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Development And Characterization Of Acellular Extracellular Matrix Scaffolds From Caprine Omasum Using SDS And Triton X-100 Decellularization Protocol

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Abstract

In the present study, a decellularization protocol for caprine omasum was successfully standardized using a combination of ionic and non-ionic detergents. The tissue was treated with 1% sodium dodecyl sulphate (SDS) under continuous agitation for up to 120 hours, followed by 1% Triton X-100 treatment for 24 hours. Samples were collected at predetermined intervals for gross evaluation, histological examination, DNA quantification, scanning electron microscopy, and hemocompatibility assessment. Progressive removal of cellular components was observed during the treatment process, and complete acellularity was achieved at 120 hours. Histological evaluation using H&E and DAPI staining confirmed effective elimination of nuclear material while maintaining the native collagen framework of the extracellular matrix. DNA quantification further demonstrated a substantial reduction in nucleic acid content, supporting successful decellularization. Scanning electron microscopic (SEM) examination revealed increased scaffold porosity and separation of collagen fibres, features that may facilitate cell attachment and infiltration. Additionally, both in vitro and in vivo evaluations indicated that the prepared scaffold possessed good hemocompatibility and biocompatibility, suggesting its potential applicability as a biological scaffold for tissue engineering and regenerative studies.

Key words: *Caprine omasum, Sodium dodecyl sulphate, Triton X-100, Decellularization, DNA quantification, Hemocompatibility, Biocompatibility.*

Introduction

Decellularized extracellular matrix (ECM) scaffolds obtained from xenogeneic tissues have gained considerable importance in the field of regenerative medicine because they retain the natural structural and biochemical characteristics of native tissues (Goyal et al., 2021). Decellularization involves the removal of cellular components from a tissue while preserving the extracellular matrix, thereby reducing immunogenicity and improving the acceptance of acellular grafts (Gulati & Cole, 1994). Preservation of important ECM components such as collagen, elastin, and glycosaminoglycans provides a favourable environment for cell attachment, proliferation, and tissue regeneration. At the same time, elimination of cellular antigens helps minimize adverse immune reactions and supports constructive tissue remodelling (Brown & Badylak, 2014). Among the various methods available, detergent-based immersion decellularization using sodium dodecyl sulphate (SDS) and Triton X-100 is widely regarded as an effective and reliable technique for a variety of tissues because of its ability to efficiently remove cellular material while maintaining the overall architecture of the ECM (Crapo et al., 2011). SDS is a strong ionic detergent capable of solubilizing lipid membranes and denaturing nucleoprotein complexes, making it highly effective for cellular removal (Gilpin & Yang, 2017). Triton X-100, a non-ionic detergent, is milder and often used to eliminate residual cellular debris while minimizing ECM damage (Mondal et al., 2022). Although SDS has been found to be more effective than Triton X-100 in removing cellular material, including the complete elimination of cellular residues, it has also been associated with disruption of the extracellular matrix (ECM), even at low concentrations such as 0.1% (v/v) (Pang et al., 2010). Triton X-100 has been identified as an effective non-ionic detergent for decellularization of several tissues and organs, including ligaments, small intestine, liver, and heart valves, due to its ability to remove cellular components while minimizing damage to the extracellular matrix (White et al., 2017).

Forestomach matrix (FM) scaffolds offer several advantages over many other biological scaffolds and have been widely explored for applications such as wound healing, tissue repair, and regenerative therapies (Lun et al., 2010). Their natural extracellular matrix composition and structural organization provide a supportive environment for cellular growth and tissue remodelling. Among the forestomach tissues, the caprine omasum appears to be a promising source for scaffold preparation because of its naturally layered structure and high collagen content. The tissue possesses good biomechanical strength, dense laminar folds, and a relatively uniform extracellular matrix architecture, which may provide added stability and support for regenerative applications compared to other ruminant tissues **(Mondal, 2022).**

Considering these characteristics, the present study was undertaken to evaluate a combined SDS and Triton X-100 decellularization protocol for caprine omasum and to investigate the biochemical, structural, and biological properties of the resulting scaffold.

Materials and Methods

Preparation of acellular matrix from caprine omasum

Caprine omasum was procured from a local slaughterhouse and transported to the laboratory in cold 1% PBS (phosphate-buffered saline) containing antibiotic amikacin (0.1mg/mL) and proteolytic inhibitor (0.2025% EDTA). In the laboratory, the caprine omasum was thoroughly cleansed with sterile 1% PBS (PH 7.4). After thorough cleaning of the caprine omasum, de-epithelialization was done in hypertonic solution 2 M NaCl for 6 h.

