Comparative Effects of Sodium Dodecyl Sulfate and Hydrogen Peroxide on Type I and III Collagen Preservation in Decellularized Bovine Pericardium Scaffolds

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Abstract

Background: Sodium dodecyl sulfate (SDS) is an anionic detergent widely used for tissue decellularization due to its effectiveness in removing cellular components. However, SDS can also reduce extracellular matrix (ECM) density, potentially compromising scaffold integrity. Hydrogen peroxide (H_2O_2), though less commonly used, also facilitates decellularization. Type I and III collagens are essential ECM components that influence the mechanical properties of scaffolds. Objective: This study aimed to evaluate the effects of SDS and H_2O_2 on the density of type I and III collagens in decellularized bovine pericardium. Methods: Bovine pericardium samples were treated with either 0.5% SDS, 3% H_2O_2, or a control solution (0.9% NaCl) for two weeks. Collagen density was assessed using immunohistochemical staining. The Kruskal-Wallis test was employed for inter-group comparisons, followed by the Mann-Whitney U test for post hoc analysis. Results: Type I collagen density was lower in SDS-treated scaffolds, with over 50% exhibiting low intensity (0 or +1), whereas 67% of H_2O_2-treated samples showed moderate density (+2). For type III collagen, the control group had the highest proportion of samples with moderate to high density (+2 and +3) (83%), followed by the H_2O_2 group (50%) and SDS group (17%). Post hoc analysis revealed significant differences between the control and SDS groups for both type I ($p = 0.037$) and type III ($p = 0.023$) collagens. Conclusion: SDS-based decellularization significantly reduces type I and III collagen densities in bovine pericardium scaffolds, whereas H_2O_2 treatment better preserves collagen content. These findings suggest that H_2O_2 may be a viable alternative for maintaining ECM integrity in decellularized tissues.

Keywords: Sodium Dodecyl Sulfate, Hydrogen Peroxide, Bovine Pericardium Scaffold, Type I Collagen, Type III Collagen

Significance | SDS significantly reduces type I and III collagen density in decellularized bovine pericardium, whereas H_2O_2 better preserves extracellular matrix integrity.

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Introduction

Pediatric and congenital heart surgery remains prevalent globally, with 96,594 surgeries reported between 2016 and 2020 in 110 hospitals across North America, according to the Society of Thoracic Surgery (Kumar et al., 2021). Data from the World Society of Pediatric and Congenital Heart Surgery indicates an annual increase in such surgeries across 19 participating countries (Louis & Kirklin, 2018). Often, these surgeries require staged operations or

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reoperations, with about 33% of surgeries from 2007 to 2011 involving at least one prior surgery using cardiopulmonary bypass (Jacobs et al., 2014).

Closing the pericardial defect with autologous pericardium patches is common in congenital defects, yet complete primary pericardial closure is challenging. Post-surgical pericardial closure can reduce epicardial adhesion, maintain spatial separation from the retrosternal space, and stabilize hemodynamics. However, it can also cause mechanical compression on grafts, such as coronary artery bypass grafting, leading to bent grafts and potential inflammatory reactions (Boyd, Tyberg & Cox, 2012).

Materials for pericardial defect closure have evolved from autologous pericardium to xenogeneic (from animals like cattle and pigs treated with glutaraldehyde) and synthetic materials like Polytetrafluoroethylene (PTFE). While xenogeneic materials are durable, they can become calcified, shrink, and stiffen over time (Neethling et al., 2013). Synthetic materials like PTFE are durable but do not grow with the patient, presenting risks of infection and rejection (Lam & Wu, 2012).

Decellularization techniques have been developed to address these issues, which remove cellular components from grafts, leaving only the extracellular matrix (Crapo, Gilbert & Badylak, 2011). This process can be performed using physical, chemical, or enzymatic methods, and its success depends on factors such as tissue thickness, lipid content, and the specific decellularization method used (Lima, Ferrasi & Kaasi, 2019).

Common decellularisation chemical agents include acidic and basic solutions, detergents, and zwitterionic solutions. Sodium dodecyl sulfate (SDS) is frequently used due to its effectiveness at eliminating cellular nuclei, but it can also damage the ultrastructure, such as collagen and the extracellular matrix. Conversely, hydrogen peroxide is recognized for its antimicrobial properties and is beneficial in maintaining the extracellular matrix as it degrades cell membranes and DNA by inducing TGF-β1 (Zhang et al., 2017).

