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Original Article

Development of decellularized mouse auricular scaffolds using sodium dodecyl sulfate immersion-agitation for microtia tissue engineering

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Abstract

Effective treatment strategies for microtia remain limited due to the side effects and shortcomings associated with current therapeutic approaches. Tissue engineering, particularly the development of biological scaffolds, has emerged as a promising alternative. However, research on auricular scaffold fabrication in murine models using sodium dodecyl sulfate (SDS) and the immersion–agitation decellularization technique remains scarce. The aim of this study was to evaluate the effects of varying SDS concentrations on the decellularization efficiency and extracellular matrix (ECM) preservation of murine auricular tissue for scaffold development. Auricular tissues from mice (n=4) were immersed in Erlenmeyer flasks containing 0.1%, 0.5%, or 1% SDS and subjected to continuous agitation until the tissues became macroscopically translucent. Qualitative assessments included macroscopic appearance and microscopic evaluation using hematoxylin–eosin and Masson's trichrome staining. Quantitative analysis involved counting residual nuclei, while semiquantitative analysis of ECM area fractions was performed using ImageJ software. Statistical comparisons were conducted using one-way analysis of variance (ANOVA), with significance defined as $p<0.05$. The results demonstrated that the decellularized scaffolds exhibited macroscopic translucency, significantly reduced nuclear content ($p=0.001$), and preserved ECM integrity ($p=0.012$). Among the tested concentrations, 0.5% SDS provided the optimal balance between effective decellularization and ECM preservation. These findings support the potential application of murine auricular scaffolds decellularized with 0.5% SDS via the immersion–agitation method for future microtia tissue engineering.

Keywords: Auricular scaffold, decellularization, immersion-agitation, microtia, sodium dodecyl sulfate

Introduction

Microtia is a rare congenital malformation characterized by partial or complete underdevelopment of the external ear, with significant implications for affected individuals [1]. The global incidence is estimated at 1–5 cases per 10,000 live births [2,3]. This condition presents not only anatomical abnormalities but also impacts physiological function and psychosocial well-being [4]. In many cases, microtia is associated with conductive hearing loss, primarily due to malformations of the external auditory canal and ossicular chain, and may predispose patients to recurrent infections [5].

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Current treatment for microtia involves surgical reconstruction employing tissue engineering techniques, particularly through the use of auricular scaffolds [6,7]. These scaffolds undergo decellularization to remove cellular components while preserving the extracellular matrix (ECM), thereby enabling their use as transplantable structures to restore auricular form and function [8]. Composed primarily of ECM components, scaffolds provide a mechanically stable framework that supports cell adhesion, proliferation, and the distribution of growth factors essential for tissue regeneration [8]. Scaffold fabrication strategies include both synthetic and biological approaches. Among these, biologically derived scaffolds—produced through decellularization—more closely mimic the native tissue microenvironment, facilitating cellular interactions and functional integration with host tissue [9].

Decellularization is a process that removes cellular components, including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), while preserving the extracellular matrix (ECM) [10]. In addition to eliminating cells, this process also removes cellular antigens, thereby minimizing the risk of immune rejection when the scaffold is used for organ transplantation [8,10,11]. Decellularization typically involves the use of chemical, enzymatic, or physical agents, often in combination with specific application techniques to enhance efficacy [12].

Tissue decellularization can be achieved using chemical and biological agents, often in combination with physical techniques to enhance efficiency [9]. Commonly used chemical agents include detergents such as sodium dodecyl sulfate (SDS), sodium deoxycholate (SD), and Triton X-100, which act by disrupting cell membranes and inducing cell lysis [9]. Biological agents, such as the enzyme deoxyribonuclease (DNase), perform a similar function by degrading nucleic acids. Physical techniques—including perfusion, immersion, and agitation—are employed to improve the penetration and effectiveness of both chemical and biological agents during decellularization [12]. Each method has its advantages and limitations; for example, chemical agents are readily accessible but may damage the ECM at higher concentrations, whereas biological agents, although highly effective, are often more difficult to procure. Physical techniques alone are insufficient and must be applied in conjunction with chemical or biological agents to achieve complete decellularization [13].

