Received: 03 December 2023 | Revised: 26 January 2024 | Accepted: 05 February 2024
DOI: 10.48309/JMPCR.2024.433692.1078

Journal of Medicinal and Pharmaceutical Chemistry Research SAMI

FULL PAPER

Study of the physical, mechanical and water absorption of bovine amnion membrane and bovine amnion sponge

Kukuh Ali Akbar[a] | Teddy Heri Wardhana[a] | Heri Suroto[a,b,*] | Gilson Khang[c]

[a]*Department of Orthopedic and Traumatology, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia*

[b]*Cell & Tissue Bank-Regenerative Medicine, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia*

[c]*Department of Polymer Science and Engineering, Chonbuk National University, Duckjin Dong, Jeonju, South Korea*

Amniotic membrane is used as a biomaterial in orthopedics, and its use keeps increasing. Another source of amnion can be found in bovine. Amniotic membrane in the sponge form is more easily applied to several types of wounds, is more durable, and can be used as an absorbent dressing for productive chronic wounds. This type of study is an analytic observational study that gives three different treatments to two different samples. The research design used is a randomized, post-test-only design. The bovine amnion was made into a membrane and sponge, and they were characterized based on thickness, water absorption, and tensile strength. The bovine amnion membrane has a tensile strength of 0.07 ± 0.006 N/mm^2, while the bovine amnion sponge has a tensile strength of 0.118 ± 0.01 N/mm^2, it has a significant result difference with a p-value of 0.001. In the absorption power test results, it was found that the bovine amnion sponge had an average absorption power of 0.84 ± 0.09 (1004 ± 122%), and the bovine amnion membrane had an absorption power of 0.80 ± 0.11 (806 ± 118%) with a p-value of 0.442. The average thickness of the bovine amnion membrane is 0.30 ± 0.01, and the thickness of the bovine amnion sponge is 0.61 ± 0.26; it also has a significant result difference with a p-value of 0.001. Bovine amnion sponges have better absorption capability and stronger tensile strength, while freeze-dried amnion membranes have a more homogeneous thickness.

***Corresponding Author:**
Heri Suroto
Email: heri-suroto@fk.unair.ac.id
Tel: N/A

KEYWORDS

Amniotic; bovine; membrane; sponge.

Introduction

Amnion membranes are widely known biomaterials and have recently been used for various clinical applications. The amnion membrane is an extracellular tissue composed of the placenta (the uterus plate) and the mother (decida) [1]. The amnion membrane has unique properties, including anti-inflammatory, antibacterial, antiviral, antifibrotic, and anti-scarring properties, and it has a very high tensile strength [2].

In recent years, amniotic membranes have been used as a biomaterial in orthopedics, and their use has kept increasing. This is because the amniotic membrane is affordable and accessible. However, amnion availability in the market is very limited because of unbalanced supply and demand. In a recent study, a freeze-dried human amniotic membrane (FdHAM) was developed.

The biological properties of FdHAM include an anti-inflammatory effect, antibacterial and antiviral effect, low antigenicity and non-immunogenicity, anti-scarring and antiadhesive effect in wound healing, angiogenesis and anti-angiogenesis properties (surface dependent), an anticancer agent with low tumorigenicity, promotes epithelialization, pain reliever, support cell adhesion, and growth. Another source of amnion can be found in bovine.

In recent years, amniotic membrane is used as a biomaterial in the orthopaedic field and its use is increasing. This is because it is affordable and readily available. The problem is that the availability of amnion in the market is very limited due to the large number of requests not proportional to the amount of production. One source of amniotic membrane other than human is from bovine. Bovine amniotic membrane in sponge form is easier to apply to several types of wounds, lasts longer and can be used as an absorbent dressing in productive chronic wounds. Bovine amnion contains collagen which can accelerate wound healing [3]. Amniotic membrane is proven to be safe to use, but its use is limited due to its sheet form. For easy application, gelatin is added to the amniotic membrane to produce a sponge shape known as amniotic sponge [1].

