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A Study To Analyse The Integrity Of Amniotic And Amnio-Chorionic Membrane With Or Without Antibiotic Treatment By Universal Testing Machine

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Abstract:
This is a study that compares the tensile strength of the amniotic and amnio-chorionic membrane obtained from 'C' section deliveries. Amniotic membrane in recent days are very much used for complex wound healing in all types of wounds, because of the collagen bundles present in them. We formulated this study to analyse the quality and integrity of collagen bundle with or without antibiotics during collection and transportation of placenta form 'C' section deliveries that is widely preferred.
We designed this study with 4 groups of samples, A-WA, A-WOA, C-WA and C-WOA with 6 samples in each group (Table-1). All the samples were processed, dehydrated, dried, cut into dumbbell shaped pieces and tested for their tensile strength. In this study the amniotic and amnio-chorionic membranes processed without antibiotics have shown high tensile strength or high membrane integrity and so can be concluded as effective in wound healing, than the counterpart.

Keywords: Amniotic membrane, Amnio-chorionic membrane, Tensile strength, Universal testing machine, Placenta, Collagen bundle

Introduction

The amniotic membrane is the inner layer of the placenta that is rich in collagen, transparent, and strong. The amniotic membrane holds the developing foetus and the amniotic fluid, so this thin membrane must possess high tensile strength and structural integrity to support the pregnancy throughout the term (1). This property of the amniotic membrane is because of the quantity, quality and the arrangement of the collagen fibres it possess with.

Anatomically the chorion that is covering the abembryonic pole of the embryo without villi is called the chorion leave and along with the inner lining amniotic membrane it is called as the placenta (2). This part of the placenta without villi is mostly used for research purpose.

If we read the microanatomy of this part of the placenta it is made up of 7 layers. The inner 3 layers, Epithelium, Basement membrane and stroma are belonging to the amniotic membrane. The stroma again will be divided into Compact layer, Fibroblast layer and Intermediate or spongy layers (3). The next 3 layers, the Reticular layer, Basement membrane and the outer trophoblasts layers are belonging to the chorionic membrane. Together they form the amnio-chorionic membrane. The outermost layer is the decidua that belongs to the mother's endometrium (4).

The compact and spongy layers of amniotic membrane is rich in collagen fibres of type I, III and the fibroblastic layer is rich in mesenchymal cells that meant to secrete collagen (5). The type I and type III collagen fibres are very much important for wound healing (6). Collagen fibres in the site of wound gives mechanical support, attract fibroblasts to secrete new collagen fibres for wound closure and also helps in stimulating new tissue

growth, autolytic debridement, re-epithelialization and angiogenesis. The quantity and integrity of the newly formed collagen determines the tensile strength of the healed skin.

The human amniotic membrane grafting is used as an adjunctive procedure across wide surgical specialties and in translational medicine for various applications including complex wound healing (7). The early application of amniotic membrane in the field of medicine is especially beneficial in healing ulcers, burns, and dermal injuries according to Iveta Schmiedova et al., 2021 (8).

The process of collection and transportation of both 'C' sectional and vaginal placenta includes a good amount of antibiotic, that according to our assumption is a necessary evil that may affect the quality, quantity and the integrity of collagen bundle present in the amniotic membrane and may interfere the time of wound healing, scarring and tensile strength of the healed wound. So we planned to compare the integrity of amniotic and amnio-chorionic membrane by analysing the tensile strength of the membranes with or without antibiotic treatment during collection and transportation, as it implies the quality of the collagen bundle meant for wound healing.

II. MATERIALS AND METHODS:

All the processes and procedures were followed using SOP and in compliance with strict ethical and laboratory guidelines. The total 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) and the laboratory work was carried out in Acadicell Innovations International Pvt. Ltd using standardized protocol, with 10,000 clean room cell culture facility set up to an industry standard.

