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In Vitro Evaluation of the Hydrolytic Biodegradation of Human Amnion Membrane in PBS: A FESEM Analysis

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Abstract
Background: *Human amniotic membrane (AM) has gained attention in regenerative medicine, especially in periodontal tissue regeneration, due to its potential for healing and minimal immunogenicity. However, limited studies exist on its hydrolytic degradation and resorption rate, particularly in the context of its use as a barrier membrane.*
Aim: *This study aimed to evaluate the resorption capacity of human amniotic membrane (AM) and its surface architecture using field emission scanning electron microscope after being subjected to hydrolytic degradation in phosphate-buffered solution (PBS) over 28 days.*
Materials and Methods: *Human AM samples were procured from Tata tissue bank Mumbai in a freeze dried form prepared under sterile conditions. Three AM samples were immersed in PBS at 37°C, and weight measurements were taken at baseline (Day 0) and after 7, 14, 21, and 28 days of degradation. Field emission scanning electron microscopy (FESEM) was used to assess the changes in surface architecture before and after degradation.*
Results: *The degradation profile showed significant weight loss in all samples, with the highest rate occurring in the first 7 days. FESEM analysis revealed that the surface architecture of the AM, including fiber bundles, underwent substantial degradation, showing fragmented and disrupted fibers by Day 28.*
Conclusion: *The findings suggest that AM undergoes significant hydrolytic degradation, with alterations in its surface architecture over a 28-day period by FESEM . The rapid degradation in the initial stages and structural changes observed make AM a promising material for use in periodontal regeneration procedures. Further studies are required to evaluate its long-term stability and mechanical properties for clinical applications.*
Keywords: *Human amniotic membrane, hydrolytic degradation, surface architecture, periodontal regeneration, scanning electron microscopy, biodegradation.*

I. Introduction

Periodontal disease, one of the most common dental conditions, can lead to the destruction of the supporting structures of the teeth, including the alveolar bone and periodontal ligament [1]. Periodontal regeneration is a complex biological process aimed at restoring these lost tissues, with a focus on promoting the healing and repair of damaged periodontal tissues. Traditional methods of periodontal treatment, such as scaling and root planing, may not be sufficient for regenerating lost tissues in advanced periodontal defects [2]. Consequently, regenerative techniques have been developed that use materials like barrier membranes, which help in tissue regeneration by guiding the migration of specific cell types, stimulating tissue growth, and preventing epithelial migration into the wound site [3].

The use of resorbable barrier membranes is a critical aspect of periodontal regenerative procedures. These membranes provide mechanical support during tissue regeneration and eventually degrade as the tissue heals, eliminating the need for removal surgery [4]. A variety of biomaterials have been explored for use as barrier membranes, including collagen, polylactic acid, and polyglycolic acid, but none have demonstrated the ideal combination of properties such as biocompatibility, biodegradability, and mechanical stability. Among these, human amniotic membrane (AM) has emerged as a promising alternative [5].

AM is a biological membrane derived from the fetal membrane of the placenta. It is composed of multiple layers, including an epithelial layer, a stromal layer, and a basal layer, and is known for its remarkable regenerative potential. AM has been shown to promote wound healing, reduce inflammation, and prevent scarring, making it an ideal candidate for various tissue engineering applications [6]. Furthermore, AM has minimal immunogenicity due to its lack of major histocompatibility complex (MHC) class II antigens, reducing the risk of immune rejection when used in allogeneic transplantation [7].

The interest in AM as a material for periodontal regeneration is rooted in its rich content of growth factors, including epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and transforming growth factor-beta (TGF-β), which are known to promote tissue healing and regeneration. These properties suggest that AM could serve as an effective scaffold in periodontal wound healing, where its ability to support cellular migration, differentiation, and proliferation is essential for tissue repair [8].

However, despite its potential advantages, the use of AM in periodontal regeneration has not been fully explored, particularly in terms of its degradation characteristics. Understanding the hydrolytic degradation of AM is crucial for its clinical application, as it needs to degrade at a rate that supports tissue healing without hindering the regeneration process. The degradation rate should align with the tissue's healing timeline to ensure that the membrane remains intact during the critical stages of regeneration and then resorbs as the tissue matures [9].

