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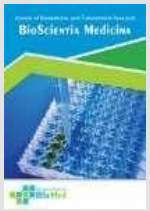

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Mechanical Properties of *Macaca fascicularis* Amniotic Membrane and Duramater: A Potential Biomaterial for Dural Defect Closure

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A B S T R A C T

Background: The amniotic membrane (AM), a versatile biomaterial with inherent stem cells and extracellular matrix, has shown promise in various tissue engineering applications. Its potential as a dural substitute, particularly in addressing dural defects and preventing cerebrospinal fluid (CSF) leakage, has garnered increasing interest. However, a comprehensive understanding of the mechanical properties of non-human primate (NHP) AM, especially in relation to human AM, remains elusive. This knowledge gap hinders the optimal utilization of NHP models, such as *Macaca fascicularis*, in translational research for dural repair. This study aimed to characterize the mechanical properties of fresh *Macaca fascicularis* AM and dura mater and to investigate the influence of fetal gender, gestational age, and parity on AM mechanics. **Methods:** Sixteen fresh preparation amniotic membranes of *Macaca fascicularis* were obtained at the elective caesarean section that was already free of several infections, and three fresh preparation of dura mater of the same species were studied. The membranes were cut in specific sizes and then loaded at the Flavigraph (Textechno, Herbert Stein GmbH & Co.KG, Moenchengladbach, Germany) machine. The Young's modulus, ultimate tensile strength, elongation at break, maximum elongation, and toughness of the amniotic membrane and dura mater were recorded and compared based on the fetal gender, gestational ages, and frequency of pregnancy. **Results:** This is the first report of mechanical properties of *Macaca fascicularis* amniotic membrane and dura mater. There are no statistically significant differences in mechanical properties of the amniotic membrane between the fetal gender, gestational age, and the frequency of pregnancy in fresh preparation amniotic membrane. The elasticity of the dura mater is seven times stiffer than the AM and the tensile strength of the dura mater is three times bigger than the AM, and the dura mater toughness is eight times bigger than the amniotic membrane. **Conclusion:** Our findings have shown the mechanical properties of Mf AM are not dependent on factors of fetal gender, gestational age, and frequency of pregnancy. This work provides an explanation of the physical properties of fresh preparation AM as the consideration to be used as allograft biomaterial in the dura mater substitution procedure.

1. Introduction

The amniotic membrane (AM), the innermost layer of fetal membranes, has garnered significant attention in the field of tissue engineering and regenerative medicine due to its unique structural and biological properties. Serving as a protective barrier and metabolic interface during fetal development, the AM is a rich source of bioactive components, including epithelial stem cells (ESCs), mesenchymal stem cells (MSCs), and a complex extracellular matrix (ECM). This intricate composition confers upon the AM a multitude of functions, such as promoting cell

adhesion, migration, and differentiation, as well as modulating inflammation and angiogenesis. These characteristics have positioned the AM as a promising biomaterial for a wide range of clinical applications, including wound healing, ophthalmic surgery, and, more recently, neurosurgical procedures. In the realm of neurosurgery, the repair and reconstruction of dural defects, which can arise from trauma, surgery, or disease, pose a significant challenge. The dura mater, the outermost layer of the meninges, plays a crucial role in protecting the brain and spinal cord, maintaining intracranial pressure, and facilitating cerebrospinal fluid (CSF) circulation. When the integrity of the dura is compromised, CSF leakage can occur, leading to complications such as infection, meningitis, and pseudomeningocele formation. Traditionally, dural defects have been repaired using autografts, allografts, xenografts, or synthetic materials. However, each of these approaches has its limitations, including donor site morbidity, risk of immune rejection, and potential for infection or foreign body reaction.[1-3]

The AM, with its inherent biocompatibility, low immunogenicity, and abundance of growth factors and cytokines, has emerged as an attractive alternative for dural repair and reconstruction. Recent studies have demonstrated the feasibility and efficacy of using AM as a dural substitute in both animal models and human patients. The AM has been shown to effectively prevent CSF leakage, promote dural regeneration, and reduce the incidence of postoperative complications. However, despite the growing body of evidence supporting the use of AM in dural repair, there remain gaps in our understanding of its mechanical properties and how these properties influence its performance as a dural substitute. The mechanical properties of the AM, including its elasticity, tensile strength, and toughness, are critical determinants of its ability to withstand the mechanical stresses and strains imposed on it during and after implantation. A dural substitute must possess sufficient strength and elasticity to resist the pulsatile forces of CSF and the movements of the brain and spinal cord, while also allowing for the growth and remodeling of the surrounding tissues. Furthermore, the mechanical properties of the AM may vary depending on factors such as gestational age, fetal gender, and processing methods. Understanding these variations is essential for optimizing the use of AM in dural repair and ensuring its long-term success.[4-6]