Collagen types I and III are essential for maintaining mammalian extracellular matrices' tensile strength and mechanical integrity (Khanna, Zamani & Huang, 2021). These proteins form a triple helix structure that determines the type of collagen formed. The effectiveness of a decellularization agent, such as SDS or hydrogen peroxide, in preserving the mechanical integrity of the tissue scaffold can be gauged by the amount of residual collagen after decellularization. This research explores the impact of these agents on collagen integrity in decellularized tissues.

Materials and Methods

This study employed a proper experimental design, specifically a controlled trial to investigate the effects of Sodium Dodecyl Sulphate (SDS) and Hydrogen Peroxide (H2O2) on the preservation of Type I and III collagen in bovine pericardium scaffolds. Three groups were established: one control group using 0.9% saline solution and two treatment groups—Group 1 treated with 0.5% SDS, Group 2 treated with 3% H2O2, and Group 3 as the control. The experiment used bovine pericardium samples randomly assigned to each group. This randomization aimed to evenly distribute potential confounding variables across groups evenly, thus reducing bias and increasing the reliability of the results. Each bovine pericardium was cut into 2x2 cm segments and subjected to their respective treatments for two weeks. The decellularization process involved repeated solution changes—initially every 24 hours for the first two days, then every 48 hours thereafter. Post-treatment, samples were incubated for four weeks to assess the efficacy of the collagen preservation in the scaffold. This phase was crucial to evaluate the structural integrity and potential for clinical application of the scaffold materials. Statistical analysis included the Kruskal-Wallis test for inter-group comparisons and the Mann-Whitney U test for post hoc analysis.

Results

This experimental study's purpose is to evaluate the effects of SDS 0,5% and H2O2 3% in preserving the type I and type III collagen in the process of preparing bovine pericardium scaffolds. The effects were studied by measuring the remaining of type I and type III collagen after the decellularization process under the immunohistochemical (IHC) staining. The color intensity found under the microscope describes the amount of remaining collagen left on the scaffold.

Type I Collagen

Under the IHC staining, type I collagen gives the brownish color. Type I collagen distribution was seen to be more intense in the control group, followed by H2O2 3% group and SDS 0,5% group (Figure 1). This result is supported by table 1. which shows about more than half of the decellularized bovine pericardium scaffold with SDS 0,5% have low intensity (0 and +1) of type I collagen remains, while on the H2O2 3% group, about two third (67%) of the sample still have moderate intensity (+2) of type I collagen left.

Statistical comparison of each group median intensity shown on the table 2. The control group (2.5) has the highest intensity, followed by H2O2 (2.0) and SDS (1.0), but the difference was not statistically significant (p=0.059).

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Head-to-head comparison of each group showed a statistically significant difference of type I collagen intensity between the control and SDS 0,5% group (2,5 vs 1,0; p=0,037) (Table 3, Table 4).

Type III Collagen

Table 1. Type I Collagen Intensity on SDS 0,5%, H2O2 3% and Control Group (replication of n=6 on every group)

Type I Collagen Intensity	**(0)**	**(+1)**	**(+2)**	**(+3)**	Total
	f	f	f	f	
Control	0	1(17%)	2(33%)	3(50%)	6
SDS 0,5%	1(17%)	3(50%)	2(33%)	0	6
H2O2 3%	0	2(33%)	4(67%)	0	6

Tabel 2. Comparison of Type I Collagen Intensity

	Control	**SDS 0,5%**	**H2O2 3%**	**P value**
Median (IQR)				
	2,5 (1)	1,0 (1)	2,0 (1)	0,059*
*Kruskall Wallis Test				

Table 3. Head-to-head type I collagen intensity median comparison of each group

Comparison	**Median (IQR)**	**P Value**
Control vs SDS 0,5%	2,5 (1)	0,037*
	1,0 (1)	
Control vs H2O2 3%	2,5 (1)	0,118*
	2,0 (1)	
SDS 0,5% vs H2O2 3%	1,0 (1)	0,212*
	2,0 (1)	

