Comparative SEM Analysis of the Ultrastructure of Human Amnion and Amnion–Chorion Membranes

Hemavathy S[1], Mary Antony Prabha[2*], Raghu Babu. P[3], Venkataramanaiah C[4], Vijayakumar Raman[5], Praveen Kumar L[6]

[1]Department of Anatomy, Melmaruvathur Adhiparasakthi Institute of Medical Sciences and Research, Melmaruvathur, Tamil Nadu, 603319, India.
[2]Department of Anatomy, Sree Balaji Medical College and Hospital, Bharath Institute of Higher Education and Research, Chennai - 600044, Tamil Nadu, India.
[3]Department of Medical Biotechnology, Faculty of Interdisciplinary Studies, Aarupadai Veedu Medical College & Hospital, Vinayaka Mission Research Foundation, (Deemed to be University), Pondicherry 607 403, India.
[4]Department of Anatomy, Bhaarath Medical College and Hospital, Bharath Institute of Higher Education and Research, Selaiyur, Chennai-600073, India.
[5]Department of Physiology, Sri Lakshmi Narayana Institute of Medical Sciences, Bharath Institute of Higher Education and Research, Osudu, Agaram Village, Puducherry - 605 502, India.
[6]Acadicell Innovations International Pvt Ltd, Crescent Innovation & Incubation Council (CIIC), Vandalur, Chennai – 600048, India.

[*]Corresponding Author: Dr. Mary Antony Prabha

Abstract

Human placental membranes, particularly the ones found during the birth of the child are the amnion and amnion–chorion membrane, are widely utilized in the wound healing and regenerative medicine because of their extracellular matrix composition and bioactive properties which are unique. But, the processing of the membrane to avoid microbial contamination using antibiotics may influence the integrity of the membrane. The current study evaluates the ultrastructural characteristics of amnion and amnion–chorion membranes treated with and without Amphotericin B and Gentamicin sulfate using scanning electron microscopy (SEM). The untreated membrane has shown an intact epithelial surface that is well organized collagenous structures, whereas in case of the treated membrane with Gentamicin Sulphate and Amphotericin B has showed structural irregularities with epithelial disruption and altered extracellular matrix organization. These structural differences are found more in the amnion chorion membrane compared to the amnion membrane separately. These findings majorly highlight the impact caused by the antibiotic usage on the placental membrane ultrastructure and emphasizes the need of optimization of the processing conditions for therapeutic applications.

Keywords: Amnion, Amnion–chorion membrane, Amphotericin B, Gentamicin Sulphate , Scanning electron microscopy, Placental membrane, Wound healing

1. Introduction

The Amnion and Chorion are the primary fetal membranes forming the amniotic sac, which protects the developing embryo and fetus. The Amnion is the innermost membrane that acts as a structural barrier and plays a key role in synthesizing prostaglandins to initiate labor. The Chorion is the outer layer, separating the Amnion from the maternal decidua and serving as an immunological buffer against maternal immune responses.(Verbruggen et al., 2017)

The amnion part which faces the is very crucial in maintaining the integrity of the fetus functions. It provides structural strength and provides mechanical properties for the sac (Gupta et al., 2015). The amnion has a single epithelial layer that has a dense basement membrane and an avascular stromal matrix that is rich in collagen fibres (Ockleford et al., 2013). The chorion which is the outer layer of the membrane is a protective layer which majorly prevents the degradation of the amnion and also shields the fetus from the maternal immune factors (Verbruggen et al., 2017). The Amnion - Chorion membrane is a composite structure comprising both the membrane fused resulting in increased thickness and mechanical strength (Richardson et al., 2017). The Amnion - Chorion membrane naturally possess anti - inflammatory, antimicrobial and low immunogenic properties making them suitable for burn management, chronic wound treatment, diabetic foot ulcers and other clinical applications (Palanker et al., 2019) (Zare-Bidaki et al., 2017).