MATERIALS

1. 50mL conical tubes
2. DPBS
3. Gentamycin sulphate
4. Amphotericin B
5. Ice pouch container
6. Sodium Chloride
7. Isopropyl Alcohol
8. Tyvek pouch
9. Hot air oven
10. Biosafety Cabinet
11. Biosafety Cabinet
12. Dumbbell cutting press
13. Universal testing machine

SAMPLE GROUPS

Table-1- showing the sample groups.

SAMPLES	GROUPS	
	AMNIOTIC MEMBRANE	AMNIO-CHORIONIC MEMBRANE
WITH ANTIBIOTICS	A-WA	AC-WA
WITHOUT ANTIBIOTICS	A-WOA	AC-WOA

Methodology:

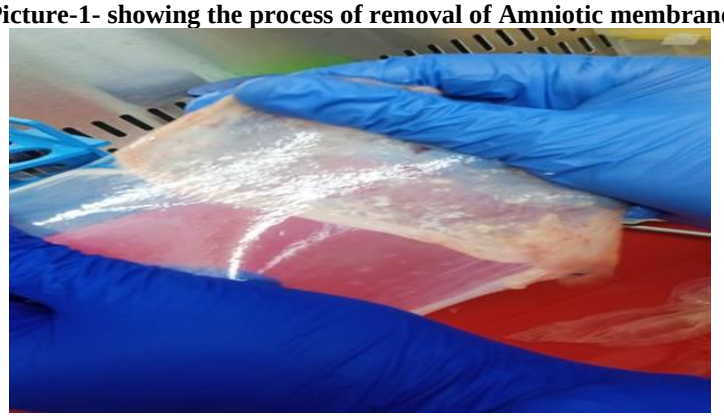
COLLECTION & TRANSPORTATION OF PLACENTA:

1. 6 'C section' placentas were collected immediately after delivery (9), following SOP.
2. The abembryonic or smooth part (10) of the placentas were separated, washed to remove the clots and were cut into 2 samples.
3. One sample is transferred to a conical tube with antibiotics (DPBS + Gentamycin Sulphate + Amphotericin B solution) and the other sample is transferred into a conical tube without antibiotics (DPBS).
4. The samples of placenta will be transported to the laboratory in an ice pouch.

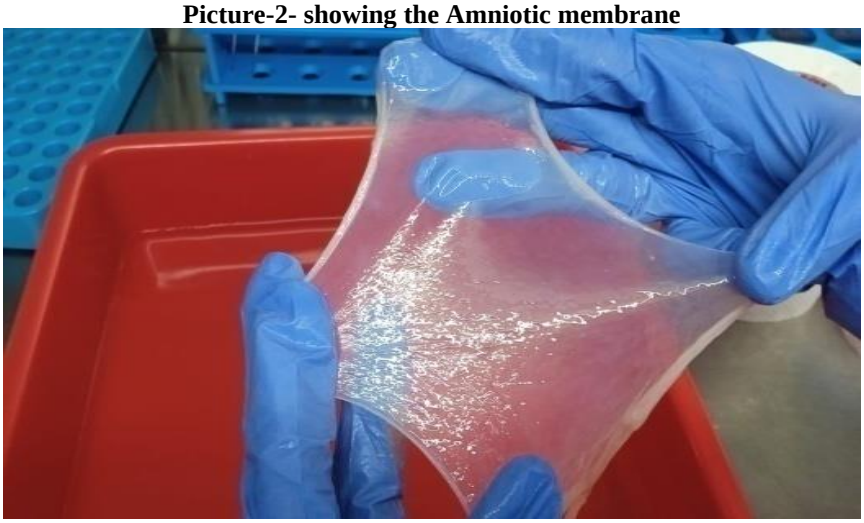
PREPARATION & PROCESSING OF AMNIOTIC AND AMNIO-CHORIONIC MEMBRANE:

1. The samples were either accepted or rejected after examination following SOP (11), and refrigerated until processing. The remaining process will be carried out in a biosafety cabinet.
2. 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. **Picture-1,2**) and the other piece was used as such as amnio-chorionic membrane (**Picture-3**).

