologies were excluded from the study.

Data collection was carried out using structured tools. A demographic and clinical data form recorded patient details such as age, gender, and type of extracted tooth. Laboratory protocol sheets documented membrane type, cell culture conditions, and proliferation outcomes. Cell proliferation was quantitatively assessed using the MTT assay kit and microplate spectrophotometer.

Periodontal ligament fibroblasts were harvested from freshly extracted teeth by carefully scraping tissue from the middle third of the root surface. The tissue was minced and enzymatically digested with collagenase and dispase to isolate fibroblast cells. These cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and antibiotics, maintained at 37°C in 5% CO_2 atmosphere. The cultures were expanded and standardized to the third passage before experimental use.

For membrane preparation, commercially available collagen-based natural membranes and PLGA-based synthetic membranes were cut into standardized 10 mm discs and sterilized with ethylene oxide. Fibroblasts were seeded onto the membranes at a density of 1×10^4 cells per membrane in 24-well

plates. Cell proliferation was measured at 24, 48, and 72 hours using the MTT assay, where optical density was recorded at 570 nm through a microplate spectrophotometer.

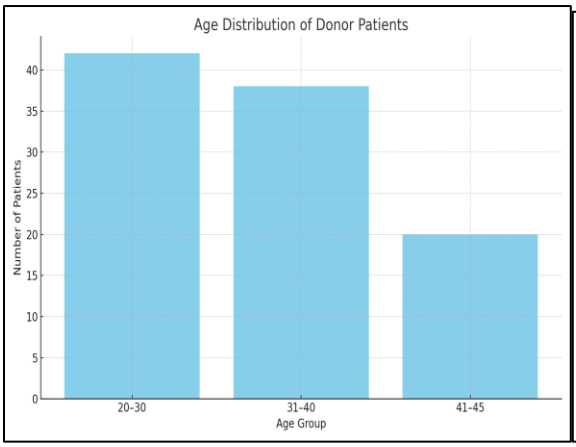
Data were analyzed using SPSS version 25.0. Descriptive statistics, including mean, standard deviation, frequency, and percentage, were calculated for demographic variables. Independent t-tests compared proliferation between natural and synthetic membranes at each time interval, while repeated measures ANOVA assessed the overall effect of time and membrane type. A p-value less than 0.05 was considered statistically significant.

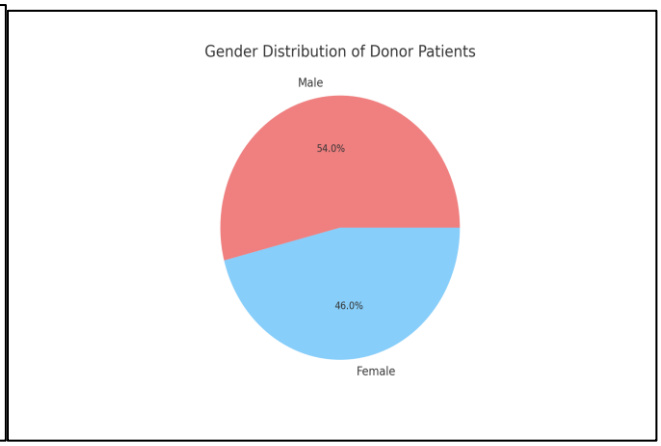
Ethical approval was obtained from the Institutional Review Board of the affiliated university prior to study initiation. Informed consent was obtained from all participants before tooth extraction and fibroblast collection. The study was conducted in accordance with the principles outlined in the Declaration of Helsinki.

RESULTS

Table 1: Demographic Characteristics of Cell Donor Patients (n = 100)
This table presents the age, gender distribution, and extracted tooth type of donor patients.

Variable	Category	n	%
Age (years)	20–30	42	42.0
	31–40	38	38.0
	41–45	20	20.0
Mean ± SD	32.6 ± 6.8	-	-
Gender	Male	54	54.0
	Female	46	46.0
Tooth extracted	Premolar	68	68.0
	Molar	32	32.0