Amniotic sponge is more easily applied to several types of wounds, is more durable, and can be used as an absorbent dressing for productive chronic wounds [3-5]. Sponge amnion is a pulverized amnion membrane with a size of about 250 μm mixed with gelatin as an adhesive material and processed by freeze-drying method using room temperature. Amnion membrane converted into a sponge shape is expected to make it easier for doctors to use it more easily. Amnion sponge is a biomaterial that is easily available, affordable, and easy to apply [1].

From everything described above, in order to evaluate the characteristics and determine the quality of the bovine amnion. This study will discuss about the characteristics of amnion sponge based on thickness, absorption capacity, biomechanical properties, and the quality of bovine amnion.

Materials and methods

This type of study is a laboratory experimental study by giving three different treatments to two different samples. The research design used is a randomized post-test-only design, where in this study, after the treatment, a measurement (posttest) is given to determine the treatment effect so that the magnitude of the impact of the treatment can be known. Female pregnant bovine should belong to inclusion criteria, no specific bovine breed was decided to become inclusion criteria.

The amnion membranes were then deep-freezed at -80 °C for at least 24 hours to make freeze-dried amnion membranes (FD-AM). The next step would be lyophilization (Lyophizer Lyovoc GT2) of the amnion membranes to a temperature of -40 °C to -50 °C that was carried out for 6-8 hours until the amnion membrane water content was 6-7%.

The amnion membranes that had been lyophilized were then cut to the desired size and packed in three layers of polyethylene plastic sealed with a vacuum sealer. This process was carried out in a laminar airflow cabinet for preparing the amnion sponge preparations; the same steps were also applied to the placenta tissue collection: amnion membranes disinfection with 0.05% NaOCl solution and washing the amnion membranes with 0.9% NaCl until placing the stretched-out amnion on top of a sterile gauze.

Amniotic sponge made of amniotic membranes, the amnion was minced into small pieces and ground with normal saline at a ratio of 1:1 until the desired texture was achieved. The mixture was then molded as amnion sponge preparations and stored in a deep freezer at -80°C for a minimum of 24 hours to make freeze dried amnion sponge before being lyophilized and, thus, packed in three layers of polyethylene plastic.

There are three evaluations in each test: thickness evaluation, absorption power evaluation, and biomechanical property evaluation. The study samples used were freeze-dried bovine amnion sponge and freeze-dried bovine amnion membrane. This study was conducted at the Tissue and Cell Bank Installation of Dr. Soetomo General Academic Hospital Surabaya and the Metallurgical Engineering Laboratory of Sepuluh Nopember Institute of Technology from January 2023 to May 2023. The tools used in the tensile strength test were Hung TA Model HT-2402, serial number T3Y0505, and thickness test using Coating Thickness Gauge.

Results

After the sample is given the treatment of 3 types of tests, the therapy results provide the data output in the form of data. Here are the data results of the tests that have been carried out. The FD-AM and amnion sponge sample can be seen in Figures 1 and 2.