Hydrolytic degradation, a process in which water molecules break the polymeric bonds in the membrane, plays a key role in determining the longevity and functionality of the material in vivo. Previous studies on AM's degradation have primarily focused on its use in ocular and wound healing applications, but there is limited research on its degradation when used in periodontal tissue regeneration. This gap in knowledge prompted the current study, which aims to evaluate the resorption capacity of AM and its surface architectural changes over time when subjected to hydrolytic degradation in phosphate-buffered solution (PBS). By assessing these characteristics, this study aims to better understand the feasibility of AM as a resorbable barrier membrane in periodontal regenerative procedures [10].

Aims and objectives: To evaluate the resorption capacity of AM and its surface architecture after being subjected to hydrolytic degradation analysis in phosphate buffer solution (PBS).

II. Materials and Methods

Preparation of Amniotic Membrane (AM)

Human AM was procured from the Tata Memorial Tissue Bank, Mumbai, after receiving ethical approval . The membranes were in freeze-dried form of size 50 x 50 mm² pieces. For analysis, the AM was removed from the package and spread over the sterlised glass slab and then membrane were prepared into triplicates of size 10 x 10 mm² and all the procedure were performed under sterile conditions in the Department of Pharmaceutical Sciences, Panjab University.

Hydrolytic Degradation Procedure

Each sample was weighed using a digital weighing machine (A&D Weighing GR-200, Japan) with an accuracy of 0.001 g. and subsequently all the samples (triplet) were immersed in PBS and incubated at 37°C. The membranes were retrieved on day seven and left to dry in a desiccator for 15 minutes, and the weight of all samples was measured. Later these samples were immersed again in sterile PBS and incubated at 37°C. These procedures were repeated on days 14, 21, and 28 to evaluate the AM degradation process for 28 days. The percentage of weight loss was calculated using the formula:

Gravimetric weight loss (%) = $(Wi - Wf)/Wi \times 100$

Wi = Initial weight of the membrane before incubating in PBS.

Wf = Final weight of the membrane after each day of assessment (7, 14, 21, and 28 days).

Surface Architecture Analysis

Field Emission Scanning electron microscopy (FESEM) was used to examine the surface morphology of the AM before and after the degradation test. One sample of AM for day zero and day 28 was retrieved for field emission scanning electron microscopy (FESEM) analysis to observe any changes in the surface architecture before and after the 28-day degradation test. The membranes were fixed using 8% formaldehyde at 4°C for 48 hours. After

that, it was washed with PBS and dehydrated in a series of graded alcohol solutions from 30%, 50%, 60%, 70%, 80%, 90%, and 100% for 10 minutes each. Then, the samples were soaked in hexamethyldisilazane (Sigma, USA) for 10 minutes and air-dried . Samples were mounted on microscope slides and observed under 2,500x and 5,000x magnification of FESEM.

Figure 1: Armanterium used in the study

III. Results

Amniotic Membrane Degradation Profile

The degradation rate of the amniotic membrane (AM) was evaluated by measuring the weight loss at day 0, 7, 14, 21, and 28 for the triplicates at different time intervals. The following data summarizes the weight loss for all three membrane samples (A, B, and C) at each stage:

Interval	Membrane A	Membrane B	Membrane C
0-7 Day	1.8mg to 0.7mg	1.5mg to 0.615mg	1.5mg to 1.1mg
7-14 Day	0.7mg to 0.52mg	0.615mg to 0.45mg	1.1mg to 0.5mg
14-21 Day	0.52mg to 0.21mg	0.45mg to 0.165mg	0.5mg to 0.2mg
21-28 Day	0.21mg to 0.11mg	0.165mg to 0.06mg	0.2mg to 0.1mg

Table 1- Showing theweight loss of amnion membrane triplet at different time interval

Time Interval	Membrane A % weight loss	Membrane B % weight loss	Membrane C % weight loss	Average % weight loss
7 day	61.11%	59%	26.67%	48.93%
14 day	71.11%	70%	66.67%	69.26%
21 day	88.33%	89%	86.67%	88%
28 day	93.89%	96%	93.33%	94.41%

Table 2- showing the percentage weight loss of amnion membrane triplet and its average at different time interval