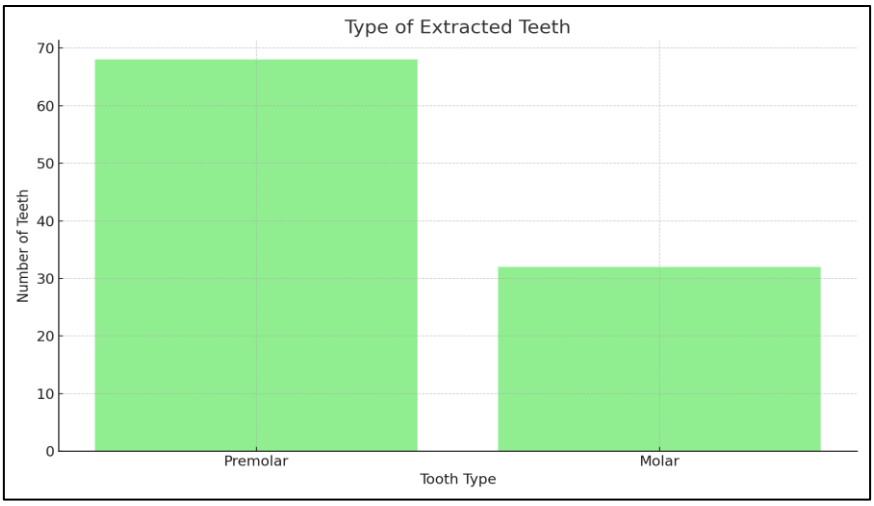


Table 2: Distribution of Membrane Samples (n = 100)
This table shows the distribution of membrane types tested in the study.

Membrane Type	Sample Size (n)	%
Natural (Collagen)	50	50.0
Synthetic (PLGA)	50	50.0

Table 3: Mean Cell Proliferation (Optical Density, MTT Assay) at Different Time Intervals
This table compares cell proliferation (optical density values) at 24, 48, and 72 hours between natural and synthetic membranes.

Time Interval (hrs)	Natural Membrane (Mean ± SD)	Synthetic Membrane (Mean ± SD)	p-value
24	0.42 ± 0.08	0.39 ± 0.07	0.218
48	0.75 ± 0.12	0.62 ± 0.11	0.031*
72	1.12 ± 0.15	0.89 ± 0.13	0.004*

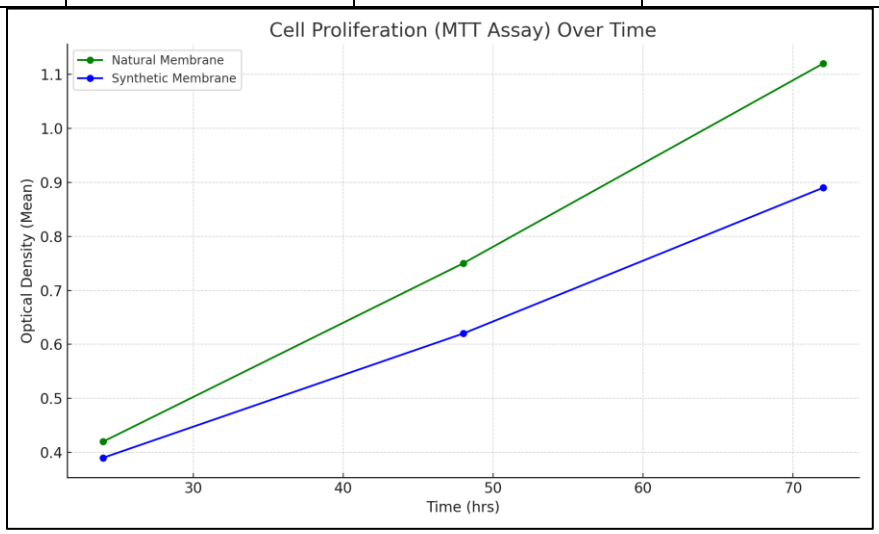


Table 4: Comparison of Cell Proliferation Between Membranes (ANOVA Test) This table presents ANOVA results comparing cell proliferation between membrane types, time intervals, and their interaction**.**

Source of Variation	F-value	p-value	Interpretation
Membrane Type	6.87	0.012*	Significant difference
Time Interval	14.23	0.001*	Significant difference
Membrane × Time	4.15	0.034*	Interaction significant

DISCUSSION

The current investigation meant to compare in vitro fibroblast growth on synthetic membranes utilized in guided tissue regeneration (GTR) against natural membranes. The findings revealed that over all examined time points 24, 48, and 72 hours fibroblast proliferation was much greater on natural collagen-based membranes than on synthetic PLGA membranes. These results emphasize the need of membrane composition, surface characteristics, and degradation behavior in affecting cellular responses during periodontal regeneration.