*. Mann Whitney Test

Table 4. Type III Collagen Intensity on SDS 0,5%, H2O2 3% and Control Group (replication of n=6 on every group)

Type III Collagen Intensity	**0**	**+1**	**+2**	**+3**	**Total**
	(f)	**(f)**	**(f)**	**(f)**	
Control	0	1(17%)	3(50%)	2(33%)	6
SDS 0,5%	0	5(83%)	1(17%)	0	6
H2O2 3%	0	3(50%)	2(33%)	1(17%)	6

Table 5. Comparison of Type III Collagen Intensity

	Control	**SDS 0,5%**	**H2O2 3%**	**P value**
Median (IQR)	2 (1) 1 (1) 1,5 (1)			0,07*

Table 6. Comparison of Type I collagen intensity for each group

Comparison	Median (IQR)	P Value
Control vs SDS 0,5%	2 (1)	0,023*
	1 (1)	
Control vs H2O2 3%	2 (1)	0,268*
	1,5 (1)	
SDS 0,5% vs H2O2 3%	1 (1)	0,211*
	1,5 (1)	

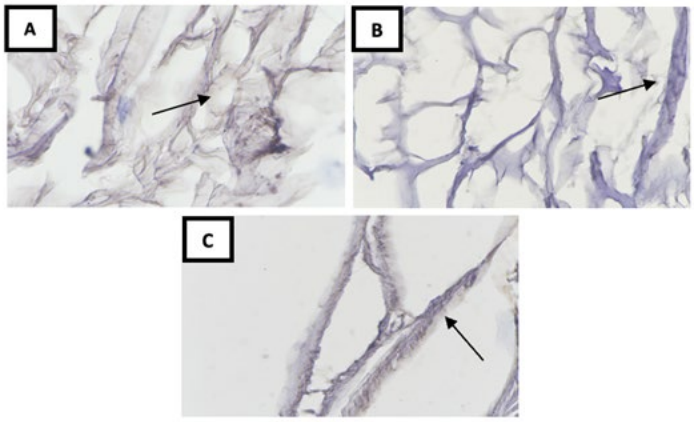

Figure 1. Bovine pericardium scaffold on control group (A), SDS 0,5% (B), and H2O2 3% (C) under the 400x magnification. Black arrow marks the type I collagen fiber on each sample.

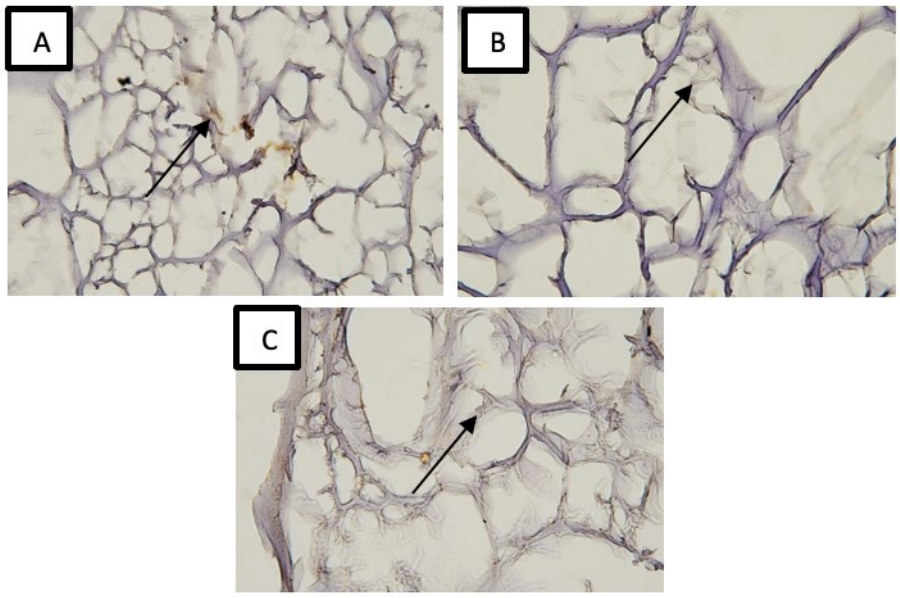

Figure 2. Bovine pericardium scaffold on control group (A), SDS 0,5% (B), and H2O2 3% (C) under the 400x magnification. Black arrow marks the type III collagen fiber on each sample.