Several studies have explored the development of auricular scaffolds using different decellularization agents. For example, Triton X-100 has been applied to generate scaffolds for human auricular reconstruction [2]. Similarly, porcine auricular cartilage has been decellularized using DNase in combination with the immersion technique [14]. However, these approaches present limitations; for instance, Triton X-100 has been shown to be less effective than SDS in removing cellular components. Other studies have employed SDS with the immersion–agitation technique to enhance decellularization efficiency [15-17]. While these methods achieved complete cell removal as confirmed by DNA quantification, they were applied to non-auricular tissues such as rat liver [15-17].

In this study, auricular scaffolds were developed using a modified decellularization approach that addressed limitations identified in previous research while accounting for local laboratory conditions. SDS was selected as the decellularization agent due to its proven efficacy in cellular removal [16], and was applied in combination with the immersion–agitation technique. The primary objective was to determine the optimal SDS concentration for effective decellularization of auricular tissue. Given the limited research on scaffold development in Indonesia, this study may serve as a foundational step toward the application of tissue-engineered scaffolds for organ transplantation in the region.

Methods

Study design

This experimental study was conducted between September 2023 and January 2024 at the Research Laboratory and Animal Breeding Unit, Faculty of Medicine and Health Sciences, Warmadewa University, and the Histology Laboratory, Faculty of Medicine, Udayana University.

Organ collection

This study utilized four male BALB/c mice, aged 7–8 weeks and weighing 25–30 g. Inclusion criteria included age and weight range, while mice with anatomical abnormalities of the auricle

were excluded. Each mouse was anesthetized via intramuscular injection of 0.2 mL of 10% ketamine into the thigh muscle [18]. Once full anesthesia was achieved, the right auricle was disinfected with alcohol, the fur was removed using forceps and scissors, and the auricle was excised using sterile tissue scissors. The harvested auricle was then placed in a sterile container filled with 25 mL of NaCl and stored at −20°C until further processing [15].

Preparation of SDS solution

A 10% stock solution of SDS was prepared by dissolving 10 g of SDS (Merck KGaA, Germany) in distilled water to a final volume of 100 mL. The solution was stirred using a magnetic stirrer at 60°C until fully homogenized. The stock solution was then diluted with distilled water (aquadest) to obtain working concentrations of 0.1%, 0.5%, and 1% SDS, as previously described [13].

Control group treatment

The right auricle of the control group mouse (n=1) was isolated using the same procedure as in the treatment group, including anesthesia with 0.2 mL of 10% ketamine [18]. Following excision, the auricle was disinfected using an alcohol swab, and the fur was removed with forceps and tissue scissors. The auricle was then placed in a container containing 25 mL of NaCl and stored at −20°C. Prior to histological processing, the auricle was thawed at room temperature (25°C) and prepared for staining.

Tissue decellularization process

The previously frozen right auricles were thawed at room temperature (25°C) until fully equilibrated. Each auricle was then immersed in an Erlenmeyer flask containing the designated concentration of SDS solution. The flasks were placed on an orbital shaker set to 100 rpm at room temperature (25°C) to facilitate agitation. SDS solutions were replaced daily at 10:00 AM to maintain optimal decellularization conditions. The process was terminated after 48 hours, upon visual observation of tissue transparency and the appearance of vascular structures, indicating successful decellularization [19]. The auricles were subsequently washed with sterile distilled water to remove residual SDS.

Histological preparation and staining

Auricles from the experimental group were washed with sterile distilled water, fixed in 10% formalin for 48 hours, dehydrated in 70%, 95%, and absolute ethanol (1 minute each, twice), and cleared in xylene (3×10 minutes). Tissues were infiltrated with molten paraffin at 58–60°C, embedded in paraffin blocks, sectioned at 5 µm, and mounted on glass slides. Slides were deparaffinized in xylene, rehydrated through graded ethanol, and rinsed with water. Hematoxylin–eosin (HE) staining was performed by immersing slides in hematoxylin and eosin (3 minutes each), followed by dehydration, xylene clearing, and coverslipping to visualize remaining nuclei.

