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Original Article

Association of Amniotic Membrane Integrity Upon Antibiotic Treatment on Wound Healing

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Abstract

Introduction: Wound healing is a complex natural process involving numerous mediators following tissue injury. In countries like India, where there is a high prevalence of both acute and chronic wounds, healing chronic wounds presents a significant challenge. Recently, amniotic membranes have become widely used in complex wound healing due to their rich collagen bundles, which contribute to the integrity of the membrane. The richness of collagen bundles may be impacted by the antibiotics used during processing and transportation of the samples. **Objectives:** This study focuses on the possibility of using amniotic and amnio-chorionic membranes for chronic wound healing without antibiotic treatment. It analyzes the integrity of the membranes with and without antibiotic treatment. **Methods:** Four sample groups were selected for this study, as shown in Table I, using amniotic and amnio-chorionic membranes. The samples were processed both with and without antibiotics. Results: The presence of rich collagen bundles was analyzed using a universal testing machine to assess membrane integrity. The amniotic and amnio-chorionic membranes processed without antibiotics demonstrated good membrane integrity. **Conclusion:** High membrane integrity suggests a rich presence of collagen bundles, which leads to faster and higher-quality wound healing.

Keywords*: Amniotic Membrane; Collagen Bundle; Universal Testing Machine*

Introduction

Wound healing is a natural process, but it becomes more complex in the case of chronic wounds. Several factors, such as immunity, infections, insufficient perfusion, and nutrition, can affect wound healing. In a highly populated country like India, the prevalence of acute and chronic wounds is 10.55 and 4.48 per 1,000 individuals, respectively, compared to the global rate of 1.89 chronic wounds per 1,000 individuals. Chronic wound healing, especially for diabetic wounds in India, is impacted by numerous factors, including lack of awareness, limited access to quality healthcare, socio-economic status, cleanliness, patient cooperation, and more, which often lead to further complications (Fathima *et al.*, 2024; Dawi *et al.*, 2025). Among these, socio-economic status ranks as the most significant factor. Poor economic status often leads individuals to neglect chronic wounds because they cannot afford to spend much time or money on treatment. Collagen can help in the healing of chronic wounds by forming a scaffold, attracting fibroblasts, and other immune cells, when the wounded tissue is unable to heal on its own.

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The amniotic membrane is a part of the placenta that becomes bio-waste after delivery. In today's world, amniotic membrane is widely used in wound healing and regenerative research studies due to its rich collagen fiber bundles. To eliminate any potential contamination, it is typically collected with antibiotics, which, in turn, can affect the quality of the fiber bundles and, consequently, the effectiveness of wound healing. As is well known, the thin amniotic membrane has high tensile strength because of its rich collagen fibers, which allow it to support the baby throughout the full term of pregnancy (Bowen *et al.*, 2022). This property of the amniotic membrane is due to the quantity, quality, and arrangement of the collagen fibers it contains.

Anatomically, the chorion covering the abembryonic pole of the embryo without villi is called the chorion laeve. Together with the inner lining, the amniotic membrane, it forms the placenta (Silini *et al.*, 2020). This part of the placenta, which lacks villi, is primarily used for research purposes.

The microanatomy of this part of the placenta consists of seven layers. The innermost three layers—the epithelium, basement membrane, and stroma—belong to the amniotic membrane. The stroma is further divided into the compact layer, fibroblast layer, and intermediate or spongy layer (Ingraldi *et al.*, 2023). The next three layers—the reticular layer, basement membrane, and outer trophoblast layers—belong to the chorionic membrane. Together, these layers form the amnio-chorionic membrane. The outermost layer is the decidua, which belongs to the mother’s endometrium (Herrick & Bordoni, 2023).

The amniotic membrane is rich in type I and type III collagen fibers, and the fibroblastic layer is highly populated with mesenchymal cells that are responsible for secreting collagen (Leal-Marin *et al.*, 2021). The integrity of type I and type III collagen fibers plays a crucial role in wound healing (Mathew-Steiner *et al.*, 2021; Kalife *et al.*, 2026). Collagen fibers at the wound site provide mechanical support, attract fibroblasts to secrete new collagen fibers for wound closure, and also help stimulate new tissue growth, autolytic debridement, re-epithelialization, and angiogenesis. The quantity and integrity of the newly formed collagen determine the quality of the healed skin.

