Comparative Analysis of Decellularization Methods on Bovine Pericardium Scaffolds for Cardiac Tissue Engineering

Komang Adhitya Arya Adiputra [1*], Heroe Soebroto [2], Ito Puruhito[2]

Abstract

Background: Congenital heart disease (CHD) involves structural heart abnormalities present since fetal development. Corrective surgery is often the ideal treatment, utilizing implants or scaffolds for tissue repair. However, repeated surgeries may be required due to declining function of grafts, leading to increased healthcare costs. Bovine pericardium is a promising scaffold material for tissue engineering due to its composition of collagen, glycosaminoglycans (GAGs), and proteoglycans. This study aimed to compare the effectiveness of bovine pericardium scaffolds using different decellularization methods: sodium dodecyl sulfate (SDS) and hydrogen peroxide (H_2O_2). Methods: An experimental study was conducted with bovine pericardium scaffolds divided into control and treatment groups. The treatment groups used decellularization with SDS (0.5%, 1%) and H_2O_2 (3%). Scaffolds were evaluated for tensile strength, strain, Young's modulus, and histological properties, including nuclear density, collagen density, and GAG content. Data were analyzed using statistical methods to compare the effectiveness of each decellularization technique. Results: Histological analysis revealed that all decellularized samples showed no nuclear density, while control samples displayed light nuclear density. Collagen density was light in scaffolds treated with SDS 0.5% and H_2O_2, while SDS 1% resulted in no collagen presence. GAG density was absent in SDS-treated samples and minimal in H_2O_2-treated samples. Tensile strength was highest in H_2O_2-treated scaffolds (24.01 N) and lowest in SDS 0.5%. SDS 1% showed the greatest tensile strain, while H_2O_2-treated scaffolds had the highest stiffness. Conclusion: The decellularization methods effectively removed cellular components from the bovine pericardium scaffolds, with varying impacts on the extracellular matrix. SDS 1% provided the most elastic scaffold, while H_2O_2 preserved tensile strength and stiffness. These findings suggest that the choice of decellularization method can be optimized based on the desired mechanical properties of the scaffold for cardiac tissue engineering applications. Further research is needed to evaluate the long-term performance of these scaffolds in clinical settings.

Keywords: Bovine pericardium, Decellularization, Sodium dodecyl sulfate (SDS), Hydrogen peroxide (H_2O_2), Tissue engineering

Significance | This study determined the effectiveness of different decellularization methods to optimize bovine pericardium scaffolds for cardiac tissue engineering.

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Editor Aman Shah Abdul Majid, Ph.D., And accepted by the Editorial Board Aug 03, 2024 (received for review May 15, 2024)

Introduction

Congenital heart disease (CHD) is a group of structural anomalies in the heart or major intrathoracic blood vessels that develop during fetal life. Defined by Mitchell et al., CHD encompasses a wide spectrum of abnormalities in the heart's macroscopic structure or

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Please cite this article.
Komang Adhitya Arya Adiputra, Heroe Soebroto et al. (2024). Comparative Analysis of Decellularization Methods on Bovine Pericardium Scaffolds for Cardiac Tissue Engineering, Journal of Angiotherapy, 8(8), 1-7, 9833

its surrounding large blood vessels (Ozeren et al., 2002; Thudt et al., 2017). CHD is a major contributor to morbidity and mortality worldwide, and its management poses significant challenges. The ideal treatment involves a one-step corrective surgery where cardiac defects are repaired by stitching or patching, damaged valves are reconstructed or replaced, arteries are widened, and blood vessel flow is normalized (Sundareswaran et al., 2012; Yin et al., 2020). However, in complex cases, multiple open-heart surgeries are often required to correct all structural defects (Rajabi et al., 2020; Trivedi et al., 2019). These repeated interventions, along with the lifelong comorbidities associated with CHD, present a substantial burden on public health systems and healthcare costs (Avolio et al., 2015; Manji et al., 2006; Razzouk et al., 2003).