Masson's trichrome (MT) staining was performed by sequentially immersing the slides in Weigert's hematoxylin, 2.5% aqueous phosphomolybdic-phosphotungstic acid, Bouin's solution, 1% aqueous Biebrich scarlet–acid fuchsin solution, and 2.5% aniline blue solution, following standard protocols [2]. After staining, the slides were rinsed under running water, then dehydrated through a graded series of ethanol (70%, 95%, and absolute) for one minute at each concentration. This was followed by clearing in xylene for three cycles of ten minutes each. Finally, the slides were mounted with coverslips. MT staining highlights collagen and ECM components in light blue, allowing visualization of ECM integrity following decellularization [20].

Data analysis

Qualitative analysis was performed to assess the macroscopic appearance of the mice's right auricles before and after decellularization. Observations were conducted daily at 10:00 AM, focusing on changes in tissue coloration until the auricle appeared transparent and clear. Daily photographs were taken, and descriptive comparisons between scaffolds were recorded.

Microscopic evaluation of HE staining was conducted to visualize residual cell nuclei, with images captured at 10× magnification using a digital microscope camera (Magcam-DC10, Magnus Theia-I, India). MT staining was similarly documented at 10× magnification to ECM integrity following decellularization.

A semi-quantitative analysis was performed to determine the ECM fractional area using MT-stained images captured at 40× magnification. Image analysis was conducted using ImageJ software (National Institutes of Health, USA), based on the percentage of blue-stained areas—indicative of collagen—converted into binary format and analyzed via brightness intensity. Five fields of view were analyzed per sample. The resulting percentage area (%area) was statistically evaluated using one-way analysis of variance (ANOVA).

In addition, quantitative analysis was conducted to assess the number of visible elastic cartilage cells. HE-stained sections were observed at 40× magnification using an Olympus CX21 microscope, and visible nuclei were manually counted across five fields of view by two independent observers. The resulting data were analyzed using one-way ANOVA followed by post hoc least significant difference (LSD) test. Statistical analyses were conducted in SPSS software version 29 (IBM, New York, USA), with a significance threshold of $p<0.05$.

Results

Morphology of mouse auricles

Macroscopically, the harvested auricles appeared white with a slight reddish hue prior to decellularization, indicative of the presence of blood vessels (**Figure 1A–B**). Following the decellularization process, the auricles became increasingly transparent (**Figure 1C–D**). All groups initially exhibited similar macroscopic characteristics.

