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# Preparation of Extra Cellular Matrix Scaffold from Bovine Amniotic Membrane

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**ABSTRACT**
Decellularization is a critical process in tissue engineering, aimed at removing cellular components from tissues while preserving the extracellular matrix (ECM). The bovine amniotic membrane (BAM) has gained interest due to its rich ECM composition and potential applications in regenerative medicine. This study, based on the work of Ballesteros *et al*. (2020) with a modification, explores the decellularization of BAM using chemical and physical method to remove cellular content while preserving the ECM structure and composition. The success of decellularization was evaluated through histological staining. Results demonstrated efficient decellularization with minimal impact on the ECM structure, making BAM a promising scaffold for tissue engineering.

**Keywords:** Bovine amniotic membrane, Decellularization

## 1. INTRODUCTION

Tissue engineering has increasingly focused on the use of biological scaffolds to support cell growth, tissue regeneration and repair. Decellularization is a critical step in creating these scaffolds by removing cellular material while retaining the structural and biochemical properties of the ECM (Gilbert *et al*., 2006). Decellularized membranes have garnered significant attention from the scientific community, as tissue engineering enables the development of biological scaffolds designed to deliver cells and proteins to damaged tissues. All decellularization methods inevitably disrupt the structure and composition of the ECM. The aim of tissue decellularization is to thoroughly eliminate cells and cellular debris while preserving the three-dimensional ultrastructure and composition of the native ECM as much as possible. Complete removal of all cellular remnants is unattainable and decellularization processes inevitably cause some degree of disruption to the matrix architecture, orientation and surface ligand landscape. (Keane *et*

*al*., 2017)

The amniotic membrane, particularly from bovine sources, has garnered attention due to its abundant ECM components such as collagen and elastin, along with its low immunogenicity and mechanical strength (Fairbairn *et al*., 2014). These properties making it an excellent candidate for applications in wound healing, corneal repair and other regenerative treatments. However, the removal of cellular debris is necessary to reduce immune responses and potential rejection in clinical applications. This study aimed to assess the effectiveness of both chemical and mechanical method of decellularization in removing cellular content from BAM while preserving the ECM's structural architecture.

This study follows the work of Ballesteros *et al*. (2020) with a modification, who proposed a decellularization protocol for BAM using both chemical and physical method. Their findings suggest that decellularized BAM may be applicable in wound healing, tissue regeneration, and other biomedical uses where the preservation of ECM integrity is crucial.

## 2.MATERIALS AND METHODS

### 2.1Harvesting of the BAM

BAM were aseptically obtained from cows that had undergone caesarean sections. The collected amnio-chorion was carefully cleaned of blood clots by washing with normal saline solution. The amnion was then separated from the chorion (fig 1).

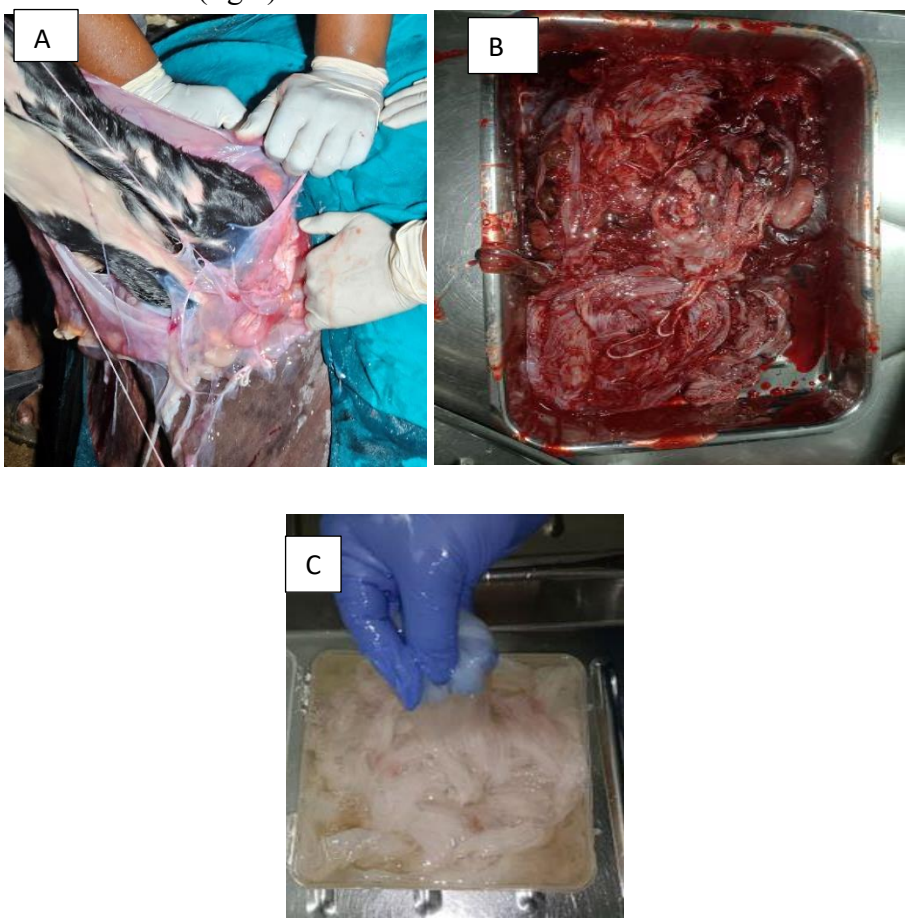


Figure 1: Harvesting of BAM; A) Collection of amnio-chorion during caesarean section
B) Blood clot removal & washing of amnio-chorion
C) Separation and washing of amnion from chorion