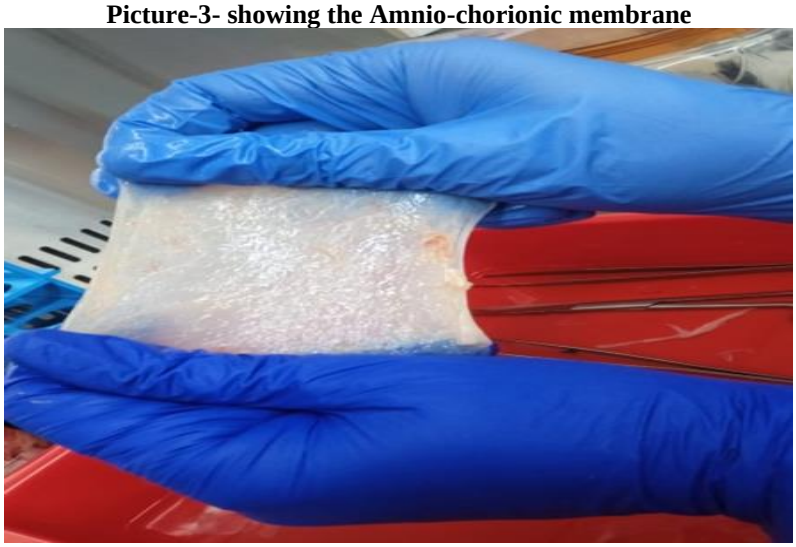
Picture-1- showing the process of removal of Amniotic membrane



Picture-2- showing the Amniotic membrane



Picture-3- showing the Amnio-chorionic membrane



1. The membranes were washed in distilled water to remove any salt deposits and were placed in a Tyvek pouch for dehydration.
2. 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 (12), **(Picture-4).**

Picture-4- showing the dumbbell shaped sample.

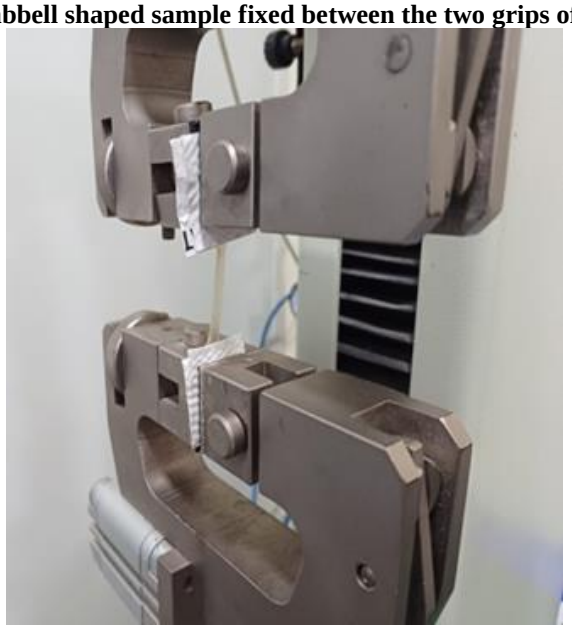
Tensile testing:

The basic idea of a **tensile test** is to place the dumbbell shaped sample between two fixtures called "grips" and to analyse the strength of the material without any deformity against a standard pulling force (13). Tensile Strength is the maximum stress that a material can withstand while being stretched or pulled before breaking.

PROCEDURE:

1. The dumbbell shaped material is placed and fixed between the two grips of universal testing machine **(Picture-5)**.
2. The thickness of the sample was analysed to check whether the added antibiotics shown any effect on the thickness of the samples.
3. A pulling force or load was given to the grip ends of the apparatus starting from ‘0’ Newton.

Picture-5- showing the dumbbell shaped sample fixed between the two grips of universal testing machine.



4. The pulling force or load was increased slowly till the point when it crosses the threshold or tensile strength of the membrane and breaks.
5. A graph was plotted by considering the pulling force or load against the displacement or change in length or stress value of the samples and the tensile strength of the sample was found.
6. The results were compared and the integrity of the samples between antibiotic and non-antibiotic groups were discussed in the light of literature using the following parameters.