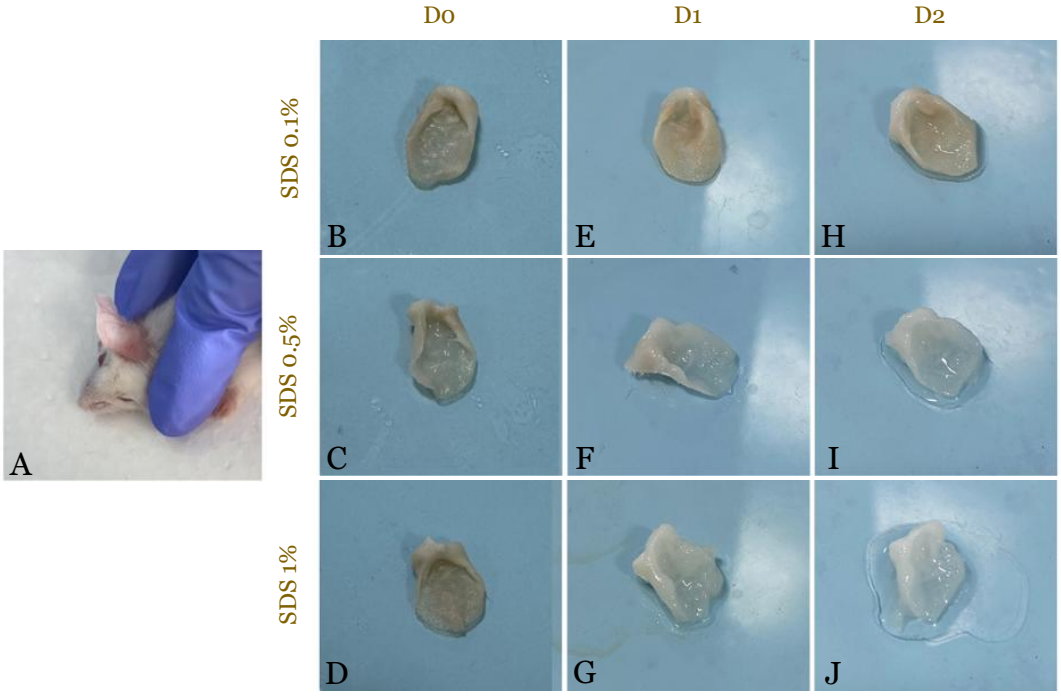

Figure 1. Mice auricula morphology before and after decellularization. (A) Living mice auricula. (B, C, D) The auricula appeared white with a slight red coloration prior to the decellularization process. (E) White and red areas were observed throughout most of the auricular surface. (F) The auricula was approximately 85% transparent, with white-red accents remaining around the base. (G) The auricula was nearly 100% transparent, with slight white-red accents around the base. (H) Transparent areas were visible only around the base. (I) The auricula appeared fully transparent. (J) The auricula was fully transparent with a slight structural disruption observed at the apex.

By the first day post-decellularization, Group 3 (treated with 1% SDS) exhibited a visibly transparent appearance, suggesting early and effective cellular removal. In contrast, Groups 1 and 2 retained some coloration, indicating the continued presence of viable cells. By the second day, both Groups 2 and 3 demonstrated complete transparency, with the 1% SDS solution achieving the most rapid and pronounced decellularization.

Elastic cartilage cell nuclei of the mouse auricle scaffold

Microscopically, several differences were observed between the decellularization and non-decellularization groups. One of them was the number of remaining nuclei (**Figure 2**). The non-

decellularized groups (**Figures 2A–B**) showed intact tissue with cells in good condition, whereas the decellularization treatment group (**Figure 2C**) showed a few remaining purple-stained nuclei. The decellularized auricle with 0.1% SDS showed cell nuclei in some lacunae, while other lacunae showed no cells. Additionally, several cell nuclei remained intact in the chondroblast section (**Figure 2C–D**).