Human amnioplasty is an adjunctive procedure used across various surgical specialties and in translational medicine for a variety of applications, including chronic wound healing (Pfister *et al.*, 2023) and the treatment of heart diseases (Pisheh *et al.*, 2024). The early application of direct natural collagen bundles in medicine is particularly beneficial for healing ulcers, burns, and dermal injuries, according to Schmiedova *et al.* (2021), and is relatively easy to obtain in the form of the amniotic membrane.

The process of collecting and transporting both cesarean and vaginal placentas involves the use of a significant amount of antibiotics, which, according to our assumption, is a necessary evil. However, this may affect the quality, quantity, and integrity of the collagen bundles present in the amniotic membrane, thus influencing wound healing time, scarring, tensile strength of the healed wound, and the quality of life of the patient, including their social activities. Quality public health requires quality wound healing, which in turn depends on quality collagen bundles. In this study, we analyzed and compared the integrity of amniotic and amnio-chorionic membranes from cesarean section deliveries, with or without antibiotic treatment, to assess their effectiveness in wound healing and the quality of life of the patients treated with them.

Materials and Methods

All processes and procedures were carried out following standard operating procedures (SOP) and in compliance with strict ethical and laboratory guidelines. The entire experimental design was approved by the Institutional Human Ethical Committee (IEC/C-P/7/2022) (Committee for the Purpose of Control and Supervision of Experiments on Human Beings), and the laboratory work was conducted at Acadicell Innovations International Pvt. Ltd. using a standardized protocol. The facility included a 10,000 clean room cell culture setup, meeting industry standards.

Materials

50mL conical tubes; DPBS; Gentamycin sulphate; Amphotericin B; Ice pouch container; Sodium Chloride; Isopropyl Alcohol; Tyvek pouch; Hot air oven; Biosafety Cabinet; Biosafety Cabinet; Dumbbell cutting press; Universal testing machine

Methodology

Collection & Transportation of Placenta (Table 1):

Six 'C section' placentas were collected immediately after delivery (Roberts *et al.*, 2019), following SOP and with written consent forms.

The abembryonic or smooth part of the placentas was separated (Marsh *et al.*, 2022), washed to remove any clots, and then cut into two pieces.

One piece was transferred to a conical tube containing antibiotics (DPBS + Gentamycin Sulphate + Amphotericin B solution), and the other piece was transferred to a conical tube without antibiotics (DPBS), while observing the highest possible hygienic methods (Table 1).

Table 1: *Showing the Sample Groups*

Samples	**Groups**	
	Amniotic Membrane	**Amnio-Chorionic Membrane**
With Antibiotics	A-WA	C-WA
Without Antibiotics	A-WOA	C-WOA

A-WA – Amniotic membrane with antibiotics; C-WA – Amnio-chrionic membrane with antibiotics; A-WOA - Amniotic membrane without antibiotics; C-WOA - Amnio-chrionic membrane without antibiotics

The samples were transported to the laboratory in an ice pouch as early as possible.

Preparation & Processing of Amniotic and Amnio-Chorionic Membrane:

The samples were either accepted or rejected after examination following SOP (Klama-Baryła *et al.*, 2020) and refrigerated until processing. The remaining process will be carried out in a biosafety cabinet.

In the laboratory, each of the samples were split into 2 equal pieces, one piece was used for the removal of amniotic membrane (the inner thin transparent membrane will be peeled off from the remaining portion. Figure 1,2) and the other piece was used as such as amnio-chorionic membrane (Figure 3).