The tissue sample was treated with 1% sodium dodecyl sulphate (SDS) for 120 h and finally treated with 1% Triton-X-100 for 24 h. In the control group the native tissue was incubated in deionized water for 120 h. These containers having tissue samples in different solutions were placed on an orbital shaker and continuously agitated at room temperature at 180 rpm.

The tissue samples were collected for histological observations using H&E Staining, DAPI fluorescent staining, scanning electron microscopic examination (SEM), DNA quantification, in buffered formalin (10%), glutaraldehyde (5%) and PBS at 0 h (native), 24 h, 48 h, 72 h, 96 h and 120 h time intervals.

Gross Observation

Macroscopic study of native and decellularized caprine omasum tissue was performed by observing the colour, consistency, swelling and stiffness of all samples. **(Yadav et al., 2022)**

Histological observation

The omasum tissue sample was transferred in phosphate buffered solution to 10% formalin for 24 hours and then embedded in paraffin. Sections with 15-20µm thickness were cut from blocks by microtome. The sections were fixed on a slide and the remaining paraffin was removed using xylene. The dehydration process was performed. Then at last, the sample was stained with Hematoxylin and then Eosin dyes (H&E) and further processed. The acellularity of the stained samples was observed and evaluated on the basis of the histological scores as per the method described by **Gangwar et al. (2015).**

DNA quantification

Caprine omasum tissue samples for DNA quantification were taken from all groups about 25 mg each and placed on dry filter paper to remove excess fluid. Each sample was homogenized separately with a mortar and pestle **(Goyal et al., 2021).** Total genomic DNA was isolated from native and decellularized scaffolds using the DNASure® Tissue mini kit following the manufacturer's instructions. The total amount of DNA was quantified using spectrophotometry (NanoDrop ND-1000, NanoDropTechnologie).

Scanning electron microscope

SEM examination was performed to evaluate structural/morphological changes and the efficiency of the decellularization methods **(Yadav et al., 2022).**

In vitro hemocompatibility (Red blood cell compatibility)

In vitro hemocompatibility of the omasum tissue samples was performed as per the method described by **Rallapalli et al. (2016).**

Determination of in vivo biocompatibility

The study was conducted on two clinically healthy adult New Zealand white rabbits of either sex. All the procedures were performed in accordance with the national guidelines for handling laboratory animals. Caprine omasum tissues from all 4 groups were implanted on the back side/ loin region (each implant on either side of the spinal region) of the New Zealand white rabbits.

Statistical Analysis

Data were analyzed using one-way ANOVA followed by Duncan's multiple range test at P<0.05.

Result and Discussion

The tissue samples collected at different time intervals during the standardization of decellularization protocols were subjected to gross and microscopic observations.

Gross Observations

There was grayish pink in color with distinct mucosal folds and a spongy soft texture of the native (N) and control (C) caprine omasum tissues. A gradual loss of the native yellowish coloration of the omasum was observed. Surface and internal examinations confirmed effective removal of cellular components with preservation of the extracellular framework.

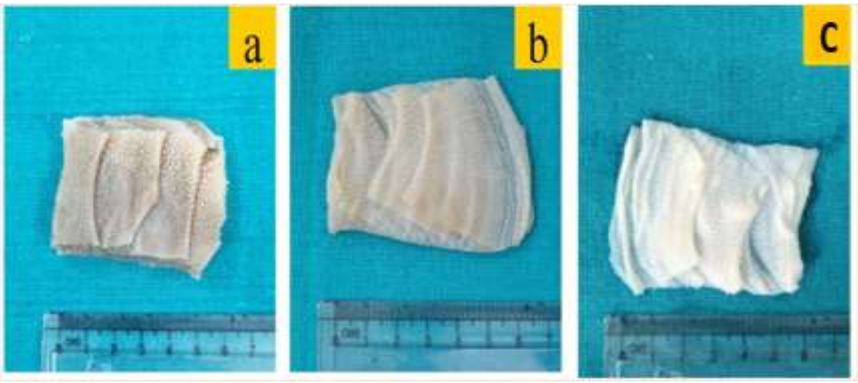

Figure 1 (a,b): Native and Control caprine omasam scaffolds, (c) Caprine omasum scaffold decellularized with 1%SDS + 1%TX100