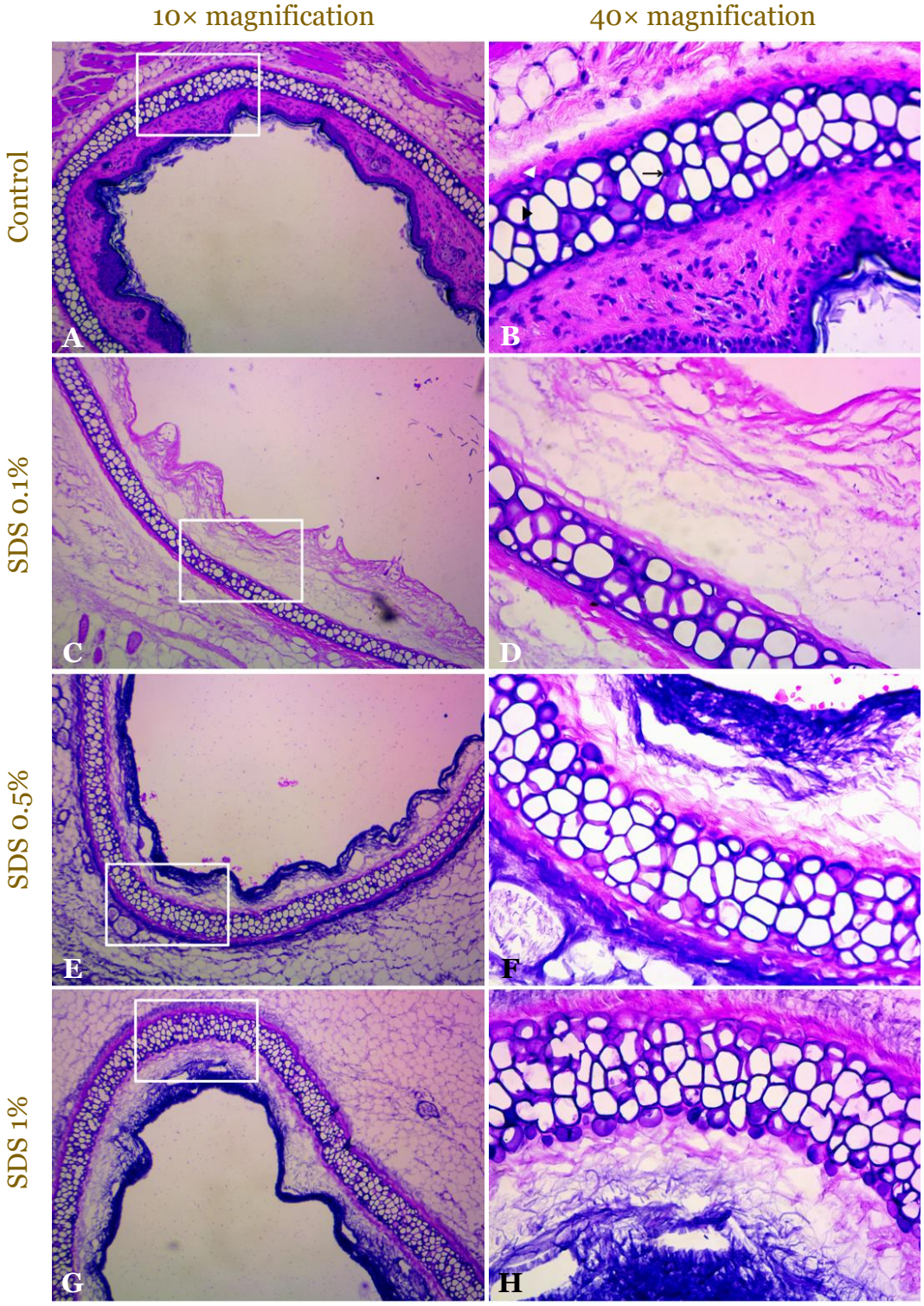

Figure 2. Elastic cartilage tissue staining. (A, B) Intact tissue with elastic cartilage cell nuclei located centrally within the lacunae. (C, D) Several elastic cartilage cell nuclei observed in the lacunae and chondroblast plate. (E, F) A small number of nuclei present in both lacunae and chondroblast plate. (G, H) A single cell nucleus observed, accompanied by disrupted lacunae. SDS: sodium dodecyl sulfate.

Group 2, or the treatment using 0.5% SDS, still had several cell nuclei in the lacunae and the chondroblast section. The number of cell nuclei was lower than that in Group 1 (**Figure 2E–F**). The decellularization process using 1% SDS resulted in the presence of only one visible cell nucleus (**Figure 2G–H**).

One-way ANOVA test showed that 0.1%, 0.5% and 1% SDS groups had mean numbers of visible cell nuclei of 9±1.87; 5.00±1.05 and 2.20±0.73 cell nuclei, respectively. The control group had an average of 13.60 ± 2.20 visible cell nuclei in each field of view. The one-way ANOVA test yielded a $p<0.001$ (**Table 1**), suggesting that mice auricles were successfully decellularized using the SDS immersion–agitation technique. Post hoc LSD analysis revealed significant differences between Group 2 (0.5% SDS) and the control ($p=0.001$), and Group 3 (1% SDS) and the control ($p=0.000$).