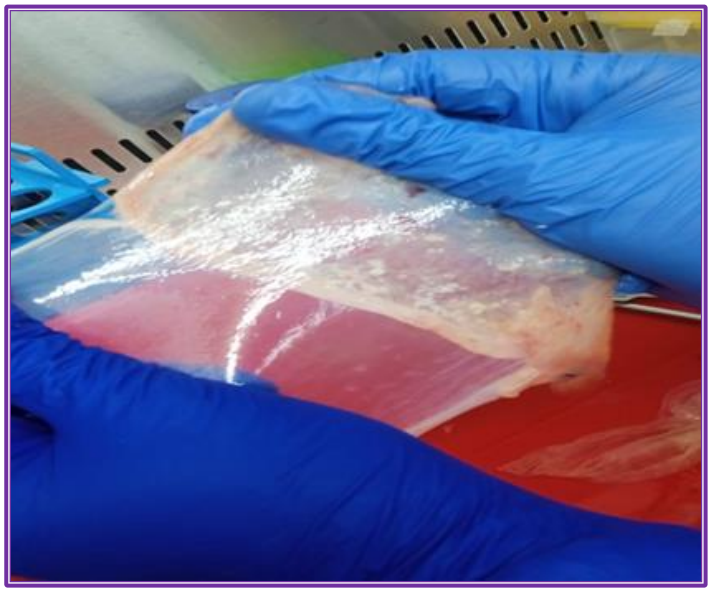


Figure 1: *Showing the Process of Removal of Amniotic Membrane*

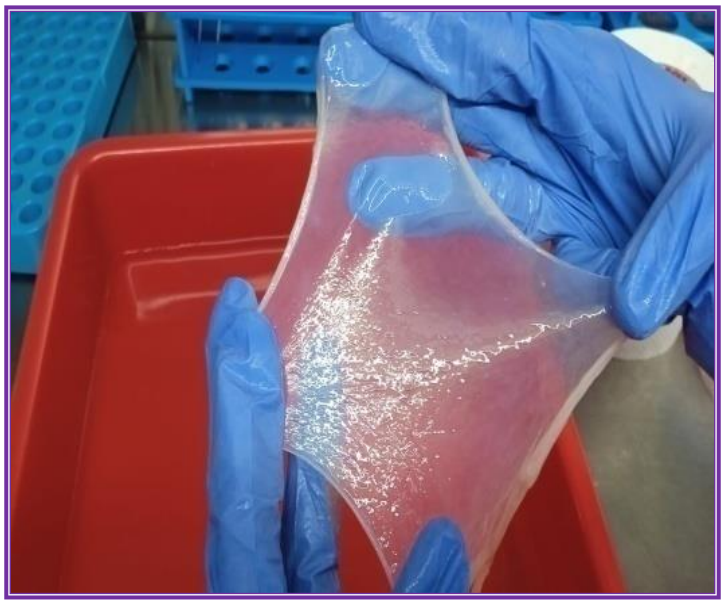


Figure 2: *Showing the Amniotic Membrane*

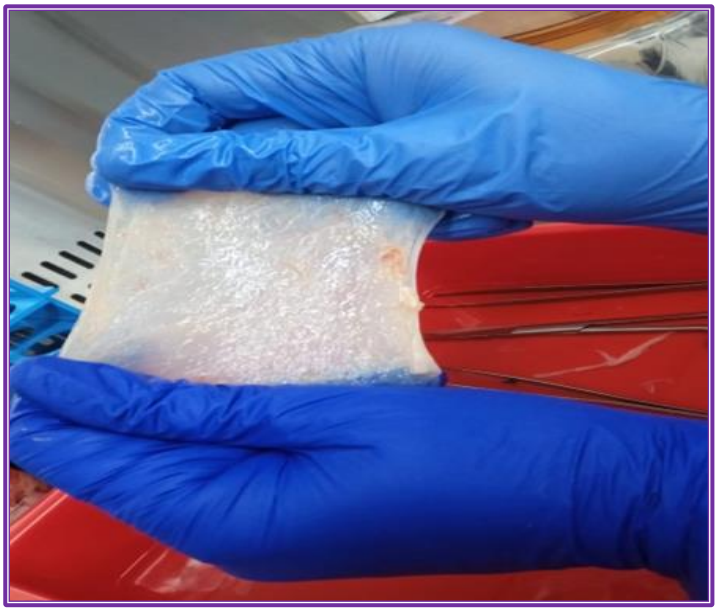


Figure 3: *Showing the Amnio-Chorionic Membrane*

The membranes were washed in distilled water to remove any salt deposits and were placed in a Tyvek pouch for dehydration. Then the membranes were dried in hot air oven at 40°C for 15 to 24 hrs for complete drying.