Non-human primates (NHPs), particularly *Macaca fascicularis*, offer a valuable model for studying the mechanical properties of AM and its potential for dural repair. NHPs share a close phylogenetic relationship with humans, and their AM exhibits similar structural and biological characteristics to human AM. Moreover, NHPs allow for controlled experimental conditions and longitudinal studies, which are not feasible in human subjects. By investigating the mechanical properties of NHP AM and comparing them to those of the dura mater, we can gain valuable insights into the suitability of AM as a dural substitute and identify potential areas for improvement.[7-9] The present study aims to characterize the mechanical properties of *Macaca fascicularis* AM and compare them to those of the dura mater. We will also investigate the influence of fetal gender, gestational age, and parity on the mechanical properties of AM. By elucidating the biomechanical characteristics of NHP AM and its potential as a dural substitute, this study will contribute to the development of more effective and reliable strategies for dural repair and reconstruction, ultimately improving patient outcomes in neurosurgical practice.

2. Methods

Mf fetal membrane was collected under sterile conditions from healthy donor pregnant monkeys following delivery by elective term caesarean section at the animal laboratory of PT. Biofarma (Persero), Bandung (n = 16). The placenta was placed in a container that contained Antibiotic-Antimycotic 100x (Gibco®, ThermoFisher). Figure 1 shows that the AM was separated manually from the chorion by blunt dissection, then filled and washed into small containers containing phosphate-buffered saline

(PBS, Sigma-Aldrich) to remove the blood and other cellular debris. After that, AM for measurement is cut into the size of the singles of 30 mm x 2 mm to achieve the machine-accepted criteria for the measurement. Three specimens of AM per placenta were directly analyzed within 6 hours of the collection after partially drying (10 min) at room temperature prior to analysis. We also measured the fresh dura mater tissue of Mf that we collected from the duraplasty procedure. The dura mater tissue was washed 5x using NaCl 0.9% solution and then placed in the clean tube containing 10 mL of NaCl 0.9% solution and then analyzed within 8 hours after collection. All Mf are healthy animals (female, specifically pathogen-free for TB, SIV, SV40, Polio type 1, 2, 3, Foamy virus, and Herpes B virus. This study was approved by the ethics committee of the Center for Primate Animal Studies in Bogor Institute of Agriculture, Animal Care and Use Ethics Commission (ACUC) No. IPB PRC-19-B001.

Figure 1. The amniotic membrane is manually separated from the placenta.

The AM thickness was measured using an automated Sylvac S228 micrometer. The Young's modulus by Flavigraph (Textechno, Herbert Stein GmbH & Co.KG, Moenchengladbach, Germany) testing machine with its Textechno Favigraph Program. The specimen (size 30 mm x 2 mm) was located in two microclamps manually. To obtain the stress-strain curves for the calculation of the tensile used the load values of 100 cN. The program showed the force/elongation curves. The Young's modulus was then counted by Microsoft® Excel software to determine the first slope of the line from the curve. The results of the stress-strain test that showed by the program were the Y-axis as the force F(g) and the X-axis as the Elongation (%). Then the Y-axis has to change into Pressure (P, MPa), using the P = F/A, which A results of the width of the material (2 mm) and thickness. The highest value of intersection (x,y) we could know that the x-values is elongation at break (EB) and y-values are the ultimate tensile strength (UTS). The last value of elongation in the x-axis is the maximum elongation (ME). UTS is a maximum of pressure or forces that can make AM start to break. Elongation at break is the substance's elongation percentage that starts to break. Maximum elongation tells the maximum elongation percentage that can be achieved by AM before a total breaking. Toughness is defined as the sum of energy that could be given to make the material start to break after the maximum tensile. The toughness value is calculated as the area under the curve of tensile strength to elongation or the extension used in the origin program.