Table 1. Comparisons of visible cell nuclei of auricles among study groups

Study groups	Mean number of cell nuclei Mean±SE	*p*-value [a]
Sodium dodecyl sulfate (SDS) 0.1%	9.00±1.87	0.001
SDS 0.5%	5.00±1.05	
SDS 1%	2.20±0.73	
Control	13.60±2.20	

[a]Analyzed using one-way ANOVA test

Extracellular matrix of the mouse auricle scaffold

MT staining results showed several differences between the undecellularized and decellularized groups. The undecellularized auricle still displayed muscle tissue, cartilage cell nuclei, and abundant collagen (**Figure 3A–B**). In the 0.1% SDS group, the ECM was still considered intact (**Figure 3C–D**). Additionally, the amount of collagen remained substantial, as indicated by the intense blue staining. The 0.5% SDS group showed favorable decellularization results, with collagen structure classified as good. In general (**Figure 3E–F**), the ECM appeared intact around the elastic cartilage, except in the spaces between lacunae.

In contrast, the 1% SDS group exhibited a notable reduction in blue staining, with only minimal collagen detected, primarily around the elastic cartilage (**Figure 3G**). Damaged lacunae were also observed following the decellularization process (**Figure 3H**). Overall, the ECM condition in the group treated with 1% SDS was not preserved.

Based on the fraction area obtained from ImageJ software, it was found that Group 1, treated with 0.1% SDS as the decellularization agent, had an average fraction area of 29.03%±2.41. Group 2, treated with 0.5% SDS, had a mean fraction area of 28.49%±2.97. The mean fraction area of Group 3, treated with 1% SDS, was 26.96%±2.01. The average fraction area in the control group was 39.99%±1.70.

The results of the one-way ANOVA test showed a significant difference in the percentage of the remaining matrix fraction area between the treatment and control groups, with $p=0.012$ (**Table 2**). Post hoc LSD tests revealed significant differences between Group 1 and control ($p=0.010$), Group 2 and control ($p=0.007$), and Group 3 and control ($p=0.003$). These results confirm that all SDS-treated groups experienced ECM loss compared to the control.

Table 2. Comparisons of extracellular matrix fraction area among study groups

Study groups	Extracellular matrix fraction area Mean (%)±SE	*p*-value [a]
Sodium dodecyl sulfate (SDS) 0.1%	29.03±2.41	0.012*
SDS 0.5%	28.49±2.97	
SDS 1%	26.96±2.01	
Control	39.99±1.70	