Tensile Strength Test Sample Preparation

The oven dried samples were placed in a dumbbell cutting press and made into dumbbell shaped samples of length 3cm (Zhang & Lu, 2018), (Figure 4).

Figure 4: *Showing the Dumbbell Shaped Sample*

Tensile Testing

The basic principle behind the tensile test is to analyze the strength of a material without deformation against a standard pulling force (Ilie ***et al.***, 2017), when the dumbbell-shaped sample is placed between two fixtures called "grips." The maximum stress a material can withstand during stretching without breaking is called tensile strength.

Procedure

1. The dumbbell shaped material is placed and fixed between the two grips of universal testing machine (Figure 5).

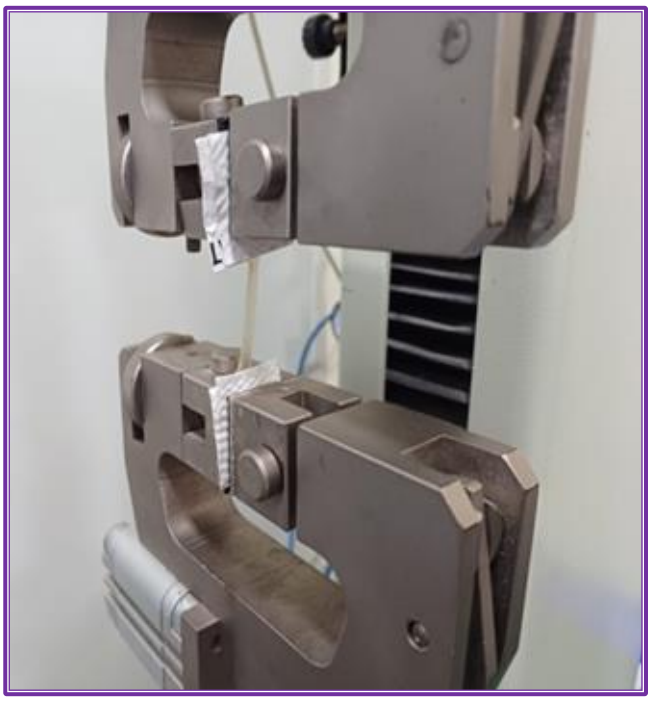


Figure 5: Showing the Dumbbell Shaped Sample Fixed Between the Two Grips of Universal Testing Machine

2. The thickness of the sample was analyzed to determine whether the added antibiotics had any effect on the thickness of the samples.

3. A pulling force or load was applied to the grip ends of the apparatus, starting from 0 Newtons.

4. The pulling force or load was gradually increased until it surpassed the threshold or tensile strength of the membrane and caused it to break.

5. A graph was plotted by considering the pulling force or load against the displacement, change in length, or stress value of the samples, and the tensile strength of the sample was determined.

6. The results were compared, and the integrity of the samples between the antibiotic and non-antibiotic groups was discussed in light of the literature using the following parameters.

Mean percentage of elongation at break between antibiotic treated and non-antibiotic treated groups.
Mean force at break between antibiotic and non-antibiotic groups.
Mean tensile strength of antibiotic and non-antibiotic groups.