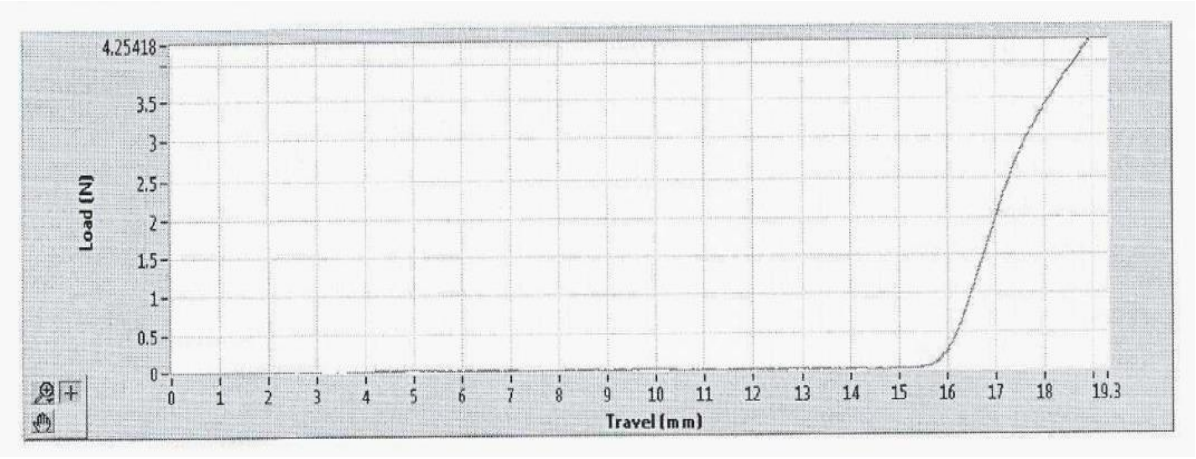
1. Mean percentage of elongation at break between antibiotic and non-antibiotic groups.
2. Mean force at break between antibiotic and non-antibiotic groups.
3. Mean tensile strength of antibiotic and non-antibiotic groups.

Results:

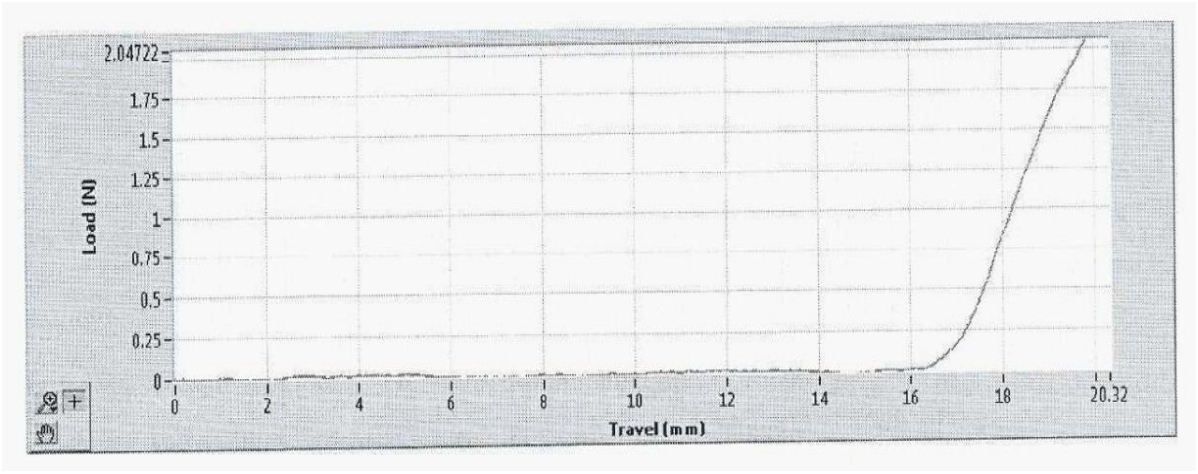
Table-2- showing the mean thickness, mean elongation % at break, mean force at break and mean tensile strength of antibiotic and non-antibiotic treated samples.