The application of antibiotics in processing is intended to decrease contaminations caused by microorganisms, though it can affect membrane structures and functions, which is significant for preserving their activity (Dasgupta et al., 2016) (Ingraldi et al., 2023). Antibiotics by default will not be able to break down structural elements such as the collagen and matrix of Amnion & Chorion properly if applied appropriately during the processing and handling (Dasgupta et al., 2016) (Palanker et al., 2019). Some of the processing methods that involve prolonged exposure of certain antibiotics or harsh methods of sterilization may reduce the viability of the epithelial cells and the mesenchymal stem cells within the membrane territory, potentially affecting their regenerative and anti - inflammatory properties. Recent studies have reported that fresh, cryopreserved, and lyophilized membranes exhibit stronger activity of antimicrobial peptides compared to membranes treated with a significant amount of antibiotics that might reduce the inherent bactericidal action of tissue membranes (Ingraldi et al., 2023).

Gentamicin Sulphate is a broad-spectrum aminoglycoside antibiotic that is commonly used because of its efficacy against Gram-negative and Gram-positive bacteria (Klama-Baryła et al., 2025). Some studies have shown that gentamicin has good retention within the amniotic membrane structure to provide continued antimicrobial activity after washing (Hofmann et al., 2023). On the other hand, long or concentrated exposure to amphotericin B may affect the tissue architecture and ECM organization of the membranes. Scanning electron microscopy allows high-resolution observation of surface morphology and ultrastructural features and thus permits evaluation of epithelial integrity and the organization of collagen fibers (Vogel et al., 2004) (Noor & Preuss, 2024).

Scanning electron microscopy provides high-resolution visualization of surface morphology and ultrastructural features, enabling assessment of epithelial integrity and collagen fiber organization (Hamid et al., 2012). This study investigates and compares the SEM-based ultrastructural characteristics of amnion and amnion–chorion membranes with and without Gentamicin sulphate treatment (120 µg/mL) and Amphotericin B (2.5µg/mL) to understand the influence of antibiotic exposure on membrane integrity.

2. Materials and Methods

2.1 Preparation of Human Amnion and Amnion–Chorion Membranes by Dehydration Method

2.1.1 Collection of Placental Tissue

The human placental membrane is collected during C-section deliveries under aseptic conditions. It consists of amnion and chorion and is placed in sterile 50 mL conical tubes with a preservation medium. Samples are grouped into two: those containing antibiotics (Gentamicin Sulphate and Amphotericin B) and the control without the antibiotics. Samples were transported under cold conditions and were kept under a temperature range of 2-8 °C for not more than 48 hours (Roberts et al., 2019) (De Oliveira Moraes et al., 2021).

2.1.2 Sample Acceptance Criteria

Each amniotic membrane was visually inspected and accepted if it was free from meconium contamination, had no yellowish or greenish discoloration, measured at least 20 × 20 cm, was free from heavy blood stains or clots, had intact containers without leakage, contained adequate preservation medium, and had proper labeling for traceability. Membranes not meeting these criteria were rejected.(Hofmann, Lafarge, et al., 2023) (Mannu et al., 2025).

2.1.3 Aseptic Handling and Transfer

To prevent contamination, all samples were placed in a pass box, with the UV light turned off prior to entry. Autoclaved samples were packed in closed stainless steel containers and exposed to UV light. Before use, the stainless steel containers were cleaned with 70% isopropanol. Membrane processing procedures involved the use of a BSC. This BSC was cleaned with 70% isopropanol and exposed to UV light for 30 minutes before and after the procedure(Gómez et al., 2025) (Ebrahim et al., 2025).