The results of measurement presented as mean, standard deviation, and median were calculated for

Young's modulus, ultimate tensile strength, elongation at break, maximum elongation, and toughness. We used the Mann-Whitney U test to test the significance of differences among the membranes based on the fetal gender. The significance of difference among the membranes to the frequency of pregnancy and the length of the pregnancy test use the Kruskal-Wallis Test. The value of $p<0.05$ was counted to be significant in this study.

3. Results

The surface structure of epithelial and stromal AM was examined by scanning electron microscopy (SEM). SEM showed that the native AM epithelial stem cells on the AM surface side (Figure 2A) than native stromal cells on the backside of AM (Figure 2B).

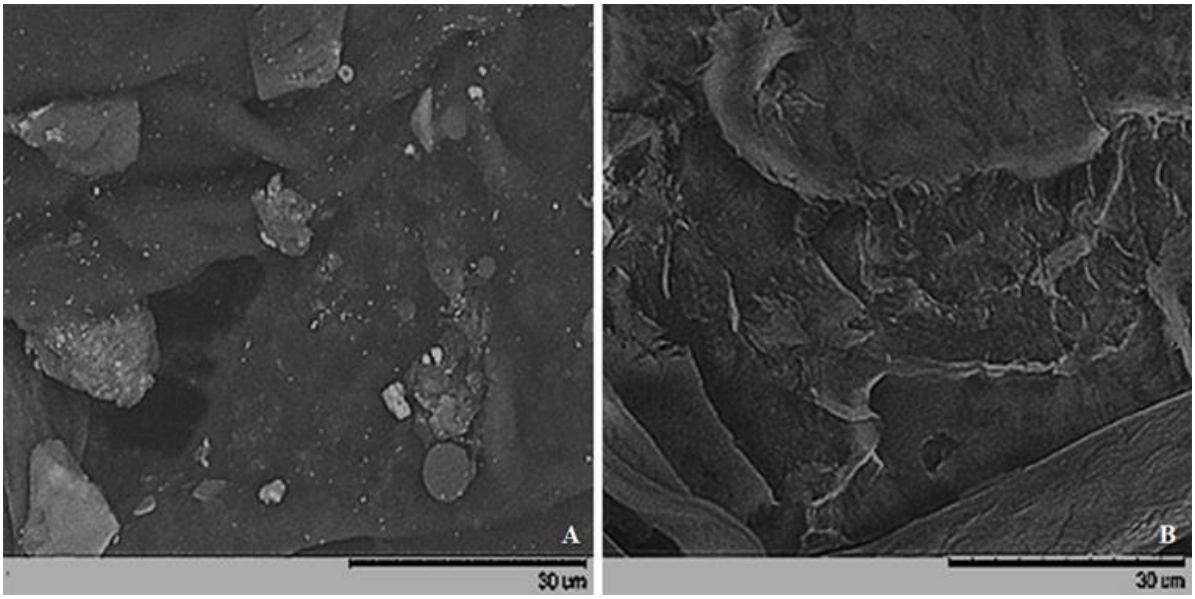

Figure 2. (A) SEM epithelial and (B) stromal surface of amniotic membrane.

The mechanical profile showed that the average thickness of Mf's AM was 0.133 mm. The mean of Young's modulus was 5.932 Mpa (median was 3.549 Mpa). Another parameter that we measured was ultimate tensile strength was 1.011 Mpa (median 0,727 Mpa), elongation at break was 27.08% (mean 3.81mm; median 3.68mm), maximum elongation 44.69% (mean 4.43mm; median 4.02mm) and the mean of toughness was 0.130 MJ/m^3 (median 0.0975 Mj/m^3). We make the comparison of the mechanical properties of Mf AM depending on the data of fetal gender, gestational age, and the frequency of the section cesarean. The results showed that there is no statistically significant difference in mechanical properties between the fetal gender, gestational age, and the frequency of pregnancy. The details of statistical data and the result of significance are given in Table 1 and Table 2. The mechanical profile from three adult male dura mater of eight years old *Macaca fascicularis* showed that the mean tissue thickness was 0.2605 mm. The mean of Young's modulus was 43.35 Mpa, the mean ultimate tensile strength was 3.0875 Mpa, the mean of elongation at break was 3.77 mm (23.64%) and the mean maximum elongation was 5.16 mm (71.94%) and the mean toughness was 0.805 MJ/m^3.