E	Sample group	Mean Thickness (mm)	Mean Elongation at Break (%)	Mean Force at Break (Newton-N)	Mean Tensile strength ("Mega pascals" MPa)
1	AC-WOA	0.04	63.133	4.254	35.451
2	AC-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

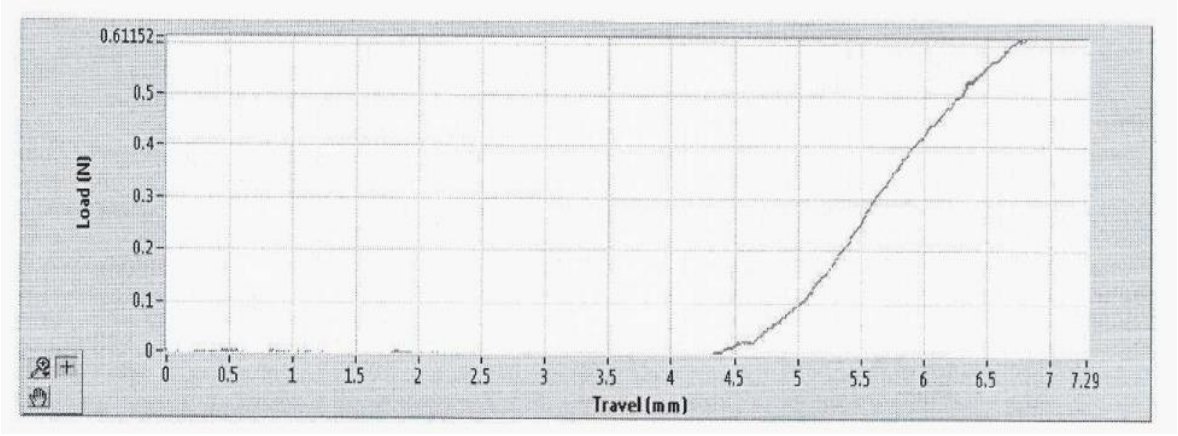
Graph-1- showing the Tensile strength of Amnio-chorionic membrane without antibiotics.



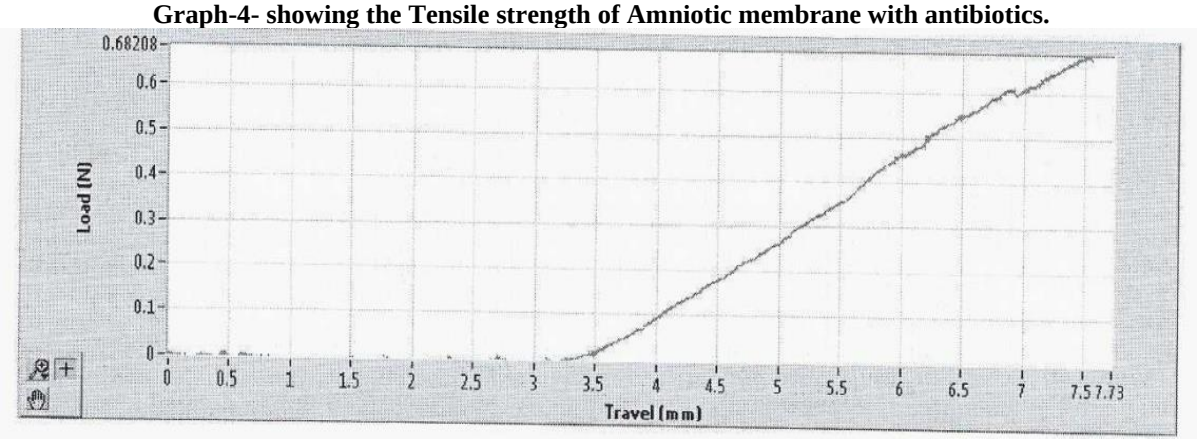
Graph-2- showing the Tensile strength of Amnio-chorionic membrane with antibiotics.