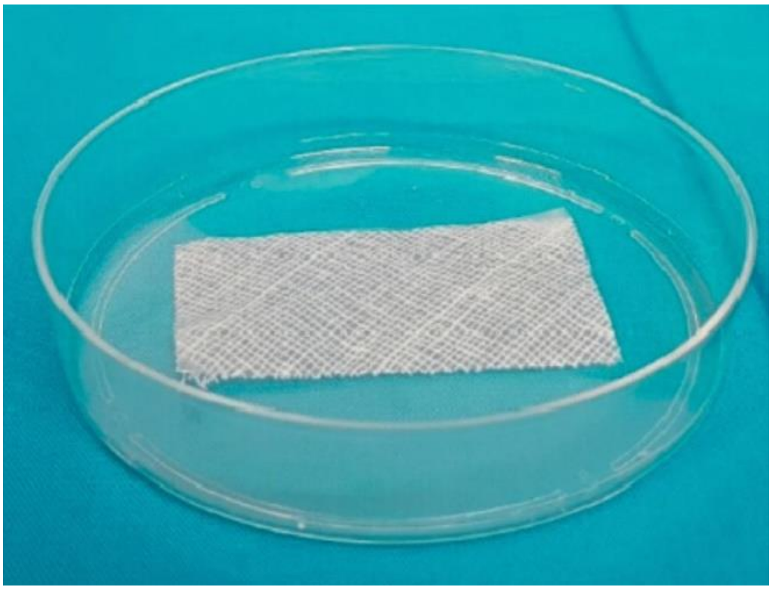

FIGURE 1 Freeze dried amnion membrane

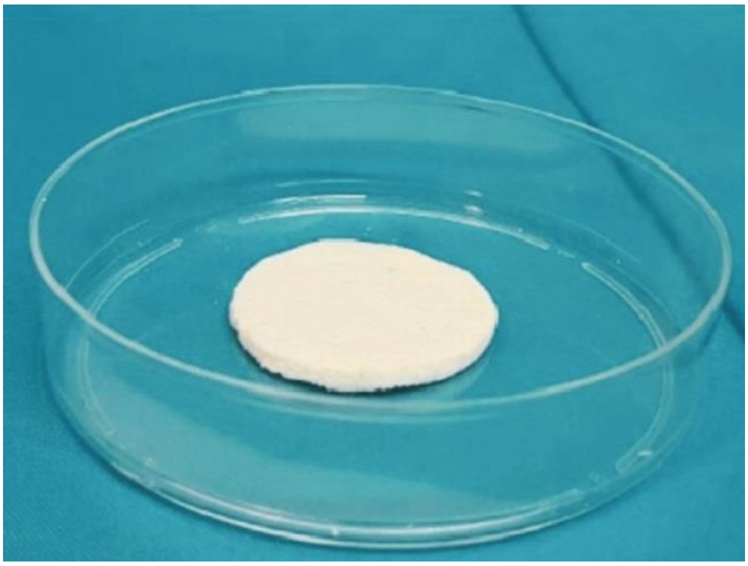

FIGURE 2 Amnion sponge

Tensile strength test

The tensile strength test results of the freeze-dried amnion sponge obtained in this study are greater than those of the amnion membrane. The bovine amnion membrane has a tensile strength of 0.07±0.006 N/mm², while the freeze-dried bovine amnion sponge has a tensile strength of 0.118±0.01 N/mm². The elongation test also shows that the freeze-dried bovine amnion sponge is more elastic than the bovine amnion membrane. The results obtained are 4.35±0.76% for the freeze-dried bovine amnion membrane and 10.50±0.39% for the freeze-dried bovine amnion sponge, as presented in Table 1.

The analytical study was initially tested for normality; the result is normally distributed with a p-value of more than 0.05, as indicated in Table 2. The independent T-test obtained a significant difference between the tensile strength of the freeze-dried bovine amnion sponge and freeze-dried bovine amnion membrane with statistically significant (p=0.001).

TABLE 1 Tensile strength bovine freeze dried amnion membrane and bovine amnion sponge size 50 mm x 5 mm

Sample	Area (mm^2)	Max Force (N)	Yield Strength (N/mm^2)	Tensile strength (N/mm^2)	Young's Modulus (E) (N/mm^2)	Elongation %
Amnion Membrane	19.55 ± 0.08	1.51 ± 0.07	0.024 ± 0.01	0.07 ± 0.006	3.71 ± 0.43	4.35 ± 0.76
Amnion Bovine	41.94 ± 0.48	4.95 ± 0.32	0.201 ± 0.26	0.118 ± 0.01	1.47 ± 0.01	10.50 ± 0.39

TABLE 2 Normality test of bovine amnion sponge and bovine amnion membrane tensile strength

Sample	Saphiro-Wilk (p) Test Result
Freeze-dried Bovine Amnion Membrane	0.084
Freeze-dried Bovine Amnion Sponge	0.106

Absorbability Test

In the absorption power test results, it was found that the freeze-dried bovine amnion sponge had an average absorption power of 0.84±0.09 (1004±122%), and the freeze-dried bovine amnion membrane had an absorption power of 0.80±0.11 (806±118%) (Figure 3). These results show that freeze-dried bovine amnion sponge has a higher absorption capacity than freeze-dried bovine amnion membrane. This can be caused by amnion, which is processed into a sponge with a cavity.