2.1.4 Membrane Cleaning and Separation

The amniotic membrane was rinsed in purified water to eliminate blood clots and stains, employing a mild massage to avoid damaging the membrane. Membranes were divided into two parts: one retained the amnion-chorion structure while the other had the amnion layer separated from the chorion. Membrane transparency indicated complete chorion removal, whereas opacity suggested its persistence.**(**Hofmann, Rennekampff, et al., 2023b) (Gómez et al., 2025b)

2.1.5 Hypertonic Saline Treatment, Dehydration and Drying

After cleaning, membranes were incubated in a 12% NaCl solution for 30 minutes at room temperature to remove residual blood clots and discoloration. They were then rinsed with distilled water to prevent salt buildup.(Hofmann, Lafarge, et al., 2023). The cleaned membranes were drained and transferred into **Tyvek pouches**. Dehydration was carried out by curing the membranes in a hot air oven at **40 °C for 15–24 hours** until completely dried (Anoop et al., 2025).

2.1.6 Gamma Sterilization

Dried amnion and amnion–chorion membranes were cut into 3 × 3 cm sizes, vacuum sealed in sterile pouches, and labeled. They were gamma sterilized at a dose of 25 kGy through gamma radiation emitted by the radioactive decay of Cobalt-60, making them bacterium-free without radioactively contamination. This method's high penetration capacity makes it suitable for sterilizing various biological materials, preparing the membranes for experimental use.

2.2 SEM ANALYSIS:

SEM imagery revealed the shape of amniotic membranes, comparing the surface morphology of antibiotic-treated and untreated human amniotic membranes in both amnion and chorion. Specimens were examined using a Hitachi SU6600 field emission scanning electron microscope, with uniform thick samples attached to aluminum stubs via an electrically sensitive adhesive strip. Additionally, a Phenom Pro desktop scanning electron microscope was used to magnify gold-sputtered specimens at 1 or 2 microns.

3. Results:

3.1 Dehydrated Amnion and Amnion Chorion Membrane:

The SEM imagery demonstrated the form of amniotic membranes, with comparison made on the amniotic membranes with and without antibiotics in terms of amnion and chorion.

The human placental membranes were successfully harvested in aseptic conditions, processed within 48 hours, and stored in cold chain storage between 2 and 8 °C. Moreover, for further processing, membranes with specific acceptance criteria for size, color, integrity, and sterility requirements were selected. Contamination of membranes was successfully eliminated under strict asepsis procedures involving pass box transfer techniques and biosafety cabinets. Blood clots on membranes were successfully eliminated under gentle washing and manual cleaning techniques without compromising the integrity of membranes, while transparency tests of membranes confirmed that amnion and chorion layers remained successfully parted. Hypertonic saline solution treatment under conditions to preserve intact integrity with negligible blood and discolored substances remained successful with membranes dried under controlled dehydration at 40 °C with negligible deformation. Moreover, sterility with negligible damage to membranes remained achieved under radiation sterilization with 25kGy doses.

Separation of Amniotic membrane, the clear layer is the amnion and the translucent layer is the amnion - chorion together.

Fig. 1 Processing of Amniotic Membrane

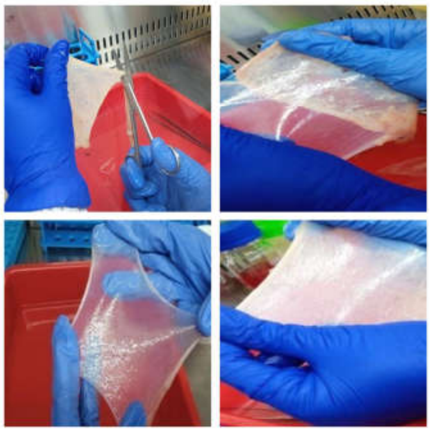

Fig. 2 Separation of Amnion and Amnion-Chorion

Fig. 3 Dehydrated Amnion - Chorion membrane (Left - without antibiotic treatment, Right - with antibiotic treatment)