### 2.2 Decellularization of the BAM

The harvested amnion was washed in phosphate-buffered saline (PBS) containing a mixture of penicillin (50 µg/mL), streptomycin (50 µg/mL) and amphotericin B (2.5 µg/mL). This was followed by another wash in cold PBS. The BAMs were subsequently subjected to a freezing cycle in liquid nitrogen (-196°C) for 22 hours, followed by thawing in a water bath at 37°C for two hours. After thawing, the membranes were immersed in Tween 80 for four hours. Following this, the BAMs were thoroughly rinsed with distilled water to remove any residual reagents and this washing process was repeated after each decellularization step to ensure complete removal of reagent residues. The membranes were then immersed in 0.1 M NaOH for one hour, followed by treatment with 0.1 M ascorbic acid for 12 hours. Afterward, the BAMs were immersed in 70% ethanol for one hour to eliminate residual nucleic acids and phospholipids and finally washed with PBS for two hours. We excluded the peracetic acid treatment from Ballesteros's decellularization protocol. Throughout the entire process, the membranes were mechanically stirred using an orbital shaker to ensure even washing. Histological studies were conducted on the final samples to confirm the absence of cellular material.

## 3. RESULT AND DISCUSSION

The histological examination of the decellularized BAM sample with H and E staining confirmed that the decellularization protocol adopted in this study with a modification of the method outlined by Ballesteros *et al.* (2020) significantly removed the cellularity of BAM matrix without any major loss in tissue architecture (fig 2). The primary goal of decellularizing xenogeneic matrices is to efficiently remove cells and nucleic acid residues while preserving the structure and composition of the ECM (Park *et al*., 2018). Complete removal of all cellular remnants is unattainable and decellularization processes inevitably cause some degree of disruption to the matrix architecture, orientation and surface ligand landscape. Considering these factors, it could be stated that chemical and physical protocol adopted in this study is successful in generating decellularized BAM derived ECM scaffold.

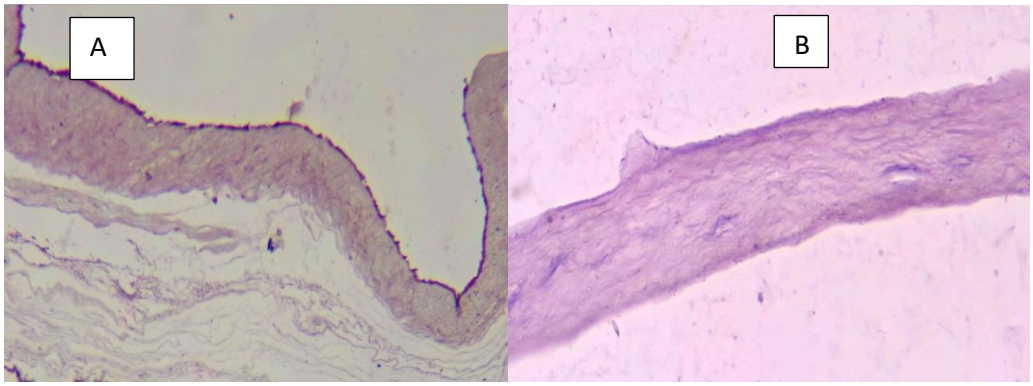


Fig 2; Histology of decellularization (H&E 400 X). A) BAM before decellularization – cellularity & epithelial layer are intact. B) BAM after decellularization-significant reduction in cellularity without major change in architecture

## 4.CONCLUSION

In an *in-vivo* environment the decellularized biological scaffolds gradually degrade, allowing space for regenerated tissue to form. However, their impact on stem/progenitor cell recruitment and the innate immune response is heavily influenced by the manufacturing processes employed with decellularization being one of the most crucial factors. The most effective decellularization techniques differ depending on

the tissue or organ, taking into account tissue-specific factors like cell density, matrix density and geometric considerations such as tissue thickness and shape. This study could be concluded that the protocol of decellularization adopted here with modification of the method developed by Ballesteros *et al,* (2020) is equally effective in removing the cellular and nuclear component from BAM while preserving the three-dimensional ultrastructure and composition of the native ECM as much as possible and this BAM derived ECM scaffold is a promising candidate in the regenerative medicine for the replacement and reconstruction of damaged or missing tissues and organs.

## 5. Conflict of Interest

Nill

## 6. Acknowledgement

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