During CHD surgeries, various materials such as implants or scaffolds are commonly used to close heart defects or replace damaged tissues (Cohen et al., 2016; Kheradvar et al., 2015). These scaffolds, which can be derived from natural or synthetic sources, often face challenges such as reduced functionality over time, necessitating further reoperations (Simon et al., 2018; Saeed et al., 2014). The search for ideal scaffold materials that offer durable tissue repair with minimal reoperation risk remains a critical issue for cardiac surgeons (Roh et al., 2010; Badylak et al., 2015). One promising approach involves the use of biological materials, particularly decellularized tissues such as bovine pericardium (Zafar et al., 2020; Thomson et al., 2018). The bovine pericardium, a multi-layered composite membrane rich in elastic fibers and an extracellular matrix primarily composed of type I collagen, glycosaminoglycans (GAGs), and proteoglycans, shows significant potential in tissue engineering applications (Avolio et al., 2015; Bhatnagar et al., 1998; Vesely, 2005).

Decellularization is a process that removes cellular components from tissues, leaving behind an extracellular matrix (ECM) that serves as a scaffold for tissue regeneration (Urciuolo et al., 2018; Mazza et al., 2017). Bovine pericardium decellularization using agents such as sodium dodecyl sulfate (SDS) and hydrogen peroxide (H_2O_2) has been proposed as a method to create effective scaffolds for cardiac repair (Wu et al., 2019; Pati et al., 2017). SDS, an anionic detergent, solubilizes cell membranes and removes cellular proteins, while H_2O_2, an oxidative agent, further aids in the destruction of cellular material (Gilbert et al., 2012; Chan et al., 2018). Both agents aim to retain the essential components of the ECM, such as collagen and elastin, which are crucial for tissue integrity and function (Li et al., 2021; Ahmed et al., 2020). The effectiveness of these decellularization methods varies depending on the agent concentration and exposure time, which can affect the mechanical properties and biocompatibility of the resulting scaffolds (Pagoulatou et al., 2012; Wollmann et al., 2019; Sierad et al., 2012).

This study aimed to evaluate the efficacy of bovine pericardium scaffolds decellularized using SDS and H_2O_2 by comparing them to untreated controls. The focus is on assessing key parameters such as tensile strength, tensile strain, and Young's modulus to determine the suitability of these scaffolds for cardiac tissue engineering (Pashneh-Tala et al., 2016; Schneider et al., 2018). By exploring the potential of decellularized bovine pericardium as a scaffold material, this study seeks to contribute to the development of improved surgical options for patients with CHD, potentially reducing the need for repeated surgeries and enhancing long-term clinical outcomes (Jenkins et al., 2020; Ma et al., 2019; Sacks et al., 2016).

2. Materials and Methods

2.1 Study Design

The experimental design employed treatment and control groups to assess the efficacy of bovine pericardium scaffolds decellularized using SDS and H2O2 compared to a control group. The control group consisted of bovine pericardium scaffolds untreated and immersed only in 0.9% NaCl solution. The treatment groups included decellularization methods with SDS 0.5%, SDS 1%, and H2O2 3%. Parameters such as tensile strength, tensile strain, and Young's modulus were evaluated.

2.2 Data Collection

The research was conducted at the RSUD Dr. Soetomo tissue bank laboratory, the physics Laboratory of UNAIR Campus C, and the Anatomical Pathology Laboratory of RSUD Dr. Soetomo. Sample selection in this study was performed using simple random sampling to determine which bovine pericardium samples were assigned to the control and treatment groups. The inclusion criteria used were pericardium from slaughtered cattle that had been certified healthy by a veterinarian at the slaughterhouse. The exclusion criteria were pericardium that was torn or damaged during the preparation phase. Bovine pericardium that met the inclusion criteria and did not meet the exclusion criteria were selected as research samples using simple random sampling. The bovine pericardium samples were then randomly assigned to the control group or treatment groups.

There were four groups in this study: the control group, the treatment with decellularization using SDS 0.5%, the treatment with decellularization using SDS 1%, and the treatment with decellularization using H2O2 3%. Fluid replacement was carried out according to the research protocol. After the decellularization method was applied, the samples were incubated for 2 weeks. After 2 weeks, the effectiveness of the bovine pericardium scaffolds in each group was assessed.