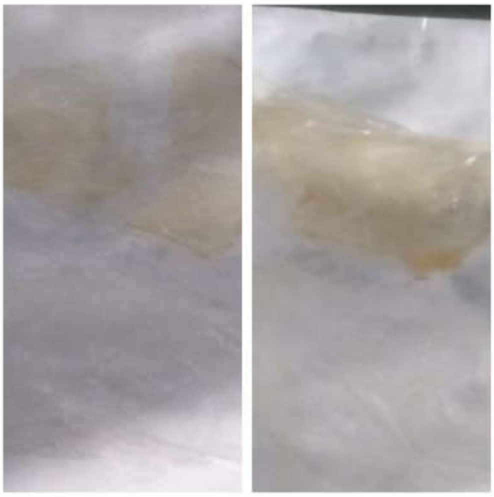

Fig. 4 Dehydrated Amnion (Left - without antibiotic treatment, Right - with antibiotic treatment)

3.2 SEM Analysis of Amnion–Chorion Membrane

The surface architecture of the untreated amnion-chorion membrane was comparatively intact and continuous. Examination of the micrographs taken by SEM showed the presence of a dense fibrous matrix with interwoven collagen fibers. The integrity and normal tissue structure were evident from the even texture and absence of irregularities on the epithelial surface.

On the other hand, the membranes that were treated with antibiotics exhibited visible alterations in surface topography. The epithelial layers of these membranes were disrupted, and these alterations were seen by the roughness and the unevenness of the surface. The effects of the antibiotics on the organization of the extracellular matrices and the epithelial layers of these membranes were evident. High cohesiveness and lack of surface degradation were evident by the presence of fine fibrillar structures.

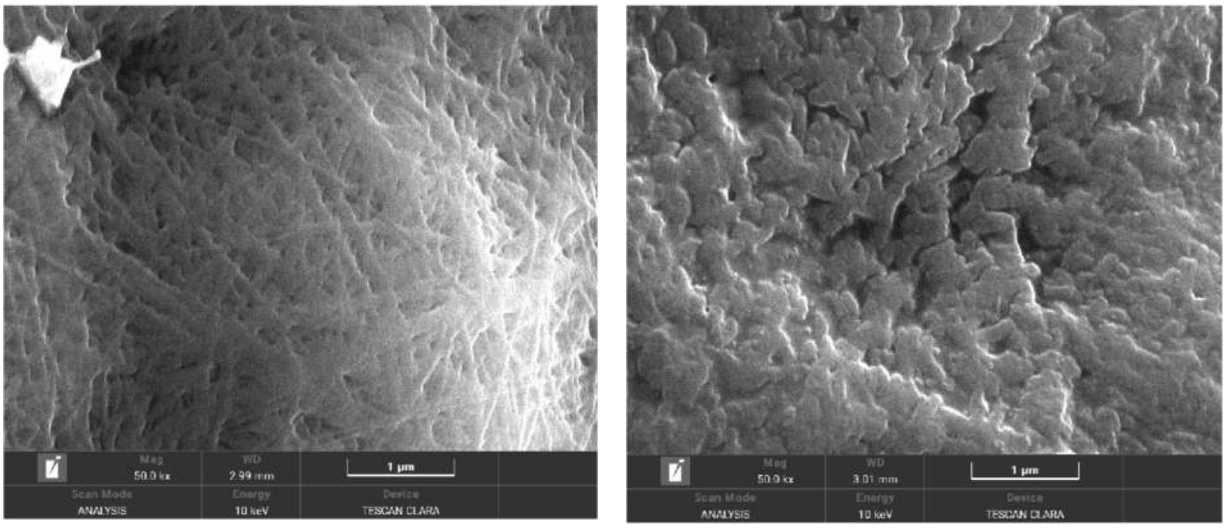

Fig 5. SEM Analysis of Amnion - Chorion (Left - With Antibiotic, Right - Without Antibiotic)

3.3 SEM Analysis of Amnion Membrane

Untreated amnion membrane SEM pictures showed a compact and smooth surface shape. Underneath, a dense and well-organized collagenous matrix seems to sustain the well-preserved epithelium layer. the amnion membranes exposed to antibiotics showed slight alterations. These included observable defects on the surface, isolated defects, and slight weakening of the collagen fibers. The amniotic epithelium is sensitive when exposed to antibiotics, as indicated by the prominent defects on the epithelial surface despite the intact structure.