Graph-3- showing the Tensile strength of Amniotic membrane without antibiotics.



Graph-4- showing the Tensile strength of Amniotic membrane with antibiotics.



MEAN PERCENTAGE OF ELONGATION AT BREAK BETWEEN ANTIBIOTIC AND NON-ANTIBIOTIC GROUPS:

Percentage of elongation at break is inversely proportional to the tensile strength of a material. In general materials with high tensile strength possess only low percentage of elongation at break as because tensile strength is the capacity of the material to withstand stretching force before breaking. So low percentage of elongation at break acknowledges high tensile strength and so high integrity of samples (14).

In this study the A-WA (30.240%) and AC-WA (79.360%) shown high percentage of elongation at break and so considered to have low tensile strength and so low integrity. But the groups without antibiotics, A-WOA

(22.667%) and AC-WOA (63.133%) shown low percentage of elongation at break and proved that their tensile strength and membrane integrity is more than antibiotic treated groups.

MEAN FORCE AT BREAK BETWEEN ANTIBIOTIC AND NON-ANTIBIOTIC GROUPS:
The force needed to break the material is directly proportional to the area and the tensile strength of the material, and is also a statement for the high integrity of the material (15). As we prepared the dumbbell samples with equal sizes of 3cm length and 3mm width, the thickness of the membrane plays a major role here.
For AC-WOA group, the mean force at break (4.254N) is greater than AC-WA (2.047N). That proved the non-antibiotic treated chorionic membrane has high tensile strength and so with integrity with collagen membrane. Between groups A-WA (0.682N) and A-WOA (0.612N), the A-WOA group has low mean force at break. This is because of the difference in the thickness of the membranes. The thickness of the A-WA (0.015mm) samples are greater than the A-WOA (0.006mm), which clearly indicated the interference of the antibiotic with the thickness of the membrane and questions its integrity.
Considering the membrane thickness the mean force at break for the A-WOA is considered greater and so the membrane is considered with greater tensile strength and integrity.

MEAN TENSILE STRENGTH OF ANTIBIOTIC AND NON-ANTIBIOTIC GROUPS:
It is considered that the tensile strength of any material is directly proportional to the integrity of the membrane or material (16). We were also provided with the automatically generated results for tensile strength based on the above parameters by the universal testing machine.
It is obvious both non-antibiotic treated groups, A-WOA (33.973MPa) and AC-WOA (35.451MPa) possess high tensile strength than the antibiotic treated groups A-WA (15.157MPa) and AC-WA(27.296MPa). That clearly stated the antibiotic group possess less tensile strength and membrane integrity.

Discussion and conclusion:

From the above discussed parameters it is evident that the antibiotic employed to preserve the amniotic and amnio-chorionic membranes (17). indeed had an adverse effect on the collagen bundle and so the integrity of the membrane that was shown bright by the above discussed parameters. Though antibiotics are necessary to preserve the placenta from microbial attack on its integrity, its presence also possess an adverse effect on its integrity too.

The above piece of research work proves that, the antibiotic used to preserve the placenta from microbes even interferes with its integrity and affects the quality of the sample we prepare for wound healing.

The integrity of the collagen fibres are very much important for wound healing especially for larger or deep wounds to cover the wound, to attract the fibroblast, for re-epithelialization and for angiogenesis (18). If the integrity of the membrane that we prepare is questionable then it may leads to improper wound closure and may leads to infection, may delay the time of healing and may leads to scare formation that affects the day to day activities and aesthetic appearance of the patient. So addition of antibiotic is like a double sided sword, or necessary evil, without it the sample may get infected by microbes and with that it may loss its integrity. With this work we conclude the aim of our study that the addition of antibiotics indeed has adverse effect on the tensile strength of the samples used and the integrity of the samples the amniotic membrane and the amnio-chorionic membrane and so may affect its quality and clinical usage. This can be modified by using either minimal level of antibiotics or by modifying and enhancing the hygienic practice during collection and transporting the samples.

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