The normality study was tested first, and the result is normally distributed with a p-value of more than 0.05 (Table 3). The independent T-test obtained a difference between the absorbability of the freeze-dried bovine amnion sponge and freeze-dried bovine amnion membrane but not statistically significance (p= 0.442).

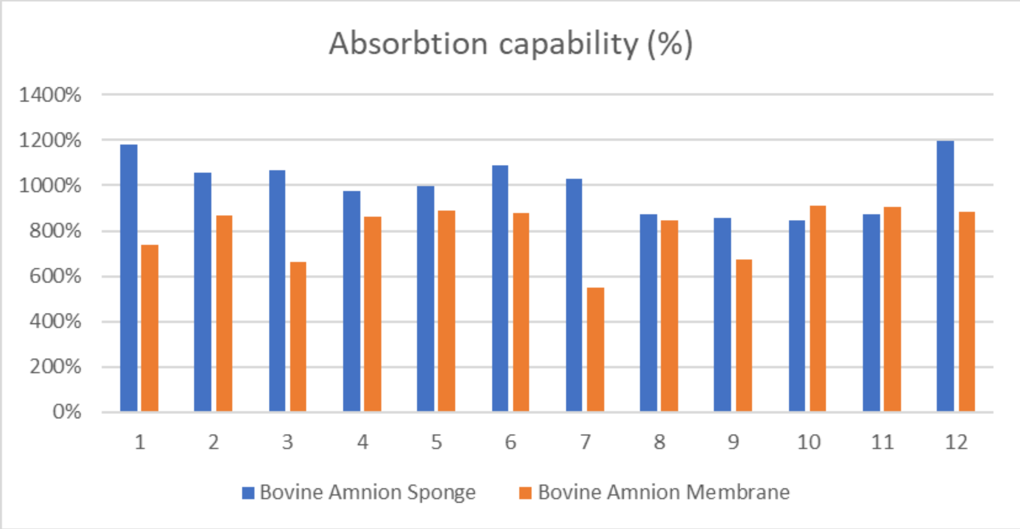

FIGURE 3 Absorption of bovine amniotic sponge and bovine amniotic membrane

TABLE 3 Normality test of bovine amnion sponge and bovine amnion membrane absorbability

Sample	Treatment	Saphiro-Wilk (p) Test Result
Bovine Amnion Membrane	Before soaking	0.200
	After soaking	0.098
Bovine Amnion Sponge	Before soaking	0.200
	After soaking	0.200

Thickness Test

The thickness test examination was carried out with coating thickness gauge at the top, middle, and bottom points. Those were then calculated as the average thickness of each composition variation. Figure 4 depicts the average thickness of the bovine amnion membrane at 0.30±0.01 and the thickness of the bovine amnion sponge at 0.61±0.26. These results show that the thickness of the amnion membrane is more homogeneous than that of the freeze-dried bovine amnion sponge.

The analytical study was initially tested for normality; the result was normally distributed with a p-value of more than 0.05 (Table 4). The independent T-test obtained a significant difference between the thickness test freeze-dried bovine amnion sponge and freeze-dried bovine amnion membrane with statically significant (p=0.001).