Results

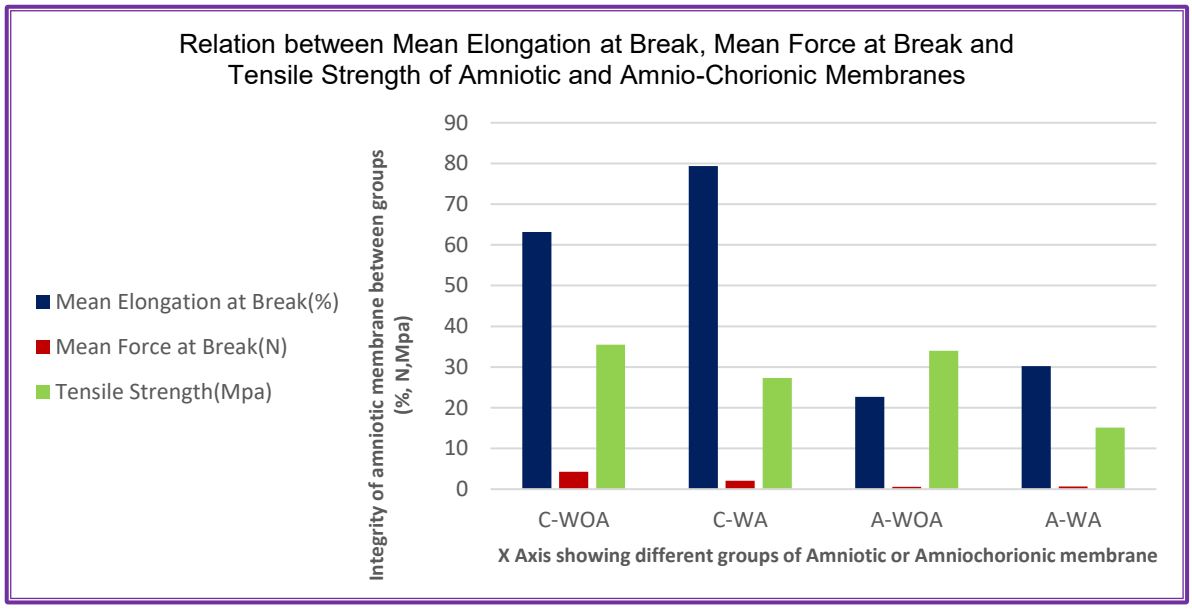
Mean Percentage of Elongation at Break between Antibiotic and Non-Antibiotic Groups:

The percentage of elongation at break is inversely proportional to the tensile strength of a material. In general, materials with high tensile strength tend to have a low percentage of elongation at break, as tensile strength refers to the material's capacity to withstand stretching force before breaking. Therefore, a low percentage of elongation at break indicates high tensile strength and, consequently, high integrity of the samples (Kar, 2022).

In this study, the A-WA (30.240%) and C-WA (79.360%) groups showed a high percentage of elongation at break, suggesting they have low tensile strength and, thus, low integrity. However, the groups without antibiotics, A-WOA (22.667%) and C-WOA (63.133%), showed a low percentage of elongation at break, indicating that their tensile strength and membrane integrity are greater than those of the antibiotic-treated groups (Table 2, Figure 6).

Table 2: *Showing the Mean Thickness, Mean Elongation % at Break, Mean Force at Break and Mean Tensile Strength of Antibiotic and Non-Antibiotic Treated Samples*

E	Sample group	Mean Thickness (mm)	Mean Elongation - at Break (%)	Mean Force -at Break (Newton-N)	Mean Tensile strength (“Mega pascals” MPa)
1	C-WOA	0.04	63.133	4.254	35.451
2	C-WA	0.025	79.360	2.047	27.296
3	A-WOA	0.006	22.667	0.612	33.973
4	A-WA	0.015	30.240	0.682	15.157



N-Newton, Mpa – Megapascals; C-WOA - Amnio-chrionic membrane without antibiotics; C-WA – Amnio-chrionic membrane with antibiotics; A-WOA - Amniotic membrane without antibiotics; A-WA – Amniotic membrane with antibiotics

Figure 6: *Showing the Relation Between Mean Elongation at Break, Mean Force at Break and Tensile Strength of Amniotic and Amnio-Chorionic Membranes with or without Antibiotic*