2.3 Statistical Analysis

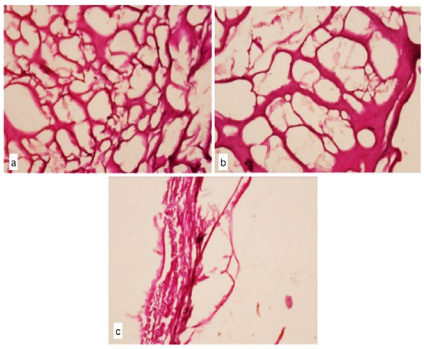

Figure 1. Histological images of bovine pericardium scaffolds stained with H&E and examined under 400x magnification show the following: (a) decellularization with SDS 0.5%, (b) decellularization with SDS 1%, and (c) decellularization with H2O2 3%. In all treatments, no residual nuclei were observed.

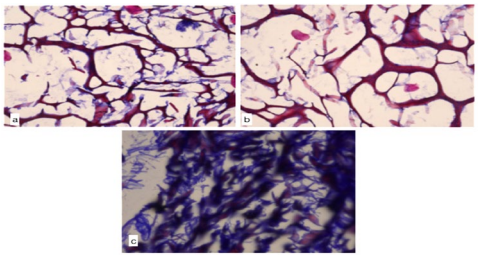

Figure 2. Histological images of bovine pericardium scaffolds stained with Masson Trichrome show the following: (a) decellularization with SDS 0.5% shows collagen fibers with light density, (b) decellularization with SDS 1% shows no visible collagen fibers, and (c) decellularization with H2O2 3% shows collagen fibers with light density.

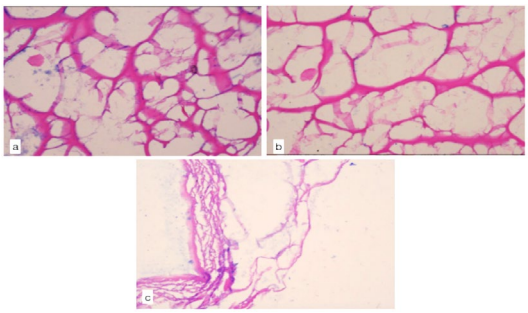

Figure 3. Histological images of bovine pericardium scaffolds stained with Alcian Blue show the following: (a) decellularization with SDS 0.5% shows no visible GAG fibers, (b) decellularization with SDS 1% shows no visible GAG fibers, and (c) decellularization with H2O2 3% shows GAG fibers with light density.