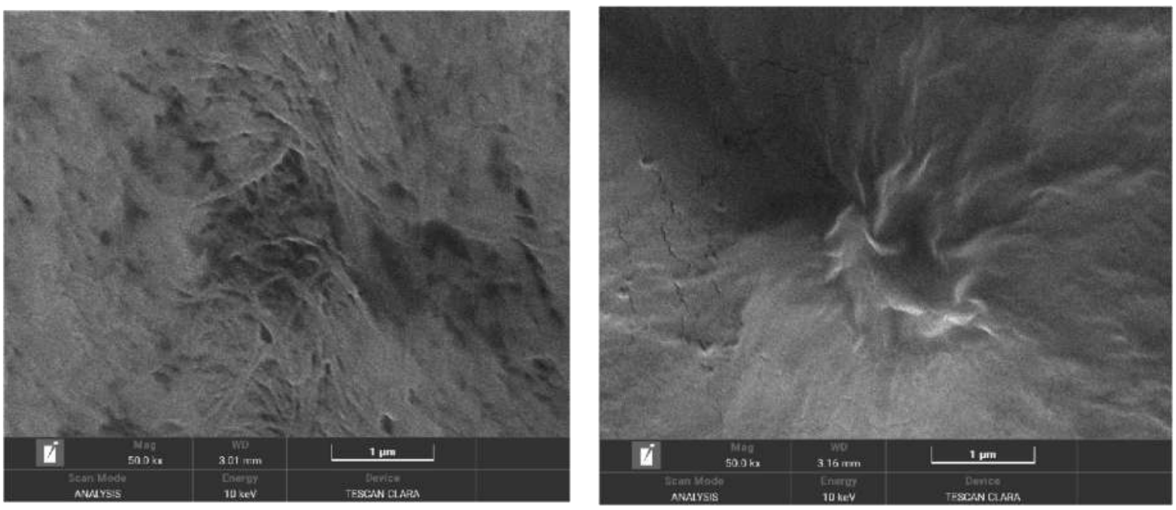

Fig 6. SEM Analysis of Amnion (Left - With Antibiotic, Right - Without Antibiotic)

4. Discussion

In the current study, it can be observed that the ultrastructural integrity of the human placental membranes is changed after exposure to 2.5 µg/mL of amphotericin-B and 120 µg/mL of gentamicin sulfate. Similar to their original structure, the untreated samples of amnion and the amnion-chorion had a structured collagen and an intact epithelial lining.

The samples treated with Gentamicin, Amphotericin B, showed morphological alterations, like disorganization in the matrix, and damage to the epithelial layers. The multilayer structure of the amnion-chorion membrane, making it perhaps more susceptible to chemicals, made such changes more pronounced (Ramuta et al., 2020) (Yadav et al., 2017).

The amnion membrane treated with antibiotics demonstrated relatively superior structural recovery with the preservation of a smoother surface topography and a more compact collagen alignment after the treatment compared to the other membranes (Galvez et al., 2025).

The suitability of the amnion membrane in biomedical applications with long-term tissue durability could be attributed to this characteristic. From these findings, one can conclude that antibiotic treatment is necessary for sterility, but in order to preserve the ultrastructure and properties of the membrane, the concentration and duration of treatment must be adjusted

5. Conclusion

The ultrastructural integrity of amnion and amnion-chorion membranes treated with and without gentamicin sulfate and amphotericin B is compared in this SEM-based investigation. Gentamycin-treated membranes displayed antibiotic-induced surface and structural changes, whereas untreated membranes showed superior epithelial and extracellular matrix structure. When compared to the amnion-chorion membrane, the amnion membrane showed more resilience to antibiotic alterations. Optimizing placental membrane processing techniques for clinical and regenerative applications requires these knowledge.

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