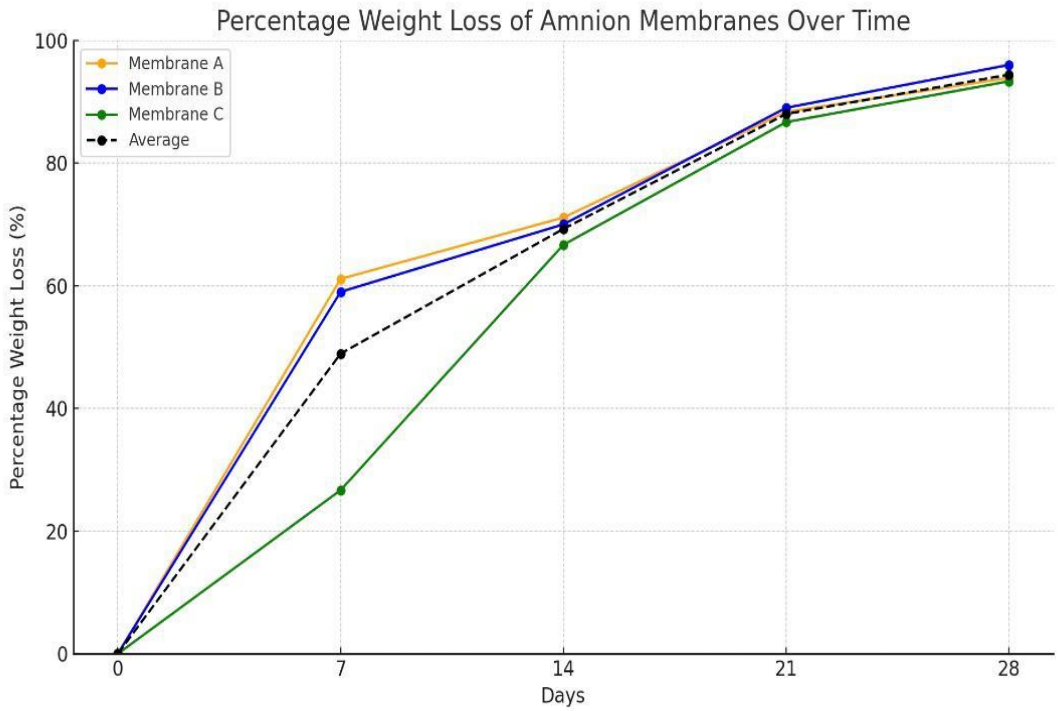

Figure 2: The degradation profile of amnion membrane based on percentage of weight loss on different days of hydrolytic degrdation

The slope of the line is very steep from day zero to day seven representing the highest percentage of weight loss, reducing about 48.93% from the initial weight. From day seven to day 14, the membranes showed an additional 20.33% weight loss followed by 18.74% and 6.41% on day 21 and 28, respectively. Overall, the membranes showed about 94.41% percentage of weight loss after 28 days of immersion in PBS. With the remaining 5.59% of weight, the AM still appeared intact physically, but with mild shrinkage in size after 28 days

Surface Architecture Analysis

To examine the structural changes in the membranes, Field emission scanning electron microscopy (FESEM) was performed on one sample of AM from both Day 0 and Day 28:

- **Day 0 (Before Degradation):**

FESEM images revealed a well-organized mosaic pattern with abundant fibers. The surface exhibited uniformity, with clear fiber bundles visible under both 2,500x and 5,000x magnification. (Figure 3)

- **Day 28 (After Degradation):**

After 28 days of hydrolytic degradation, FESEM images showed noticeable structural alterations. The previously well-ordered fiber network was disrupted, and fibers appeared fragmented, indicating significant degradation of the membrane material. (Figure 4)

These findings confirm that AM undergoes structural and morphological changes during hydrolytic degradation, aligning with the observed weight loss.