Table 1. Comparison of mechanical properties of *Macaca fascicularis* amniotic membrane based on the fetal gender.

Mechanical property	**Thickness (mm)**		**Modulus young's (MPa)**		**Ultimate tensile strength (MPa)**		**Elongation at break (%)**		**Maximum elongation (%)**		**Toughness (MJ/m³)**	
Fetus gender	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Mean	0.13	0.14	8.25	4.125	1.096	0.945	20.99	31.81	41.74	46.99	0.113	0.1433
(SD)	0.057	0.058	13.12	3.254	1.377	0.599	13.63	24.41	33.7	23.59	0.094	0.096
Median	0.136	0.143	3.33	3.77	0.71	1.069	13.38	23.21	27.63	47.41	0.088	0.147
P-value[a]	**0.791**		**0.874**		**0.791**		**0.186**		**0.56**		**0.397**	

[a]Mann-Whitney U test.

Table 2. Comparison of mechanical properties of *Macaca fascicularis* amniotic membrane based on the duration of pregnancy and frequency of the section cesarean.

Characteristic	**Mechanical property**	**Thickness (mm)**			**Modulus young's (MPa)**			**Ultimate tensile strength (MPa)**			**Elongation at break (%)**			**Maximum elongation (%)**			**Toughness (MJ/m³)**		
Duration of the pregnancy (days)		**127**	**137**	**147**	**127**	**137**	**147**	**127**	**137**	**147**	**127**	**137**	**147**	**127**	**137**	**147**	**127**	**137**	**147**
	Mean	0.0833	0.144	0.166	2.28	5.33	12.61	0.374	0.955	2.03	19.281	27.23	37.03	31.68	45.78	58.81	0.052	0.13	0.233
	(SD)	0.06	0.036	0.075	1.877	3.79	20.83	0.351	0.609	1.77	7.165	23.96	22.96	10.53	27.02	44.26	0.05	0.09	0.019
	Median	0.094	0.146	0.19	2.38	3.77	0.69	0.368	1.069	1.29	18.9	22.97	48.23	27.01	36.51	67.68	0.05	0.093	0.24
	P-value[b]	**0.072**			**0.476**			**0.122**			**0.75**			**0.588**			**0.063**		
Frequency of pregnancy		**1st**	**2nd**	**3rd**	**1st**	**2nd**	**3rd**	**1st**	**2nd**	**3rd**	**1st**	**2nd**	**3rd**	**1st**	**2nd**	**3rd**	**1st**	**2nd**	**3rd**
	Mean	0.147	0.134	0.12	3	13.55	2.03	1.012	1.68	0.45	28.11	16.13	35.35	41.63	27.26	61.78	0.15	0.14	0.11
	(SD)	0.081	0.038	0.05	2.61	13.42	1.57	0.69	1.4	0.3	14.13	5.43	29.69	17.93	20.15	32.09	0.1	0.1	0.088
	Median	0.172	0.14	0.13	3.33	8.85	1.58	1.3	1.43	0.55	23.34	13.38	25.1	36.51	20.35	63.47	0.147	0.093	0.095
	P-value[b]	**0.452**			**0.051**			**0.133**			**0.194**			**0.117**			**0.846**		

[b]Kruskal-Wallis Test.

4. Discussion

The AM has been used in any area of the medical field of tissue engineering as the material graft and transplantation. Some studies showed the use of AM as the scaffold for tissue regeneration or promote tissue differentiation.[10] Two study groups already report the advantage of the AM in dural closure and substitute.[1,2] They conclude that the mechanism of AM could be used as the scaffold of dura mater tissue regeneration as well as had the safety mechanical barriers to prevent the leakage of CFS. In order to observe the ability of Mf AM as a potential biomaterial for dural defect closure, the use of Mf as an NHP animal model, a physical barrier, and the cellular content of the AM is important. The ideal graft or mechanical barrier that could be used as a dura mater substitute or closure of the defect, the material must fulfill some profile criteria. Our study provides evidence of mechanical properties that naturally belong to AM to act as the physic-mechanical barrier in the dura mater substitute procedure.