Mean Force at Break Between Antibiotic and Non-Antibiotic Groups

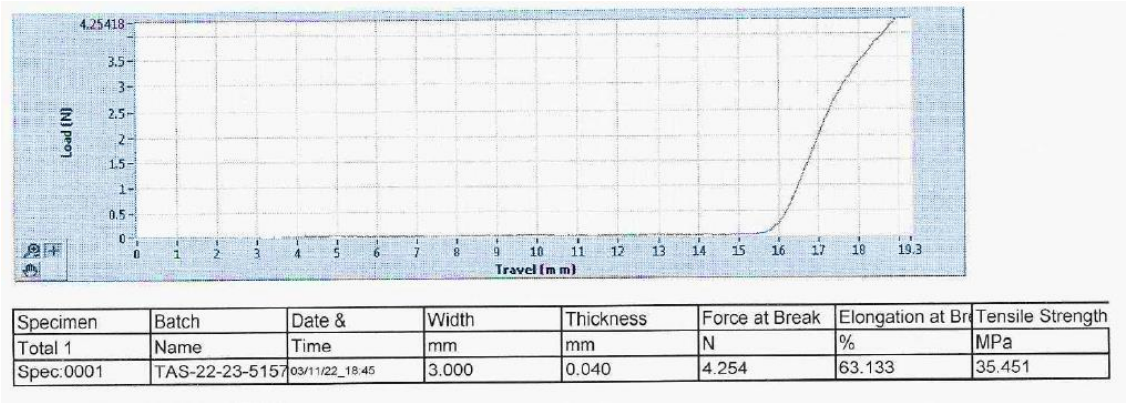
The force needed to break the material is directly proportional to both the area and the tensile strength of the material, and it also reflects the high integrity and collagen content of the material (Bezek *et al.*, 2022). Since we prepared the dumbbell samples with equal sizes of 3 cm in length and 3 mm in width, the thickness of the membrane plays a major role here.

For the C-WOA samples, the force required to break the membrane (4.254 N) is greater than for the C-WA samples (2.047 N). This indicates that the non-antibiotic treated chorionic membrane has higher tensile strength and, therefore, greater integrity and collagen content. Between the A-WA (0.682 N) and A-WOA (0.612 N) groups, the A-WOA group required a lower mean force to break. This difference is due to the variation in the thickness of the membranes. The thickness of the A-WA (0.015 mm) samples is greater than that of the A-WOA (0.006 mm) samples, which clearly suggests that the antibiotic interfered with the thickness of the membrane, raising questions about its integrity.

Considering the membrane thickness, the mean force at break for the A-WOA group is considered greater, indicating that the membrane has higher tensile strength and integrity.

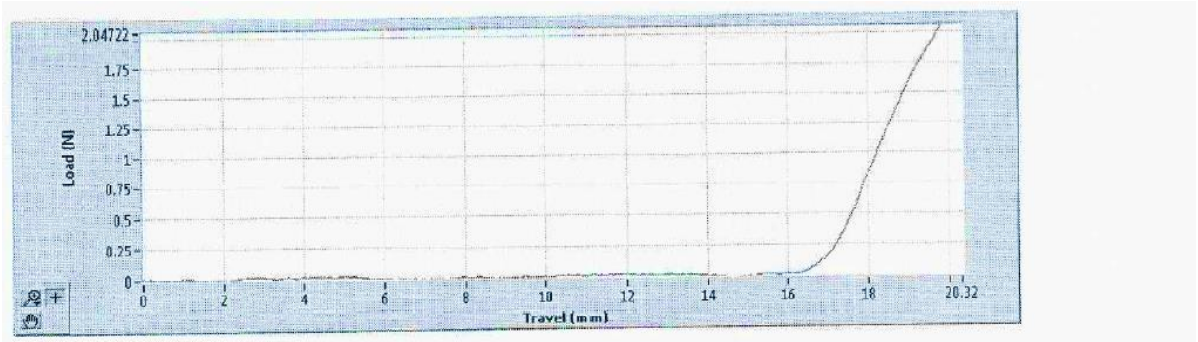
Mean Tensile Strength of Antibiotic and Non-Antibiotic Groups (Graph-1, 2, 3, 4):

Graph 1: *Showing the Tensile Strength of Amnio-Chorionic Membrane Without Antibiotics*