Histological Observations

Histological examination using hematoxylin and eosin (H&E) staining revealed the gradual removal of cellular components from the caprine omasum during the decellularization process. Native and control tissues showed normal histological architecture with densely packed nuclei and compactly arranged collagen fibres, indicating intact cellularity and preserved tissue structure. Similar findings in native extracellular matrices have been reported by Gilbert et al. (2006) and Crapo et al. (2011), where the presence of abundant nuclei and organized collagen bundles represented normal tissue morphology As the duration of detergent treatment increased, a progressive decline in cellularity was observed in the decellularized scaffolds. In scaffolds, nuclear material gradually decreased during SDS treatment and complete removal was achieved by 120 hours. This finding suggests effective penetration and action of SDS within the dense lamellar arrangement of the omasum. Similar observations have been reported in earlier studies where SDS efficiently disrupted cellular membranes and facilitated removal of intracellular contents from biological tissues (Singh et al., 2023). Comparable preservation of collagen structure following detergent-based decellularization has also been described in porcine and bovine extracellular matrix scaffolds (Brown & Badylak, 2014). The present findings indicate that the optimized concentration of SDS and Triton X-100 was sufficient to remove cellular material while maintaining the structural integrity of the extracellular matrix. DAPI staining further supported the histological observations. Native and control tissues exhibited intense blue fluorescence due to the presence of abundant nuclear DNA, whereas fluorescence gradually decreased in scaffolds treated with SDS and Triton X-100. Complete loss of fluorescence in later stages confirmed successful removal of nuclear material from the scaffold. Similar reductions in DAPI fluorescence have been widely accepted as indicators of efficient decellularization in previous scaffold studies (Gilbert et al., 2009; Keane et al., 2012). Effective removal of cellular remnants is essential because residual DNA and cell debris may trigger inflammatory or immunogenic reactions after implantation (Ahmad et al., 2025). Overall, the histological and fluorescence findings suggest that the developed protocol successfully produced a biocompatible acellular scaffold while preserving the native extracellular matrix architecture, thereby making it suitable for future tissue engineering and regenerative medicine applications.

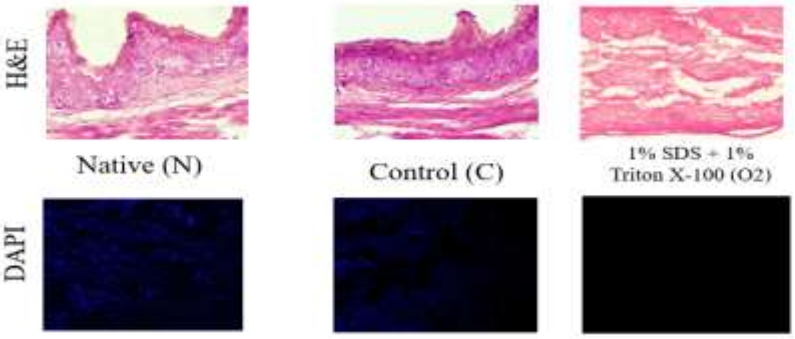

Figure 2: Native and Control caprine omasum after delamination showing cellularity, loose muscular layer, and collagen fibers, no cellular debris between the spaces of thick collagen fibers in omasum scaffold decellularized with 1%SDS + 1%TX100

DNA quantification

Native and control tissues exhibited high DNA concentrations of 91.51 ± 0.504 ng/µl and 90.10 ± 0.728 ng/µl, respectively, with no significant difference between them, indicating the presence of intact cellular and nuclear material within the tissue matrix. Similar levels of DNA content have been reported in untreated native tissues where cellular components remain preserved (Yadav et al., 2022). In contrast, scaffolds treated with 1% SDS followed by Triton X-100 showed a significant reduction in DNA content, with values decreasing to 23.68 ± 0.713 ng/µl ($P<0.01$). The marked decline in nucleic acid concentration indicates effective removal of cellular remnants and nuclear debris from the omasal tissue. The residual DNA concentration observed in the present study (23.68 ± 0.713 ng/µl) was markedly lower than that of native tissue and was comparable to the substantial DNA reductions reported in decellularized caprine aortic, urinary bladder, and esophageal scaffolds prepared using detergent or Sapindus mukorossi based protocols (Goyal et al., 2021; Singh et al., 2023; Yadav et al., 2022). These findings indicate effective removal of cellular and nuclear components while preserving extracellular matrix architecture. These findings correlate well with the H&E and DAPI staining results, which demonstrated progressive loss of nuclei following detergent treatment. Previous studies have similarly reported that SDS-based decellularization protocols are highly effective in reducing residual DNA content in extracellular matrix scaffolds (Keane et al., 2015).