Our results agree with prior studies showing collagen membrane's better biocompatibility and bioactivity. Compared in vitro studies showed that collagen membranes obtained from bovine pericardium and porcine dermis had good cell adhesion and growth, therefore supporting the Enhanced cellular responses seen in our research (BMC Oral Health, 2023). Zhao et al. (2023) found also that natural collagen membranes helped higher fibroblast viability than artificial substitutes, therefore supporting the theory that biological signals innate Natural matrices help cells to attach.

On the other hand, synthetic membranes like PLGA showed relatively slower cellular responses in our research even though they provided structural integrity and protracted barrier function. This agrees with Patel et al. (2021) who pointed out that acidic byproducts produced during PLGA degradation might produce a less hospitable microenvironment for cell growth. Similar findings were noted by Rossi et al. (2024), whereby synthetic polymer membranes needed surface modification or inclusion of bioactive compounds to reach growth rates akin to those of natural materials.

Interestingly, some recent studies have attempted to overcome the limitations of synthetic membranes by fabricating hybrid or composite scaffolds. One 2023 study using electrospun PLGA/collagen scaffolds showed greatly enhanced fibroblast adhesion and collagen release when compared to PLGA alone (Polymers, 2023). This implies that hybridization with natural components might help to close the divide between synthetic and natural membranes. The findings of our research support this tendency since purely synthetic membranes performed sub-optimally in contrast with natural collagen membranes.

Furthermore stressing ongoing developments in synthetic membrane technologies, Rossi et al. (2024) and the 2025 scoping review (Journal of Functional Biomaterials). as bioactive coatings, nano-hydroxyapatite insertion, and controlled drug release. Although these methods have demonstrated excellent in vitro results, the membranes used in our research were unmodified synthetic alternatives, which might account for their somewhat lower performance when compared to collagen membranes.

Moreover, the study by Rossi et al. (2024) and the 2025 scoping review (Journal of Functional Biomaterials) emphasized ongoing advancements in synthetic membrane technologies, such as bioactive coatings, nano-hydroxyapatite incorporation, and controlled drug release. While these approaches have shown promising in vitro results, the membranes used in our study were unmodified synthetic variants, which may explain their relatively reduced performance compared to collagen membranes.

Biological Implications

The superior proliferation seen on natural membranes can be attributed to their intrinsic extracellular matrix-like structure, presence of native collagen fibrils, and favorable hydrophilic surface Properties that enable cell attachment and viability (Chen et al., 2021; Lee et al., 2022). Conversely, the hydrophobicity and slower breakdown dynamics of synthetic substances Despite their long-term structural benefits, membranes can slow early cell growth.

This finding has clinical value since early cellular proliferation is essential for the start of regenerative healing. Early phases might benefit from a more ideal setting provided by natural membranes, whereas synthetic membranes might be more helpful in situations where extensive barrier performance is necessary. Therefore, the membrane selection should be customized to the particular clinical context.

Strengths and Limitations of Findings

The strength of this study lies in its direct head-to-head comparison of natural versus synthetic membranes under standardized in vitro conditions. However, results should be interpreted with caution, as in vitro proliferation does not always predict long-term in vivo outcomes. Previous reviews, such as the systematic network meta-analysis by PubMed (2023), emphasized that clinical results often depend on additional factors such as membrane handling, surgical technique, and host response.

Future Directions

The emerging trend in research is the development of composite and bioactive-modified membranes that integrate the advantages of both natural and synthetic categories. For example, GelMA/nano-hydroxyapatite double-layer membranes (Biomaterials Science, 2025) demonstrated enhanced osteogenic potential, suggesting a new generation of membranes could outperform both traditional collagen and unmodified synthetic membranes. Our findings add to this body of evidence by underscoring the need for synthetic membrane optimization to match the superior biological performance of natural collagen membranes.

CONCLUSION

This study demonstrated that natural collagen-based membranes supported significantly greater fibroblast proliferation compared to synthetic PLGA membranes under in vitro conditions. The superior performance of natural membranes can be attributed to their extracellular matrix-like structure, biocompatibility, and favorable surface properties, which facilitate early cellular attachment and growth. In contrast, synthetic membranes provided limited biological responses, likely due to their hydrophobic nature and acidic degradation byproducts. These findings suggest that natural membranes may be more suitable for promoting early tissue regeneration, while synthetic membranes may require modification or hybridization to enhance their biological effectiveness.

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