Our results have shown that there is no statistically significant difference in Young's modulus, tensile strength, elongation at break, maximum elongation, and the toughness between the fresh preparation of AM that protects the male or female fetus intrauterine. There are no statistically significant differences in mechanical properties between the gestational age (among 127-, 137- or 147 days) and the frequency of pregnancy in fresh preparation Mf AM. There is no previous report of mechanical properties of AM from Macaque species, so we cannot compare our results in the same NHP species. The normal gestational age for *Macaca fascicularis* is 155-165 days. Births before 155 days are known as preterm and if less than 140 days are premature.[12] Our sample group consists of two groups of premature (127 days, n=4; 137 days, n=9) and one group of preterm (147 days, n=3).

The average thickness of Mf AM used in this study was 0.133 mm and there is no statistically significant difference (p=0.072) in thickness among the three groups of gestational age. This same phenomenon was also found in Young's modulus (p=0.476), tensile strength (p=0.122), elongation at break (p=0.75), maximum elongation (p=0.588), and toughness (p=0.063). Benson-Martin et al (2006)[12] report that human AM has a higher Young's modulus in preterm samples (3.60 Mpa) than in term samples (2.29 Mpa). Our results showed the average of Young's modulus in preterm Mf AM was 12.61 MPa. Young's modulus is a measure of elasticity and is determined by elastin that is present in amnion.[12,13] The higher value of Young's modulus means that the material is stiffer.

Another study by George et al., (2018)[13] measured the mechanical properties of human AM and showed Young's modulus was 0.645 Mpa, tensile strengths were 0,156 Mpa, elongation at break was 17 mm and the thickness was 0.46 mm; they also reported the average thickness of human AM was 0.43 mm, Young's modulus was 0.65 Mpa, tensile strength was 0.16 MPa and elongation at break was 17.33 mm.14 Our study showed the average of Mf AM Young's modulus was 5.932 Mpa (median was 3.549 Mpa), tensile strength was 1.011 MPa (median 0,727 Mpa), elongation at break was 3.81 mm (median 3.68 mm) and thickness of Mf AM was 0.133 mm. Chuck et al (2004) reported the dry preparation of human AM to have a higher Young's modulus compared to the moist preparations. Rehydrated gamma-irradiated preparations had the lowest mean Young's modulus than dry and moist preparation.[14] This means that low-dose electron beam-irradiated human AM has the best elasticity and provides more advantages in clinical applications as the material of tissue reconstructions. It concluded that different methods in sample preparation will affect the Young's modulus results.

Fresh amniotic membrane (AM) possesses distinct advantages in clinical applications, particularly in the context of dura mater tissue substitution, due to its rich repertoire of bioactive components. These include an array of growth factors, stem cells, and anti-angiogenic and anti-inflammatory proteins, which collectively contribute to the intricate process of dural healing and regeneration. The utilization of fresh amniotic membrane (AM) in dural repair, as opposed to preserved or processed alternatives, represents a strategic approach to harnessing the full spectrum of its regenerative potential. This preference stems from the critical need to preserve the delicate balance and bioactivity of labile molecules intrinsic to the AM, particularly growth factors, which play pivotal roles in orchestrating the complex dural healing cascade. The concept of "freshness" in the context of AM utilization underscores the temporal sensitivity of its bioactive components. Growth factors, cytokines, and other signaling molecules embedded within the AM's intricate extracellular matrix are susceptible to degradation and denaturation upon prolonged storage or exposure to harsh processing methods. Fresh AM, harvested and utilized within a short timeframe, circumvents these challenges, ensuring the preservation of these molecules in their native, biologically active state.[14,15]

This preservation of bioactivity is paramount for eliciting the desired therapeutic effects in dural repair.