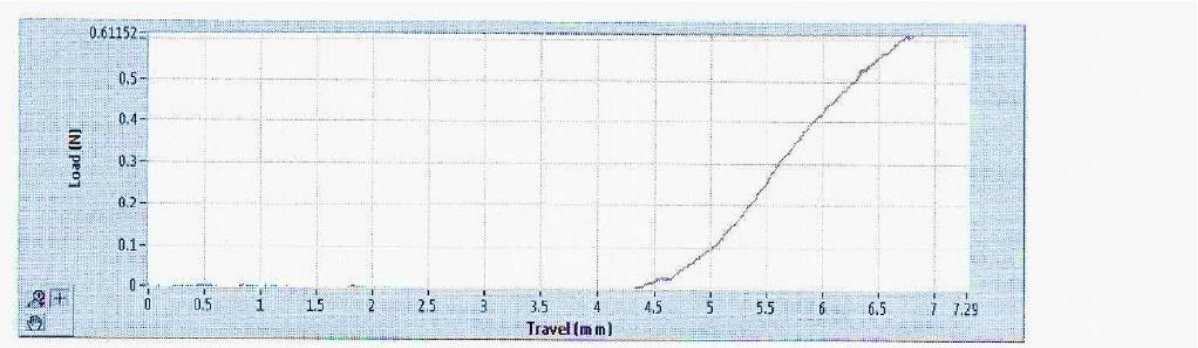
Specimen	Batch	Date &	Width	Thickness	Force at Break	Elongation at Br	Tensile Strength
Total 1	Name	Time	mm	mm	N	%	MPa
Spec:0001	TAS-22-23-5157	03/11/22_18:45	3.000	0.040	4.254	63.133	35.451

Graph 2: *Showing the Tensile Strength of Amnio-Chorionic Membrane with Antibiotics*



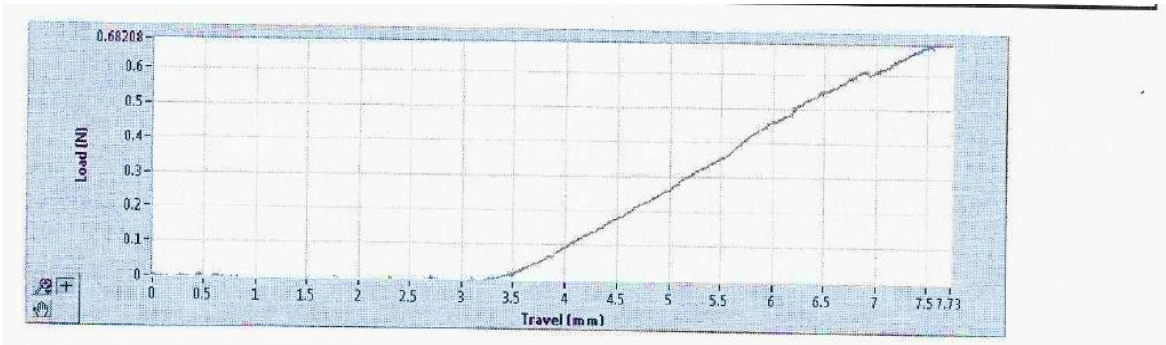
Specimen	Batch	Date &	Width	Thickness	Force at Break	Elongation at Br	Tensile Strength
Total 1	Name	Time	mm	mm	N	%	MPa
Spec:0001	TAS-22-23-5157	03/11/22_17:45	3.000	0.025	2.047	79.360	27.296

Graph 3: *Showing The Tensile Strength of Amniotic Membrane Without Antibiotics*



Specimen	Batch	Date &	Width	Thickness	Force at Break	Elongation at Br	Tensile Strength
Total 1	Name	Time	mm	mm	N	%	MPa
Spec:0001	TAS-22-23-5157	03/11/22_18:21	3.000	0.006	0.612	22.667	33.973

Graph 4: *Showing the Tensile Strength of Amniotic Membrane with Antibiotics*



Specimen	Batch	Date &	Width	Thickness	Force at Break	Elongation at Br	Tensile Strength
Total 1	Name	Time	mm	mm	N	%	MPa
Spec:0001	TAS-22-23-5157	04/11/22_11:10	3.000	0.015	0.682	30.240	15.157

It is understood that the tensile strength of any material is directly proportional to the integrity of the membrane and, therefore, the collagen content of the material (Walkden, 2020). We were also provided with automatically generated results for tensile strength based on the above parameters by the universal testing machine.