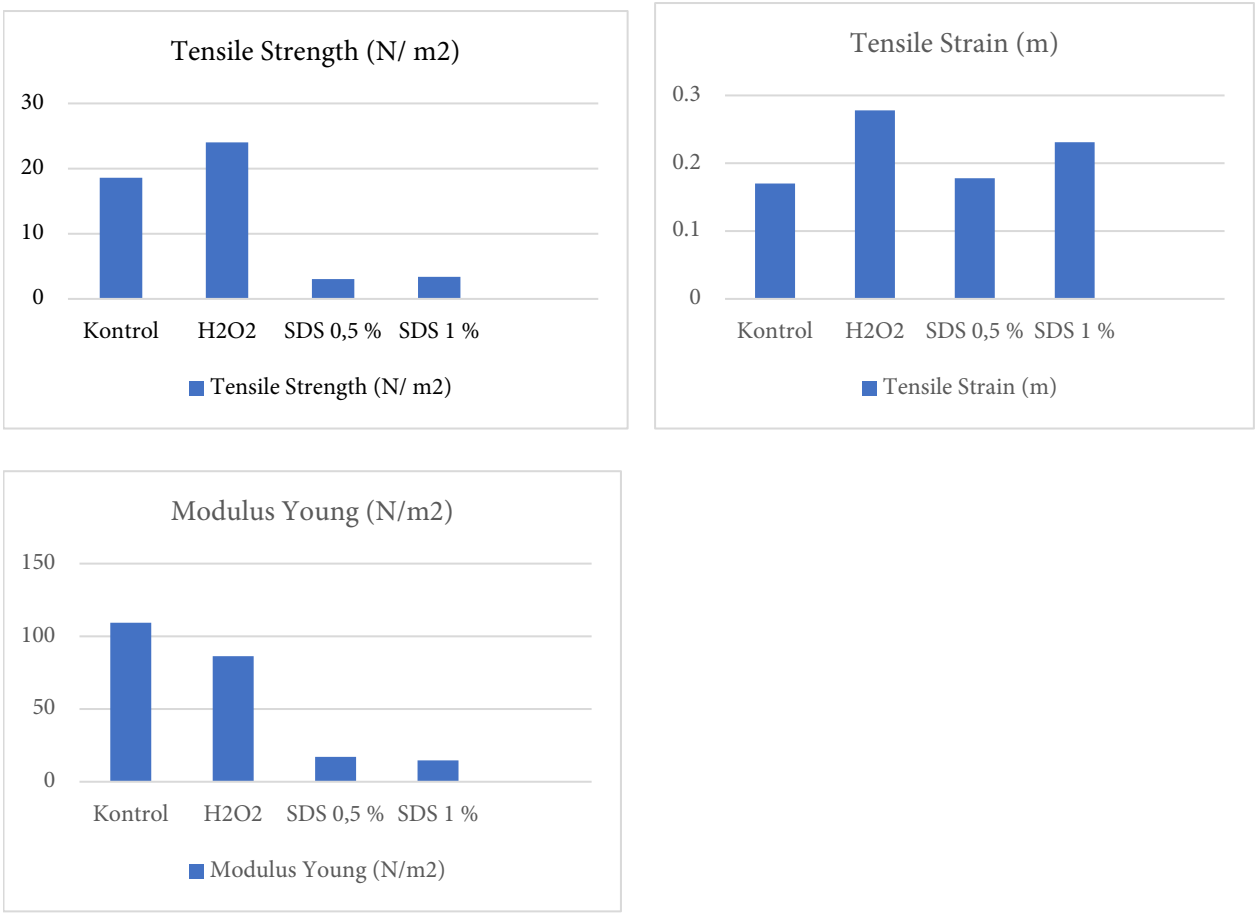

Figure 4. Tensile Strength, Tensile Strain and Modulus Young Graph

The data in this study consisted of numerical data and images. Numerical data were tabulated using spreadsheet software. Ordinal data were presented in the form of frequencies, while numerical data were presented as means and standard deviations when available. The image data from the microscope preparations were graded and described by a specialist in anatomical pathology.

3. Results

3.1 Nuclear Histology

Eosin staining was performed to assess nuclear density in each of the 4 treatment samples. In the samples treated with SDS 0.5%, all 4 samples (100%) showed no nuclear density. In the samples treated with SDS 1%, all 4 samples (100%) also showed no nuclear density. In the samples treated with H2O2 3%, all 4 samples (100%) showed no nuclear density as well. In the control samples, all 4 samples (100%) exhibited light nuclear density (Figure 1).

3.2 Collagen Histology

Masson Trichrome staining was performed to assess collagen density in each of the 4 treatment samples. In the samples treated with SDS 0.5%, all 4 samples (100%) showed light collagen density. In the samples treated with SDS 1%, all 4 samples (100%) showed no collagen. In the samples treated with H2O2 3%, all 4 samples (100%) exhibited light collagen density. In the control samples, all 4 samples (100%) displayed high collagen density (Figure 2).

3.3 GAG Histology

Alcian Blue staining was performed to assess GAG (glycosaminoglycan) density in each of the 4 treatment samples. In the samples treated with SDS 0.5%, all 4 samples (100%) showed no GAG density. In the samples treated with SDS 1%, all 4 samples (100%) also showed no GAG density. In the samples treated with H2O2 3%, 3 out of 4 samples (75%) exhibited light GAG density, while 1 sample (25%) showed no GAG. In the control samples, all 4 samples (100%) showed light GAG density (Figure 3).