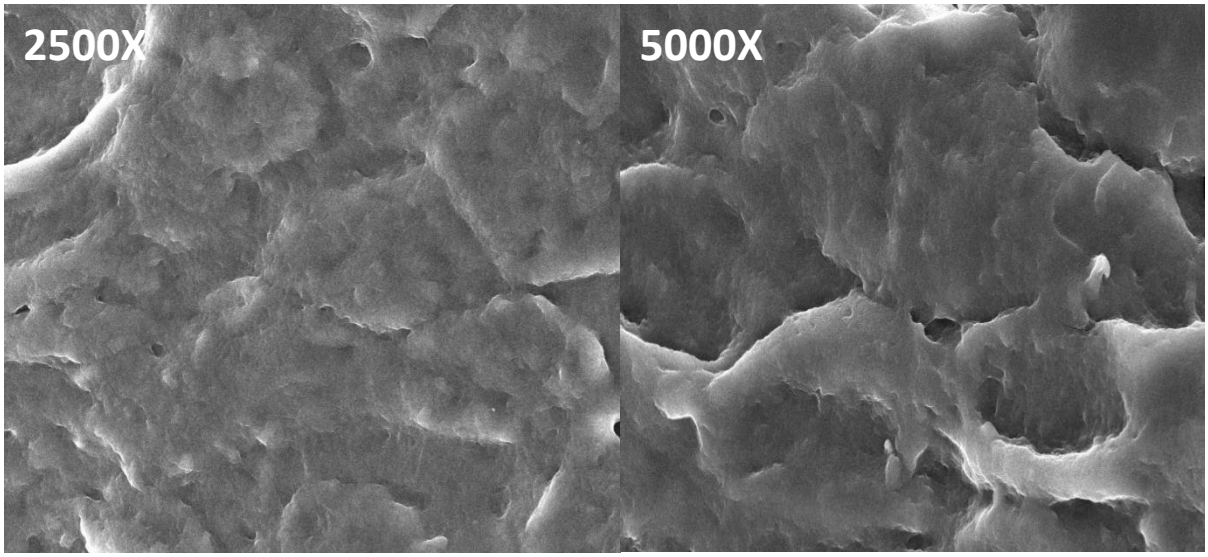

Figure 3: FESEM analysis of human amnion membrane at day 0 of hydrolytic degradation showing the well arranged mosaic pattern and abundant fibers of amnion membrane

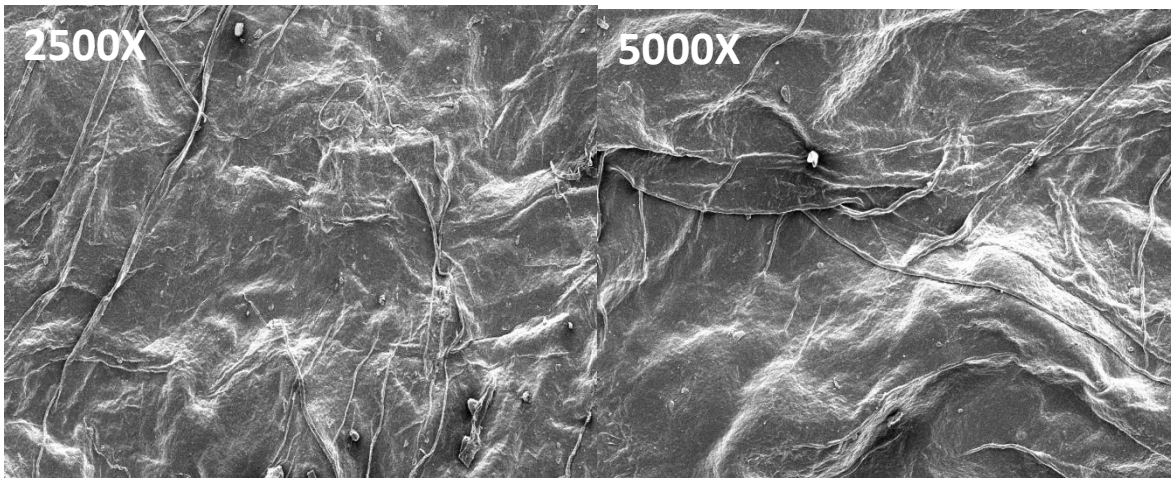

Figure 4: FESEM analysis of human amnion membrane at day 28 of hydrolytic degradation showing porous with less dense irregularly arranged collagen fibers of Amnion membrane seen at day 28

IV. Discussion

The results of this study demonstrate that human AM undergoes significant degradation when immersed in PBS and incubated at 37°C, with a notable weight loss observed in the initial phase of degradation. These findings are consistent with the research of Ingraldi AL et al. (2020) [11] which suggests amniotic membrane's potential in regenerative medicine. The observed structural changes in AM, including fiber disruption, may influence its mechanical properties and its ability to serve as a barrier membrane for periodontal regeneration.

The steep degradation rate in the first seven days suggests that AM could be beneficial in clinical applications requiring rapid resorption, such as periodontal tissue regeneration. However, long-term degradation patterns must be studied further to understand its stability and efficacy over extended periods [12].

V. Limitations

This study's limitations include the absence of clinical trials, the inability to replicate precise clinical conditions in vitro, and the lack of detailed analysis of mechanical properties post-degradation. The short-term nature of this evaluation and the limited sample size also pose constraints on generalizing the findings.