It is clear that both non-antibiotics treated groups, A-WOA (33.973 MPa) and C-WOA (35.451 MPa), possess higher tensile strength than the antibiotic-treated groups, A-WA (15.157 MPa) and C-WA

(27.296 MPa). This clearly indicates that the antibiotic-treated groups have lower tensile strength and membrane integrity, and consequently, lower collagen content, which negatively affects the quality of wound healing.

Discussion

Wound healing in chronic wounds is a complex and crucial process, as it follows a cycle of repeated infection and inflammation, affecting the patient's quality of life in society (Adebanji *et al.*, 2025; Frasier *et al.*, 2024). The amniotic membrane has already been shown to play a role in chronic wound healing, and in this study, we analyzed the possibility of achieving quality wound healing at a low cost, contributing to better community life. Quality and rapid wound healing are essential for anyone recovering from a period of intense trauma, and the amniotic membrane is one such solution (Parmar *et al.*, 2025). Given that public health prioritizes quality of life, it is crucial to provide quality wound healing using collagen-rich amniotic membranes. Some researchers have suggested using fresh amniotic membranes in chronic wound healing (Gupta *et al.*, 2025), as they retain all growth factors and collagen fibers intact. However, fresh amniotic membranes have their limitations, such as contamination, availability, and being in a non-preservable form.

From the above-discussed parameters, it is evident that the antibiotics used to preserve the amniotic and amnio-chorionic membranes (Ramuta *et al.*, 2021) indeed had an adverse effect on the collagen bundles and, therefore, the integrity of the membrane, as demonstrated by the parameters discussed above. While antibiotics are necessary to preserve the placenta from microbial attack on its integrity, their presence also has an adverse effect on the membrane's integrity, which may delay the wound healing process (Schmiedova *et al.*, 2024).

The integrity of collagen fibers is crucial for wound healing, especially for larger or deeper wounds, as it helps cover the wound, attract fibroblasts, promote re-epithelialization, and support angiogenesis (Sorg *et al.*, 2017). If the integrity of the membrane we prepare is questionable, it may lead to improper wound closure, increase the risk of infection, delay the healing process, and result in scar formation that affects the patient's daily activities and aesthetic appearance. Therefore, the addition of antibiotics is like a double-edged sword, or a necessary evil. Without it, the sample may become infected by microbes, but with it, the membrane may lose its integrity. Through this work, we conclude the aim of our study: the addition of antibiotics does indeed have an adverse effect on the tensile strength and integrity of the amniotic and amnio-chorionic membranes, potentially impacting their quality and clinical usage.

This can be improved by accepting only C-section placentas with minimal microbial load (Inchingolo *et al.*, 2024), using either minimal levels of antibiotics or by enhancing hygienic practices during collection and transportation of the samples, as we have done in this study, to ensure better membrane integrity and quality collagen preservation for effective wound healing.

While non-antibiotic treated amniotic membranes have the advantage of better membrane integrity and quality collagen, they also have their own limitations (Guzeldemir-Akcakanat *et al.*, 2025). It is essential to maintain a high degree of cleanliness throughout the procedure, from collecting the placenta to using it in wound healing. This process is time-consuming and requires precision. In the future, this could be automated and integrated with AI to ensure precision and time efficiency.

Conclusion

In conclusion, the above research work demonstrates that the antibiotics used to preserve the placenta from microbes can interfere with its integrity and affect the quality of the sample prepared for wound healing. Our procedure is simpler and more cost-effective than preparing artificial collagen wound healers like Integra and Biobrane and is highly effective because it is derived from human bio-waste. Unlike Alloderm, a human-derived product that requires a surgical procedure for harvesting and results in its own scar formation, this approach can serve the community well in terms of quality public health.

Conflict of Interest

The authors declare that they have no competing of interest.

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