3.4 Tensile Strength, Tensile Strain and Modulus Young

Tensile strength measures the amount of force per unit area that a material can withstand before tearing. According to the data from this study, different decellularization methods showed varying tensile strengths. Decellularization with H2O2 yielded the highest tensile strength at 24.01 N, while decellularization with SDS 0.5% resulted in the lowest tensile strength (Figure 4).

Tensile strain measures the change in length of a material relative to its original length that can be achieved under force before tearing. In this study, all samples exhibited an increase in tensile strain after decellularization. The longest tensile strain was achieved by H2O2 (0.278 m), while the shortest tensile strain was observed in the bovine pericardium decellularized with SDS 0.5%.

Elastic modulus measures the stress per strain that a material can achieve. The smaller the value of the elastic modulus, the more elastic the material. In this study, the elastic modulus of the decellularized samples decreased compared to the native samples. Bovine pericardium decellularized with SDS 1% had the lowest elastic modulus compared to other materials. This indicates that bovine pericardium decellularized with SDS 1% is more elastic than those decellularized using other methods. Meanwhile, bovine pericardium decellularized with H2O2 showed the highest stiffness compared to other materials. The decellularization process reduced the elastic modulus.

4. Discussion

The effects of different decellularization methods, particularly SDS (Sodium Dodecyl Sulfate) and H_2O_2 (hydrogen peroxide), highlights their effectiveness and impact on tissue characteristics. Both methods were successful in eliminating cellular components, including nuclei, from tissue samples. In this study, all samples treated with either SDS (1%) or H_2O_2 (3%) showed no nuclear density, suggesting complete removal of cellular nuclei. In contrast, control samples retained light nuclear density, indicating the preservation of cellular material.

Previous research has corroborated these findings. Studies using H&E and DAPI staining reported a significant reduction in visible nuclei in pericardial tissue following decellularization with SDS. Additionally, the extracellular matrix (ECM) of the decellularized pericardium maintained its collagen and elastin bundles, similar to native pericardium, with a notable increase in tissue thickness and a significant reduction in DNA content ($p < 0.001$) (Wollmann et al., 2019). These observations confirm the efficiency of SDS and H_2O_2 in removing cellular components while preserving some ECM integrity.

The underlying mechanisms of these decellularization methods are key to understanding their effectiveness. SDS, an anionic detergent, solubilizes cell membranes and removes cellular proteins effectively, while H_2O_2 acts as an oxidative agent, destroying cellular material through oxidation. The use of SDS at concentrations between 0.5% and 1% has shown varying levels of efficacy in removing cells, with higher concentrations yielding more efficient decellularization. However, these higher concentrations may also damage ECM structures, particularly collagen and glycosaminoglycans (GAGs). Conversely, H_2O_2, while effective in decellularization, may cause less damage to ECM structures and is often used alongside other agents to optimize cell removal while preserving ECM integrity (Pagoulatou et al., 2012).

In this study, samples treated with SDS 0.5% exhibited light collagen density, whereas samples treated with SDS 1% showed no collagen. Samples treated with H_2O_2 3% demonstrated light collagen density, suggesting that SDS, particularly at higher concentrations, is more aggressive in removing collagen from tissues. The reduction in collagen content has implications for tissue strength and

mechanical properties, which are crucial for maintaining tissue functionality post-decellularization.

Histological analysis further demonstrated a reduction in most cellular components, but also indicated a reduction in some ECM components and an increase in the thickness of decellularized tissue. Courtman et al. observed a threefold increase in tissue thickness following decellularization (Courtman et al., 1994). In contrast, Mirsadraee et al. found no significant changes in the histological structure of human pericardium after SDS-based decellularization (Mirsadraee et al., 2006). Wollmann et al. reported that the collagen concentration per mg of dry weight of human pericardial tissue before and after decellularization showed a slight increase ($p = 0.716$), suggesting minimal degradation of collagen during the process (Wollmann et al., 2019).