Growth factors, in particular, act as potent signaling molecules that guide and regulate the intricate cellular processes involved in tissue regeneration. Two key players in this context are fibroblast growth factor-2 (FGF2) and transforming growth factor beta-1 (TGFβ1), each with distinct yet complementary roles in the dural healing cascade. FGF2, a member of the fibroblast growth factor family, is renowned for its mitogenic and chemotactic effects on fibroblasts, the principal cells responsible for synthesizing and maintaining the structural integrity of the dura mater. Upon dural injury, FGF2 acts as a beacon, attracting fibroblasts to the site of damage and stimulating their proliferation. This rapid expansion of the fibroblast population provides the necessary cellular workforce for the subsequent deposition of collagen and other ECM components, ultimately leading to the formation of new connective tissue and the closure of dural defects. Beyond its mitogenic role, FGF2 also orchestrates the migration of fibroblasts, guiding them toward the areas in need of repair. This directed cell movement ensures efficient and targeted tissue regeneration, minimizing the formation of excessive scar tissue and promoting a more organized and functional dural repair. TGFβ1, a pleiotropic cytokine with diverse functions in tissue repair and regeneration, exerts its influence on multiple fronts during dural healing. One of its primary roles is the modulation of the inflammatory response, a critical early phase in the healing cascade. While inflammation is essential for clearing debris and initiating repair, its dysregulation can lead to chronic inflammation and impaired healing. TGFβ1 helps to maintain a balanced inflammatory response, promoting the resolution of inflammation and preventing excessive tissue damage. In addition to its anti-inflammatory effects, TGFβ1 also plays a crucial role in angiogenesis, and the formation of new blood vessels. Adequate blood supply is essential for delivering oxygen and nutrients to the healing tissues, supporting cell proliferation, and ECM deposition. TGFβ1 stimulates the growth of new blood vessels, ensuring proper tissue perfusion and facilitating the regeneration process. Furthermore, TGFβ1 directly influences ECM deposition and remodeling. It stimulates the production of collagen and other ECM components by fibroblasts and regulates the activity of matrix metalloproteinases (MMPs), enzymes responsible for ECM degradation. This delicate balance between ECM synthesis and degradation is crucial for achieving a well-organized and functional dural repair. The utilization of fresh AM, with its preserved growth factors and other bioactive molecules, ensures the optimal activation and coordination of these intricate cellular and molecular processes involved in dural healing. The timely release of FGF2 and TGFβ1 at the site of injury sets in motion a cascade of events, starting with fibroblast recruitment and proliferation, followed by ECM deposition, angiogenesis, and ultimately, the restoration of dural integrity and function. In contrast, preserved or processed AM may exhibit reduced bioactivity due to the degradation or denaturation of growth factors and other labile molecules. This can compromise the efficacy of dural repair, leading to delayed healing, incomplete tissue regeneration, and an increased risk of complications.[15,16]

The amniotic membrane (AM) harbors a heterogeneous population of stem cells, including epithelial stem cells (ESCs) and mesenchymal stem cells (MSCs), which significantly contribute to its remarkable regenerative potential. These stem cells possess the unique ability to self-renew and differentiate into a diverse array of specialized cell types, making them indispensable players in tissue repair and regeneration. In the context of dural repair, the presence of these stem cells within the AM offers a promising avenue for promoting the restoration of dural architecture and function. ESCs, located on the outermost layer of the AM, are characterized by their ability to differentiate into various epithelial cell types, including keratinocytes and corneal epithelial cells. While their primary role in the AM is to provide a protective barrier against the external environment, ESCs also exhibit remarkable plasticity and can contribute to the regeneration of other tissues. In

dural repair, ESCs can potentially differentiate into fibroblasts, the key cells responsible for producing collagen and other ECM components that provide structural integrity to the dura mater. This differentiation capacity allows ESCs to actively participate in the healing process, promoting the formation of new dural tissue and restoring the dural barrier. MSCs, residing within the stromal layer of the AM, are multipotent stem cells capable of differentiating into a wide range of cell types, including fibroblasts, osteoblasts, chondrocytes, and adipocytes. This remarkable differentiation potential makes MSCs highly attractive for tissue engineering and regenerative medicine applications. In the context of dural repair, MSCs can contribute to the regeneration of multiple tissue types, including bone, cartilage, and connective tissue, which may be necessary in cases of complex dural defects involving adjacent structures. Moreover, MSCs possess potent immunomodulatory and anti-inflammatory properties, which can help to create a favorable microenvironment for tissue repair. By suppressing the inflammatory response and promoting angiogenesis, MSCs facilitate the recruitment of other reparative cells and the delivery of nutrients and oxygen to the injured site, thereby accelerating the healing process.[14,16]