Residual DNA content observed in the present study was below the widely accepted benchmark of 50 ng DNA/ mg dry tissue weight, which is considered a critical criterion for successful and safe decellularization (Goyal et al., 2021). Similar synergistic effects of ionic and non-ionic detergents have been documented in previous decellularization studies involving gastrointestinal and soft tissue scaffolds (Faulk et al., 2014). The DNA quantification results confirmed successful decellularization of the caprine omasum and further validated the histological findings.

Table 1: Mean and SD values of DNA content (ng/µl) after decellularization

S.N.	Tissue samples/Protocol	DNA (ng/µl)
1	Native	91.51 ± 0.504^{a}
2	Control	90.10 ± 0.728^{a}
4	1%SDS + 1%TX100	23.68 ± 0.713^{c}

Scanning electron microscopy

Scanning electron microscopic examination revealed distinct structural differences between native tissues and decellularized scaffolds. Native tissues exhibited densely packed collagen fibers with minimal pore formation and extensive cellular coverage throughout the surface. The compact arrangement of fibers and limited porosity observed in the native tissue are characteristic features of intact extracellular matrices where cellular components occupy the inter-fibrillar spaces. Similar ultrastructural features have been described in untreated biological tissues by Yadav et al. (2022) and Crapo et al. (2011). In contrast, scaffolds treated with 1% SDS followed by 1% Triton X-100 showed marked ultrastructural alterations characterized by extensive porosity, increased separation of collagen fibres, and improved pore interconnectivity. Similar relationships between enhanced porosity and improved regenerative potential have been documented in previous tissue engineering studies by Singh et al. (2023). These findings indicate effective removal of cellular and membranous components from the tissue matrix. The development of interconnected pores following decellularization is considered advantageous because it may facilitate nutrient diffusion, vascular ingrowth, and migration of host cells into the scaffold. Similar increases in scaffold porosity after detergent-based decellularization have been reported in several extracellular matrix studies involving gastrointestinal and soft tissues (Faulk et al., 2014; Keane et al., 2015).

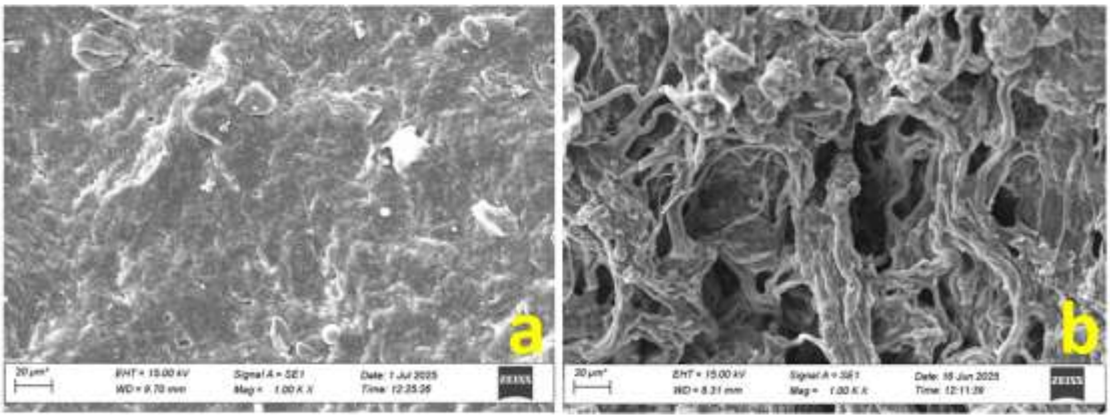

Figure 3: (a) The ultrastructure of native omasum samples showed very little porosity and a compact collagen arrangement. (b) sample treated with SDS & Triton combination, the collagen fibres had large spaces present between them and structure appeared more porous.

In vitro hemocompatibility

Native and control tissues produced severe hemolysis values of 69.36 ± 0.600% and 69.50 ± 0.957%, respectively, indicating poor blood compatibility due to the presence of intact cellular and antigenic components. High hemolytic activity in native tissues has been associated with membrane instability and increased immunogenicity when exposed to blood components (Rallapalli et al., 2016).

In contrast, scaffolds treated with 1% SDS followed by 1% Triton X-100 showed a significantly lower hemolysis value of 1.92 ± 0.159%, which was well below the accepted 5% threshold for safe biomaterials. The marked reduction in hemolysis suggests effective removal of cellular constituents and antigenic residues during decellularization, thereby improving blood compatibility of the scaffold. Similar findings have been reported in previous extracellular matrix studies where successful decellularization minimized erythrocyte damage and reduced adverse blood reactions (Crapo et al., 2011). Surface properties such as fibre organization, porosity, and interface roughness are known to influence erythrocyte interaction and hemolytic response. A biocompatible scaffold surface helps reduce thrombosis, inflammation, and immune-mediated rejection following implantation (Brown & Badylak, 2014).