[a]Analyzed using one-way ANOVA test

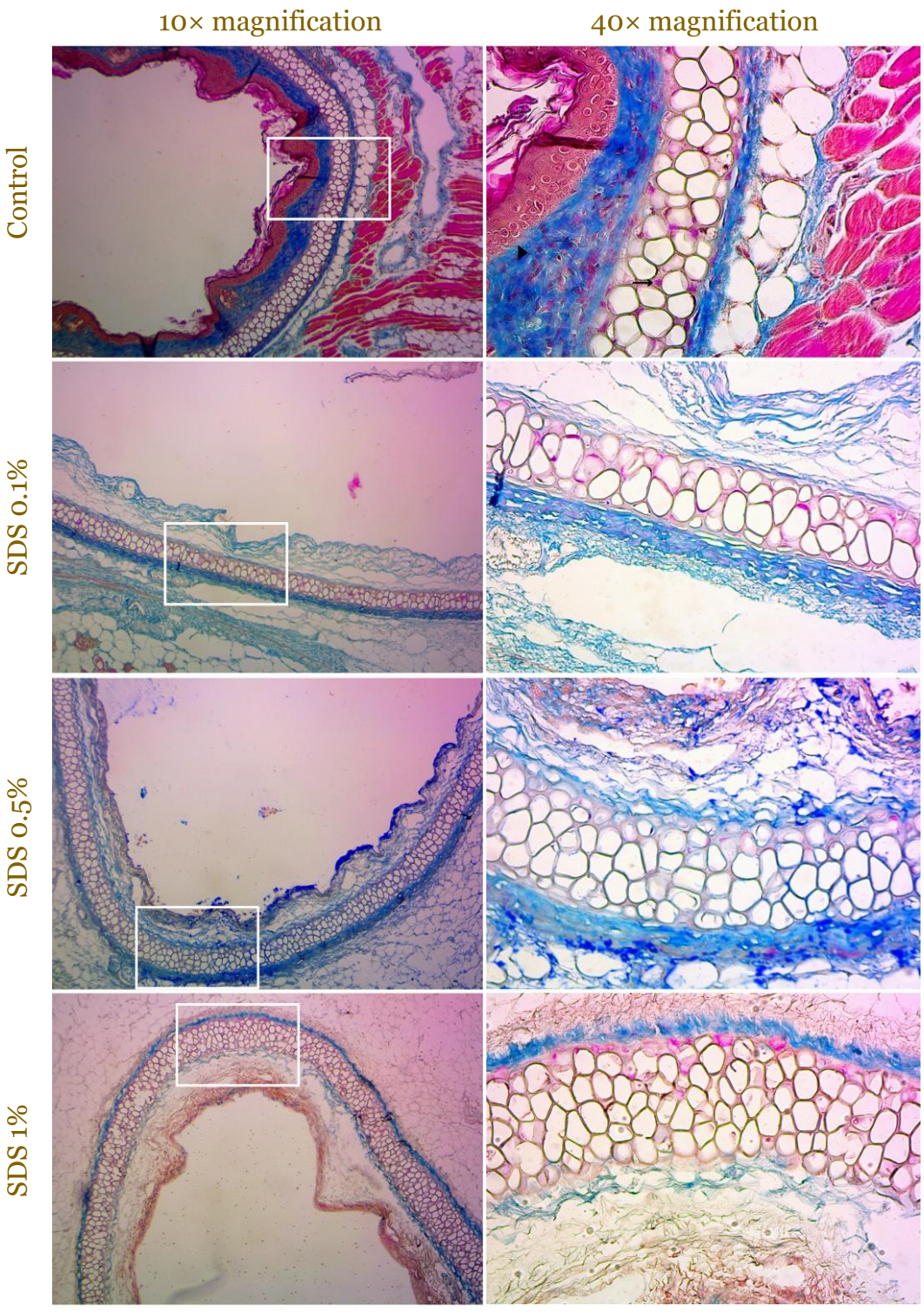

Figure 3. Elastic cartilage tissue staining. (A, B) Intact tissue with abundant extracellular matrix (ECM). (C, D) Extensive ECM observed surrounding the cartilage plate. (E, F) Intact ECM visible around the cartilage. (G, H) Disrupted and minimal ECM. SDS: sodium dodecyl sulfate.

Discussion

The decellularization process in this study was carried out for 48 hours, based on observations in Group 3, which began to show signs of transparency at that time point [19]. Transparency is indicative of cell removal, which occurs through contact with SDS. As an anionic detergent, SDS disrupts cellular membranes by emulsifying the phospholipid bilayer [9]. It also disrupts the nuclear envelope, ultimately leading to complete cell lysis and degradation of intracellular components, including genetic material [16].

Group 3, treated with 1% SDS, exhibited the most rapid and pronounced transparency among all treatment groups. This finding is consistent with previous studies using rat liver tissue and mouse heart and lung tissue, in which macroscopic transparency was observed following SDS-mediated decellularization [21]. However, this result contrasts with studies involving human auricular tissue, where macroscopic changes were limited to a shift from pink to light yellow, with minimal transparency [2]. This discrepancy is likely attributable to tissue thickness; human auricles are approximately 6 mm thick, whereas mice auricles in this study were less than 1 mm. Thicker tissues tend to retain more ECM even with similar levels of cellular clearance.