In addition to their differentiation capacity, stem cells within the AM exert their regenerative effects through paracrine signaling. These cells secrete a diverse array of growth factors, cytokines, and chemokines, which act as signaling molecules to regulate various cellular processes involved in tissue repair. These secreted factors can stimulate cell proliferation, migration, and differentiation, promote angiogenesis, modulate the immune response, and enhance ECM deposition. By creating a favorable microenvironment for tissue regeneration, stem cell-derived paracrine factors play a crucial role in the success of dural repair. The presence of both ESCs and MSCs within the AM creates a synergistic effect, maximizing its regenerative potential. ESCs, with their ability to differentiate into fibroblasts and provide a protective barrier, contribute to the initial closure of dural defects and the prevention of CSF leakage. MSCs, on the other hand, with their multi-lineage differentiation capacity and immunomodulatory properties, promote the long-term regeneration and remodeling of the dural tissue, ensuring a durable and functional repair.[15]

The amniotic membrane's (AM) inherent anti-angiogenic and anti-inflammatory properties are of paramount importance in facilitating optimal dural healing. These properties contribute significantly to a well-orchestrated and balanced repair process, minimizing the risk of complications and promoting functional tissue regeneration. Inflammation is an indispensable part of the initial wound healing response, serving to eliminate debris, recruit immune cells, and initiate tissue repair. However, when inflammation becomes chronic or excessive, it can impede the healing process and lead to adverse outcomes. In the context of dural healing, uncontrolled inflammation can result in the formation of excessive scar tissue, which can impair dural function and contribute to neurological complications. The AM, acting as a natural immunomodulator, helps to regulate the inflammatory response during dural healing. It contains a variety of anti-inflammatory mediators, including cytokines, growth factors, and bioactive lipids, which suppress the production of pro-inflammatory molecules and promote the resolution of inflammation. This immunomodulatory action of the AM creates a favorable microenvironment for tissue repair, preventing excessive scarring and promoting a more organized and functional dural regeneration.[16,17]

Angiogenesis, the formation of new blood vessels, is a critical process in tissue regeneration, providing oxygen and nutrients to the healing site and facilitating the removal of waste products. However, uncontrolled or excessive angiogenesis can lead to the formation of aberrant blood vessels, which can be leaky, fragile, and prone to hemorrhage. In the context of dural healing, dysregulated angiogenesis can contribute to the formation of vascularized scar tissue and compromise the integrity of the blood-brain

barrier. The AM, through its anti-angiogenic factors, helps to maintain a delicate balance in angiogenesis during dural healing. It contains several molecules, such as endostatin, angiostatin, and thrombospondin-1, which inhibit the proliferation and migration of endothelial cells, the building blocks of new blood vessels. This anti-angiogenic action of the AM ensures that new blood vessels are formed in a controlled and regulated manner, providing adequate tissue perfusion while minimizing the risk of aberrant vessel growth and associated complications. The AM's anti-angiogenic and anti-inflammatory properties work in concert to create an optimal environment for dural healing. By modulating the inflammatory response and regulating angiogenesis, the AM promotes a balanced and organized repair process, minimizing the risk of excessive scarring and aberrant vessel growth. This results in a more functional and durable dural repair, reducing the likelihood of complications and improving patient outcomes. Furthermore, the AM's anti-inflammatory and anti-angiogenic actions may also contribute to its neuroprotective effects. Inflammation and angiogenesis are implicated in the pathogenesis of various neurological disorders, including traumatic brain injury, stroke, and neurodegenerative diseases. By modulating these processes, the AM may help to protect the brain and spinal cord from further damage and promote neurological recovery.[15,16]

The amniotic membrane's (AM) potential in dura mater tissue substitution is significantly bolstered by the synergistic relationship between its physical attributes, bioactive molecules, and cellular constituents. This multifaceted nature positions the AM as an active participant in the dural healing process, surpassing the capabilities of traditional, passive repair materials. The AM's inherent elasticity and tensile strength are crucial in providing a robust mechanical barrier against cerebrospinal fluid (CSF) leakage. CSF leakage is a major concern in dural repair, as it can lead to serious complications such as infection, meningitis, and pseudomeningocele formation. The AM's ability to withstand the pulsatile pressures and mechanical stresses exerted by the CSF and surrounding tissues is paramount in maintaining the integrity of the dural repair and preventing these complications. The elasticity of the AM allows it to conform to the contours of the dural defect and accommodate the natural movements of the brain and spinal cord. This adaptability minimizes the risk of tension or stress on the repair site, promoting a more stable and secure closure. Additionally, the AM's tensile strength ensures that it can resist tearing or rupture, even under significant mechanical loads. This resilience is particularly important in the early stages of healing when the repair site is most vulnerable to disruption.[16,17]