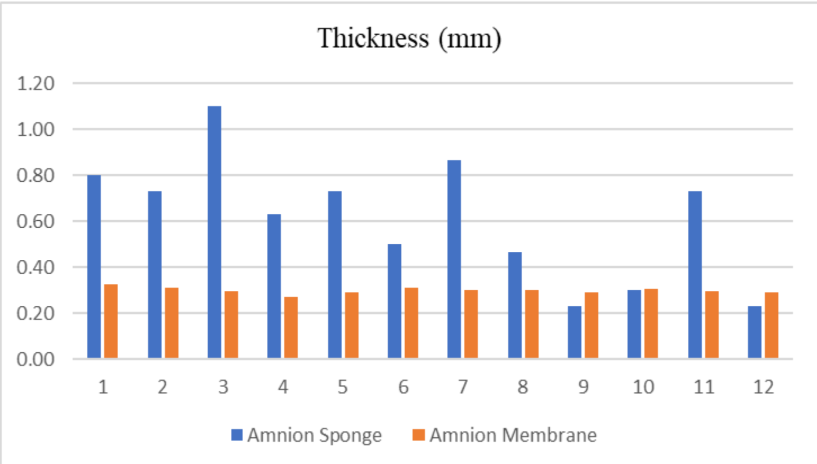

FIGURE 4 Thickness of bovine amnion sponge and bovine amnion membrane

TABLE 4 Normality test of bovine amnion sponge and bovine amnion membrane thickness

Sample	Saphiro-Wilk (p) Test Result
Freeze-dried Bovine Amnion Membrane	0.434
Freeze-dried Bovine Amnion Sponge	0.575

Discussion

Amnion can be sourced from humans or bovines (cows). However, human amnion is currently limited, as the number of human donors is less than the demand. Therefore, a bovine-derived amnion was created as a solution to this problem. Amnion derived from bovine has the potential to be a solution because the production process can be more efficient, and ethical issues are not a problem in its production [3,6].

One of the requirements of an ideal wound dressing is to protect the wound from external friction and trauma. This study tested the physical properties of freeze-dried bovine amnion membrane and freeze-dried amnion sponge. The results showed that the thickness of the freeze-dried amnion sponge was more heterogeneous than the freeze-dried amnion membrane. This can occur because when the freeze-dried amnion sponge is prepared, the washing process is soaked in sodium hypochlorite 0.05% for 10 minutes, and then the freeze-dried amnion sponge is inserted into the water bath shaker, which has been filled with sterile physiological NaCl. Freeze-dried amnion Sponge that has been washed, blended, and placed on sterile gauze with the chorion side facing the gauze, and then cut according to the desired size. This process is carried out in a ''laminar airflow cabinet''. Amnion Sponge is placed in a sterile tray and stored in a deep freezer for 24 hours before freeze-drying [7]. This can cause the thickness of the amnion sponge to be heterogeneous. This study also measured the physical and mechanical characteristics of the bovine freeze-dried amnion sponge and freeze-dried bovine amnion membrane using tensile testing. The results were the tensile strength of bovine amnion membrane samples up to 0.078-0.092 MPa and freeze-dried amnion sponge ranging from 0.095-0.105 MPa. This indicates that the mechanical strength of a freeze-dried bovine amnion sponge is better than that of a bovine amnion membrane. Tensile strength is defined as the breaking strength of a material divided by its cross-sectional area, and it is the most accurate physiological measure to assess the ability of a wound dressing to withstand tension [6,8].

This study shows that the freeze-dried bovine amnion sponge has an absorbency of 1004±122%. In contrast, the bovine amnion membrane has 806±118% absorbency. Based on these results, freeze-dried bovine amnion sponge has better water absorption properties than bovine amnion membrane. The hollow structure of freeze-dried amnion sponge can also increase absorption. Good absorption will minimize fluid, blood, or pus that can become a breeding ground for microorganisms [9,10].

The study conducted by Yang *et al.* showed that wound dressings using human-derived amnion sponges have been developed to accelerate diabetic wound healing. This study also found that Amnion Sponge has better absorption power because the hollow scaffold improves blood coagulation and swelling. This study shows that dressings using amnion sponges have better biocompatibility and air permeability, absorbency, and mechanical properties [11].