The effects on GAG content were also notable. Samples treated with SDS (both 0.5% and 1%) showed no GAG density, whereas samples treated with H_2O_2 3% exhibited light GAG density in 75% of cases, with one sample showing no GAG. In the control samples, light GAG density was observed in all cases. This aligns with Mirsadraee's findings, which noted a decrease in GAG content in decellularized human pericardium. Similarly, Mendoza-Novelo et al. reported a reduction in GAG content in pericardial tissue following decellularization with tridecyl alcohol ethoxylate surfactant and reversible alkaline swelling (Mendoza-Novelo et al., 2011). However, another study by Mirsadraee et al. observed a slight increase in hydroxyproline and GAG content in tissue post-decellularization, attributing the increase to a relative rise in the ratio of these molecules to total dry weight due to the loss of soluble proteins and cellular components (Mirsadraee et al., 2006).

The study also examined the mechanical properties of decellularized scaffolds, particularly tensile strength and strain. Most decellularization processes resulted in decreased tensile strength compared to native scaffolds, except those decellularized with H_2O_2. This decrease is likely due to ECM degradation, as observed in studies on bovine pericardium where SDS-based decellularization led to swelling, denaturation, and an irreversible decrease in tensile strength compared to native tissue (Mendoza-Novelo et al., 2011; Neethling et al., 2014).

Conversely, tensile strain measurements indicated an increase in tensile strain for all decellularized scaffolds compared to native scaffolds, with the scaffold treated with SDS 1% showing the highest increase. This increase in extensibility is consistent with findings from a study on porcine pericardium, where overall extensibility increased significantly after decellularization with SDS, Trypsin, and Triton X-100 (Liao et al., 2008). However, the elastic modulus of decellularized bovine pericardium scaffolds decreased compared to native scaffolds, indicating that decellularization renders the scaffold more flexible. The reduction in elastic modulus is consistent with other studies that observed a loss of stiffness in decellularized tissues, resulting in a nonlinear moment-curvature relationship compared to the linear response of native tissues (Liao et al., 2008; Neethling et al., 2014).

Overall, the findings of this study, in conjunction with existing literature, indicate that both SDS and H_2O_2 are effective decellularization agents, with distinct effects on ECM preservation and mechanical properties. SDS is more aggressive in removing cellular components, including nuclei, but may damage ECM structures, particularly collagen and GAGs, at higher concentrations. H_2O_2 appears to be less damaging, providing a balance between effective cell removal and ECM preservation. However, the choice of decellularization method should be carefully considered based on the intended application of the scaffold, as different methods can result in varying mechanical properties and ECM integrity. Further research is needed to optimize these processes to balance effective decellularization with the preservation of ECM components and mechanical strength, which are critical for the functional application of tissue-engineered scaffolds.

5. Conclusion

The study compared the effectiveness of two decellularization methods—SDS (0.5% and 1%) and H_2O_2 (3%)—on bovine pericardium scaffolds, assessing parameters like nuclear removal, collagen, and GAG density, as well as mechanical properties. Both SDS concentrations and H_2O_2 effectively removed cellular components, including nuclei, but had varying impacts on extracellular matrix integrity. SDS 1% showed the highest cellular clearance but also caused more significant collagen loss, whereas SDS 0.5% and H_2O_2 3% maintained better collagen density. In terms of mechanical properties, decellularization reduced the elastic modulus, with SDS 1% yielding the most elastic scaffolds, while H_2O_2 3% preserved tensile strength the best. The findings suggest that while SDS is effective in decellularization, its concentration needs careful optimization to minimize ECM damage, and H_2O_2 could offer a balanced approach for scaffold preparation, maintaining both cellular removal efficiency and mechanical integrity for cardiac tissue engineering applications.

Author contributions

K.A.A.A., H.S., and I.P. conceived and designed the study, collected the data, analyzed and interpreted the results, and prepared the manuscript.

Acknowledgment

The authors thank the Department of Thoracic, Cardiac, and Vascular Surgery, Faculty of Medicine, Universitas Airlangga - Dr. Soetomo General Academic Hospital for their active support.

Competing financial interests

The authors have no conflict of interest.

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