Beyond its role as a physical barrier, the AM's cellular components, including fibroblasts and collagen, actively contribute to the dural healing process. Fibroblasts, the primary cells responsible for synthesizing and maintaining the ECM, play a crucial role in dural regeneration. Upon implantation, the AM's fibroblasts integrate with the host tissue, proliferate, and secrete collagen and other ECM components, thereby facilitating the formation of new dural tissue. Collagen, the main structural protein of the ECM, provides tensile strength and structural support to the dura mater. The AM's abundant collagen content serves as a framework for the deposition of new ECM, guiding the organization and maturation of the regenerating dural tissue. Moreover, the AM's collagen fibers interact with the host's fibroblasts, stimulating their activity and further promoting ECM synthesis. The AM's rich array of bioactive molecules, including growth factors, cytokines, and chemokines, orchestrates a complex cascade of events that drive dural healing and regeneration. These molecules act in concert to promote cell migration, proliferation, and differentiation, modulate inflammation, and stimulate angiogenesis. Growth factors, such as FGF2 and TGFβ1, play pivotal roles in this process. FGF2 stimulates fibroblast proliferation and migration, while TGFβ1 regulates the inflammatory response and promotes ECM deposition. Other growth factors

present in the AM, such as epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF), further contribute to cell proliferation, angiogenesis, and tissue remodeling. The AM's anti-inflammatory and anti-angiogenic properties are also crucial in ensuring a balanced and controlled healing response. Excessive inflammation can lead to scarring and fibrosis, while uncontrolled angiogenesis can result in aberrant vessel growth and potential complications. The AM's anti-inflammatory and anti-angiogenic factors help to regulate these processes, promoting a more organized and functional repair.[17]

The synergistic interplay between the physical properties, bioactive molecules, and cellular constituents of fresh AM creates a dynamic and conducive environment for dural regeneration. The AM not only acts as a passive barrier but also actively participates in the healing process, stimulating and guiding the host's regenerative response. This multifaceted approach to dural repair offers significant advantages over traditional methods, which often rely on passive materials that lack the inherent regenerative capacity of the AM. By providing a mechanical barrier, promoting cell migration and proliferation, modulating inflammation, and facilitating angiogenesis, the AM fosters a more rapid and complete dural healing process. This translates to reduced risk of complications, improved functional outcomes, and faster recovery times for patients undergoing neurosurgical procedures. AM has been used as a graft in dural closure and or substitution.[1,2] Its mechanical properties as the membrane barrier achieved the capability in the prevention of CSF leakage and cell integrations to the native dura mater cells component. On the side, some native cells of AM and dura mater i.e. fibroblast and collagen play major roles in dural healing and defect closure.[15,17-19] It means that the fresh preparation of AM that contains extracellular matrix, cellular content, and growth factors is the better suitable preparation of AM as the biomaterial graft in dura mater substitute. In order to use AM in a duraplasty procedure, the neurosurgeon will consider choosing the materials that have the highest advantages to reduce the inflammation reaction and improve the proliferation and differentiation of the host tissue. The main consideration depends on the AM's ability to support and mediate the fusion with the host in addition to mechanical properties.

From our study, we know that the dura mater tissue of Mf is seven times more stiff than AM and three times more strength tensile than AM. The capability of this membrane as the biomaterial graft for dural replacement or substitute depends on its mechanical properties, the native cellular content of stem cells, growth factors, and extracellular matrix. In vivo, investigation in duraplasty procedure using the AM will provide the efficacy and advantages as a potential biomaterial graft for dural substitute and or defects closure. This work provides a clear focus to consider the mechanical properties of the AM for future application of allograft material for dural defect closure and or substitutions.

5. Conclusion

The mechanical properties of Mf AM are not dependent on fetal gender, gestational age, and frequency of pregnancy. The elasticity of the dura mater is seven times stiffer than the AM and the tensile strength of the dura mater is three times bigger than the AM. This work provides an explanation of the physical properties of fresh preparation AM as the consideration for use as allograft biomaterial in dural substitution. Our work suggests, in regards to different thicknesses between AM and dura mater, that this could be solved by stacking the AM into several layers, i.e., stacking up the AM with the stromal surface facing down the dura mater, prior to use.

6. References

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