S.NO.	Tissue samples/Protocol	Haemolysis (%)
1.	Native (N)	69.36 ± 0.600^a
2.	Control (C)	69.50 ± 0.957^a
4.	1%SDS + 1%TX100	1.92 ± 0.159^b

Table 2: Mean±SE of degree of haemolysis (%) produced by native (N), control (C) and decellularized caprine omasum scaffolds

Determination of in vivo biocompatibility

Subcutaneous implantation studies in New Zealand White rabbits provided important insights into the in vivo biocompatibility and regenerative potential of the prepared omasal scaffolds. On day 20 post-implantation, native and control tissues showed severe acute inflammatory reactions characterized by necrosis, cellular debris, and dense infiltration of inflammatory cells. Persistence of cellular material was further confirmed by positive DAPI staining, while gross examination revealed noticeable graft shrinkage. These findings indicate poor tissue compatibility and may be attributed to the presence of residual cellular antigens capable of triggering host immune responses. Similar inflammatory reactions in untreated xenogeneic tissues have been reported previously in decellularization studies (Badylak et al., 2009; Gilbert et al., 2006). In contrast, scaffolds treated with 1% SDS followed by 1% Triton X-100 exhibited minimal inflammatory response along with early host cell infiltration at the scaffold periphery by day 20. Reduced inflammatory reaction suggests efficient removal of immunogenic cellular components during decellularization. Early migration of host cells into the scaffold is considered a favourable indicator of biocompatibility and tissue remodelling potential (Brown & Badylak, 2014). By day 40, native and control tissues exhibited extensive necrosis and poor integration with surrounding tissues, confirming their unsuitability for implantation. However, the decellularized scaffolds demonstrated excellent integration with host tissue along with prominent vascularization and extensive cellular repopulation throughout the matrix. Similar findings have been reported in extracellular matrix scaffolds where preserved collagen architecture promoted constructive tissue remodelling and vascular ingrowth **(Faulk et al., 2014; Keane et al., 2015).** DAPI staining further confirmed the presence of host-derived nuclei within the scaffold, indicating successful recellularization and active tissue remodelling.

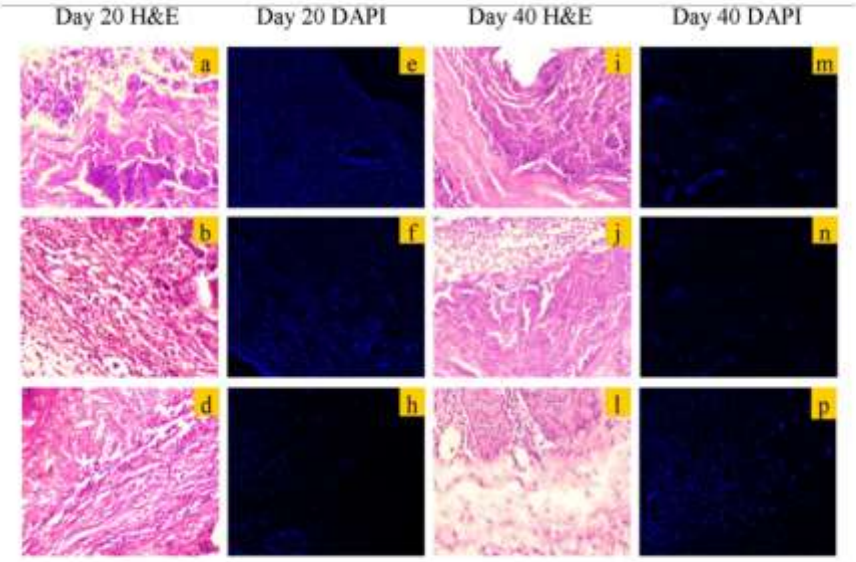

Figure 4: Micrograph of biopsy samples retrieved on 20th day and 40th day H&E and DAPI stained native (N), control (C) and decellularized caprine omasum scaffold.

Conclusion

Treatment of caprine omasum with a combination of 1% SDS and 1% Triton X-100 resulted in complete decellularization within 120 h while preserving extracellular matrix architecture. Decellularized scaffolds were hemocompatible in vitro, and subcutaneous implantation in New Zealand White rabbits confirmed their biocompatibility.

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