Thickness is associated with the structural strength of a material. One structural aspect that we can study here is the arrangement of collagen fibers in tissue. Two features of collagen fibrils and fibers in tissue are the wavy fibers and the number of orientations or alignments of the fibrils. This is in line with the research conducted by Hannah C. Wells *et al*., who mentioned that the nature of bovine amnion, which has wavy fibers, is a vital property found in highly elastic tissues. This structure is caused by fibrillar collagen. Fibers in the amnion are more numerous than in the dermis but may not be as numerous as in the pericardium [12,13].

Preserved preparations of amniotic grafts, such as freeze-dried amnion membrane and amnion sponge, exhibit reduced levels of growth factors compared to fresh amnion grafts. Suroto *et al.* identified lower levels of TGF-β and b-FGF in the amnion sponge compared to the amnion membrane, indicating an inevitable decline in growth factors in preserved amniotic grafts. However, it is crucial to note that a statistically significant decrease in growth factor levels does not necessarily render the grafts unsuitable for clinical use. Despite having lower growth factor levels, preserved amniotic grafts have demonstrated clinical potency comparable to non-preserved amnion membranes in various studies [7].

Both bovine amnion membrane and bovine amnion sponge exhibit favorable safety profiles. With its superior porosity and larger cavity, the bovine amnion sponge proves more conducive to cell proliferation. Nonetheless, further investigation is warranted to explore the preservation capability of the bovine amnion sponge, particularly concerning moisture content levels exceeding 10% [14,15].

Conclusion

The Bovine Amnion Sponge group was better at absorption test, elongation and tensile strength tests. While Freeze Dried Amnion Membrane group was better at the thickness test.

Limitations

This study was limited by minimal total sample and short period.

Acknowledgements

The authors wish to express our gratitude to the Cell and Tissue Bank Regenerative Medicine of Dr. Soetomo General Academic Hospital for its support in the conduct of this study.

Funding

This study received no external funding.

Authors' Contributions

Kukuh Ali Akbar: contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript. Teddy Heri Wardhana: contributed to the implementation of the research, to the analysis of the results and to the writing of the manuscript. Heri Suroto: contributed to the implementation of the research, to the analysis of the results and to the writing of the manuscript. Gilson Khang: contributed to the analysis of the results and to the writing of the manuscript

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Orcid:

Kukuh Ali Akbar:
https://orcid.org/0009-0009-2885-2356
Teddy Heri Wardhana:
https://orcid.org/0000-0002-6936-8730
Heri Suroto:*
https://orcid.org/0000-0002-9384-897X
Gilson Khang:
https://orcid.org/0000-0002-6452-5653

References

[1] E. Munadziroh, F.A. Razak, B. Abbas, H. Soekartono, T.H. Agustantina, M.D. Surboyo, Characterization of Bovine Sponge Amnion (BSA) by a Novel Process for Dental Treatment, *Journal of International Dental and Medical Research*, **2022**, *15*, 479-84. [Google Scholar], [Publisher]

[2] N. Hilmy, N. Yusof, A.A. Nather, *Human amniotic membrane: basic science and clinical application*. World Scientific, **2017**. [Google Scholar], [Publisher]

[3] D.W. Indrawati, E. Munadziroh, T.I. Sulisetyawati, P.M. El Fadhlallah, Sponge amnion potential in post tooth extraction wound healing by interleukin-6 and bone morphogenetic protein-2 expression analysis: An animal study, *Dental Research Journal*, **2019**, *16*, 283. [Google Scholar], [Publisher]

[4] Y. Rizal, M. Edward, T.M. Martanto H. Suroto, Composite characterization of freeze-dried human amnion membrane and human adipose tissue-derived stromal cells for soft tissue engineering, *Bali Medical Journal*, **2023**, *12*, 1543-1548 [Crossref], [Google Scholar], [Publisher]