Type III Collagen also showed a brownish color under the 400x light microscope magnification, with IHC staining methods. Type III collagen is found to be more intense in the control group, followed with H2O2 3% group and SDS 0,5% group (Figure 2)

Based on the intensity scoring, the control group have the most amount of sample with high type III collagen intensity (+2 and +3) with 5 samples (83%), followed by H2O2 3% group (n=3; 50%) and SDS 0,5% group (n=1; 17%). Most of the SDS 0,5% samples showed a low type III collagen intensity in the end of the study (n=5; 83%). Control group has the highest median type III collagen, followed by H2O2 3% and SDS 0,5 % group on the second and third place. However, statistical analysis using the Kruskal Wallis Test of each group median intensity shown on the table 5 below, showed no statistically significant difference (p>0,05). Meanwhile, a head-to-head comparison of each group showed a significant difference between control and SDS 0,5% group (p=0,023) (Table 6).

Discussion

The comparative study on the effects of Sodium Dodecyl Sulphate (SDS) and Hydrogen Peroxide (H2O2) on Type I and Type III collagen within bovine pericardium scaffolds has provided critical insights into their respective impacts on the structural integrity of extracellular matrix components. These components are fundamental in maintaining the mechanical and elastic properties of scaffolds used in tissue engineering. SDS, an ordinary detergent used in decellularization, significantly degrades the collagen structure, potentially compromising the scaffold's mechanical integrity. In contrast, H2O2, while also affecting collagen content, does so to a lesser extent, suggesting a more conservative impact on the overall matrix structure (Guruswamy-Damodaran & Vermette, 2018).

SDS operates by disrupting both hydrophobic and electrostatic bonds within the collagen structure. This dual action effectively removes cellular content and unravels collagen's triple helix structure, leading to significant losses in collagen density and structural integrity. This effect was particularly evident from the immunohistochemical (IHC) staining results, which showed a significant reduction in collagen density in samples treated with SDS. Over 50% of these samples displayed low-density collagen, indicating extensive denaturation and degradation of the scaffold's structural proteins (Zhang et al., 2021).

In comparison, H2O2-treated scaffolds demonstrated a lower reduction in collagen levels. Hydrogen peroxide, primarily functioning through oxidative mechanisms, targets cellular components and genetic material, sparing the macroscopic structure of collagen to a greater extent. This selective action results in less damage to the collagen network, preserving a significant amount of its original structure and functionality. This preservation is crucial for maintaining some degree of mechanical integrity in the scaffold, which is essential for its performance in biological environments (Nashchekina et al., 2021).

The quantitative data derived from IHC staining provided a clear visual and semi-quantitative representation of the differential impacts of these agents on collagen density. While SDS significantly reduced the presence of high-density collagen, H2O2 preserved moderate levels of collagen density across the treated scaffolds. This outcome suggests that while both agents reduce overall collagen content, the preservation of collagen structure is more pronounced with H2O2, highlighting its potential suitability for applications where structural integrity is paramount.

The study analyzed the interaction mechanisms of SDS with collagen, further elucidating its impact. At lower concentrations, SDS disrupts the collagen structure predominantly through electrostatic interactions. However, as the concentration increases, hydrophobic interactions take precedence, leading to more profound structural disruptions. This knowledge points to the possibility that optimizing SDS's concentration and application conditions could have detrimental effects on collagen integrity (Zhang et al., 2021).

Conclusion

This study highlighted the necessity of considering scaffolds' application-specific requirements when selecting a decellularization agent. Bovine pericardium scaffolds decellularized using SDS 0.5% showed the lowest density of type I and type III collagens compared to the H2O2 and control group. For instance, while SDS is highly effective for complete decellularization, its aggressive nature makes it less suitable for applications requiring the preservation of mechanical properties. Conversely, H2O2, with its less destructive impact on collagen, compromises decellularization efficiency and structural preservation, making it a potentially more appropriate choice for specific clinical applications.

Author contributions

Z.P. conceptualized the project and developed the methodology. H.S. conducted a formal analysis and drafted the original writing. D.J. contributed to the methodology. Y.E.S. and P., conducted investigations, provided resources, visualized the data, and contributed to reviewing and editing the writing.

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Competing financial interests

The authors have no conflict of interest.

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