VI. Conclusion

The study concludes that human AM exhibits a suitable degradation profile for use as a barrier membrane in periodontal regeneration. The observed structural alterations after 28 days of hydrolytic degradation suggest that AM may be an effective material for supporting periodontal tissue healing. Future research should focus on the clinical application of AM and a more detailed analysis of its mechanical properties post-degradation.

References

[1]. Sedghi LM, Bacino M and Kapila YL (2021) Periodontal Disease: The Good, The Bad, and The Unknown. Front. Cell. Infect. Microbiol. 11:766944. doi: 10.3389/fcimb.2021.766944

[2]. Liang Y, Luan X, Liu X. Recent advances in periodontal regeneration: A biomaterial perspective. Bioact Mater. 2020 Feb 28;5(2):297-308. doi: 10.1016/j.bioactmat.2020.02.012. PMID: 32154444; PMCID: PMC7052441.

[3]. Alauddin MS, Abdul Hayei NA, Sabarudin MA, Mat Baharin NH. Barrier Membrane in Regenerative Therapy: A Narrative Review. Membranes (Basel). 2022 Apr 20;12(5):444. doi: 10.3390/membranes12050444. PMID: 35629770; PMCID: PMC9143924.

[4]. Abtahi S, Chen X, Shahabi S, Nasiri N. Resorbable Membranes for Guided Bone Regeneration: Critical Features, Potentials, and Limitations. ACS Mater Au. 2023 Jun 23;3(5):394-417. doi: 10.1021/acsmaterialsau.3c00013. PMID: 38089090; PMCID: PMC10510521.

[5]. Ren Y, Fan L, Alkildani S, Liu L, Emmert S, Najman S, Rimashevskiy D, Schnettler R, Jung O, Xiong X, Barbeck M. Barrier Membranes for Guided Bone Regeneration (GBR): A Focus on Recent Advances in Collagen Membranes. Int J Mol Sci. 2022 Nov 29;23(23):14987. doi: 10.3390/ijms232314987. PMID: 36499315; PMCID: PMC9735671.

[6]. Sanders FWB, Huang J, Alió Del Barrio JL, Hamada S, McAlinden C. Amniotic membrane transplantation: structural and biological properties, tissue preparation, application and clinical indications. Eye (Lond). 2024 Mar;38(4):668-679. doi: 10.1038/s41433-023-02777-5. Epub 2023 Oct 24. PMID: 37875701; PMCID: PMC10920809.

[7]. Ayala García MA, González Yebra B, López Flores AL, Guaní Guerra E. The major histocompatibility complex in transplantation. J Transplant. 2012;2012:842141. doi: 10.1155/2012/842141. Epub 2012 Jun 20. PMID: 22778908; PMCID: PMC3388305.

[8]. Huang TH, Chen JY, Suo WH, Shao WR, Huang CY, Li MT, Li YY, Li YH, Liang EL, Chen YH, Lee IT. Unlocking the Future of Periodontal Regeneration: An Interdisciplinary Approach to Tissue Engineering and Advanced Therapeutics. Biomedicines. 2024 May 14;12(5):1090. doi: 10.3390/biomedicines12051090. PMID: 38791052; PMCID: PMC11118048.

[9]. Ling KE, Roslan SM, Taib H, Berahim Z. Biodegradability of Amniotic Membrane as Potential Scaffold for Periodontal Regeneration. Cureus. 2023 Sep 17;15(9):e45394. doi: 10.7759/cureus.45394. PMID: 37854737; PMCID: PMC10580300.

[10]. Vaid R, Yildirim E, Pasquinelli MA, King MW. Hydrolytic Degradation of Polylactic Acid Fibers as a Function of pH and Exposure Time. Molecules. 2021 Dec 13;26(24):7554. doi: 10.3390/molecules26247554. PMID: 34946629; PMCID: PMC8706057.

[11]. Ingraldi AL, Allen T, Tinghitella JN, Merritt WC, Becker T, Tabor AJ. Characterization of Amnion-Derived Membrane for Clinical Wound Applications. Bioengineering (Basel). 2024 Sep 24;11(10):953. doi: 10.3390/bioengineering11100953. PMID: 39451330; PMCID: PMC11504399.

[12]. Zheng, Z., Zong, Y., Ma, Y. *et al.* Glucagon-like peptide-1 receptor: mechanisms and advances in therapy. *Sig Transduct Target Ther* **9**, 234 (2024). https://doi.org/10.1038/s41392-024-01931-z