[5] H. Elkhenany, A. El-Derby, M. Abd Elkodous, R.A. Salah, A. Lotfy, N. El-Badri, Applications of the amniotic membrane in tissue engineering and regeneration: the hundred-year challenge, *Stem Cell Research & Therapy*, **2022**, *13*, 8. [Crossref], [Google Scholar], [Publisher]

[6] T. Zhang, Y. Zhang, J. Yang, P. Wen, H. Li, N. Wei, et al., Dynamic measurement of amnion thickness during loading by speckle pattern interferometry, *Placenta*, **2021**, *104*, 284–294. [Crossref], [Google Scholar], [Publisher]

[7] H. Suroto, D.M. Aryawan, C.A. Prakoeswa, The Influence of the Preservation Method and Gamma Irradiation Sterilization on TGF-β and bFGF Levels in Freeze-Dried Amnion Membrane (FD-AM) and Amnion Sponge, *International journal of biomaterials,* **2021**, *2021*, 1–9. [Crossref], [Google Scholar], [Publisher]

[8] J.E. Ireton, J.G. Unger, R.J. Rohrich, The Role of Wound Healing and Its Everyday Application in Plastic Surgery, *Plastic and reconstructive surgery Global open,* **2013**, *1*, 1–10. [Crossref], [Google Scholar], [Publisher]

[9] E. Rezvani Ghomi, S. Khalili, S. Nouri Khorasani, R. Esmaeely Neisiany, S. Ramakrishna. Wound dressings: Current advances and future directions, *J Appl Polym Sci*, **2019**, *136*, 47738. [Crossref], [Google Scholar], [Publisher]

[10] T.Ž. Ramuta, M.E. Kreft, Human Amniotic Membrane and Amniotic Membrane–Derived Cells, *Cell transplantation,* **2018**, *27*, 77–92. [Crossref], [Google Scholar], [Publisher]

[11] Y. Yang, Y. Zhang, Y. Yan, Q. Ji, Y. Dai, S. Jin, Y. Liu, J. Chen, L. Teng, A Sponge-Like Double-Layer Wound Dressing with Chitosan and Decellularized Bovine Amniotic Membrane for Promoting Diabetic Wound Healing, *Polymers*, **2020**, *12*, 535. [Crossref], [Google Scholar], [Publisher]

[12] H.C. Wells, K.H. Sizeland, N. Kirby, R.G. Haverkamp. Structure and strength of bovine and equine amniotic membrane, *Biology*, **2022**, *11*,1096. [Crossref], [Google Scholar], [Publisher]

[13] Z. Jahanafrooz, B. Bakhshandeh, S. Behnam Abdollahi, E. Seyedjafari, Human amniotic membrane as a multifunctional biomaterial: recent advances and applications, *Journal of Biomaterials Applications*, **2023**, *37*, 1341–1354. [Crossref], [Google Scholar], [Publisher]

[14] D. Oktavia, H. Suroto, T.H. Wardhana, G. Khang, Characterization of Toxicity, Porosity, and Moisture Content of Bovine Freeze-dried Amnion and Bovine Amnion Sponge, *Biomolecular and Health Science Journal,* **2023**, *6*, 54-58. [Crossref], [Google Scholar], [Publisher]

[15] I.G.B.A. Hariastawa, J.A. Sutantio. Future of bovine amniotic membrane: bovine membrane application on wound healing, surgery and prospect of use for urethral reconstruction in Bovine Science: Challenges and Advances, *IntechOpen,* **2021**. [Google Scholar], [Publisher]

How to cite this article: Kukuh Ali Akbar, Teddy Heri Wardhana, Heri Suroto*, Gilson Khang, Study of the physical, mechanical and water absorption of bovine amnion membrane and bovine amnion sponge. *Journal of Medicinal and Pharmaceutical Chemistry Research*, 2024, 6(7), 865-873. **Link:** https://jmpcr.samipubco.com/article_189956.html