The transparency observed macroscopically corresponds with the reduction in remaining cellular content. Previous research showed that 1% SDS can reduce DNA content to approximately 0.95%, indicating that ~99.05% of cellular DNA is removed during the process [22]. This DNA loss serves as a proxy for effective cell removal. Another study reported that SDS can eliminate up to 90% of cells [23]. In a decellularization study using pancreatic tissue, DNA quantification revealed DNA remnants of 2.03% with 1% SDS, 2.28% with 0.5% SDS, and 2.31% with 0.1% SDS, suggesting a concentration-dependent increase in cell clearance [24]. This supports the interpretation that DNA loss—linked to reduced nuclear visibility—is a key indicator of successful decellularization [25].

HE staining was employed to assess cellular presence and distribution. Hematoxylin stains basophilic structures such as nuclei blue-purple, while eosin stains acidophilic components like cytoplasm and collagen pink [20]. In this study, HE staining revealed fewer elastic cartilage cell nuclei in the decellularized groups compared to the control. This outcome reflects SDS's ability to disrupt cell membranes and induce oxidative stress, both of which contribute to nuclear degradation and cell death [12,26]. SDS-induced oxidative stress can inhibit DNA replication and purine metabolism, further driving cell death [12,26].

Among the SDS concentrations tested, Group 3 (1% SDS) exhibited the lowest number of remaining elastic cartilage cell nuclei, whereas Group 1 (0.1% SDS) showed the highest. This is consistent with prior findings that increasing SDS concentration enhances cell lysis by disrupting hydrogen bonds and damaging nuclear integrity [27]. In a comparative study of blood vessel scaffolds, fewer nuclei were observed in tissue treated with 1% SDS than with 0.5% SDS [28]. Similarly, another study using 0.1% and 1% SDS showed that 1% SDS left only 50% of nuclei compared to 90% with 0.1% SDS, further supporting the concentration-dependent effect [29]. Higher SDS concentrations not only remove more cells but also reduce immunogenic antigens [30].

The treatment groups included three SDS concentrations, namely 0.1%, 0.5%, and 1%, which showed different results in the images of remaining cell nuclei after the decellularization process. Group 3, which used 1% SDS, had the fewest HE images of elastic cartilage cell nuclei. Group 1, or the group with 0.1% SDS as the decellularization agent, had the most cell nuclei. Previous research explained that increasing the concentration of SDS would increase cell lysis [31]. Cell lysis occurs due to the disruption of hydrogen bonds, which induces cell lysis during decellularization with SDS [27]. As a result, in the microscopic image, fewer cell nuclei were observed in this study. Increasing the concentration of SDS reduced the number of cell nuclei, which can be evaluated from the staining image [28]. A previous study compared histological images of scaffolds resulting from decellularization of blood vessel tissue using 0.5% and 1% SDS [28], and found that the number of visible cell nuclei was lower in the 1% SDS group compared to the 0.5% SDS group.

The number of cell nuclei showed that the group treated with 1% SDS as a decellularization agent had the fewest remaining cell nuclei. Another study used two different SDS concentrations to complete decellularization [29]. The concentrations used are 0.1% and 1%. The number of cell nuclei remaining when using 0.1% SDS is 90%, while using 1% SDS leaves 50% cell nuclei. This shows that increasing the concentration of SDS used for the decellularization process will eliminate more cell nuclei. Increasing the SDS concentration will increase the effectiveness of the decellularization process, which not only removes cells properly but also causes specific antigens to disappear [30].

Statistical analysis confirmed these findings. The one-way ANOVA test revealed a significant difference in the number of cell nuclei between groups (p=0.001). Post hoc LSD analysis revealed

significant differences between Group 2 (0.5% SDS) and the control (p=0.001), and Group 3 (1% SDS) and the control (p=0.000), confirming effective decellularization in these groups.

MT staining was used to evaluate ECM integrity, in which collagen appears light blue, muscle and keratin appear red, and nuclei appear dark blue [20]. The decellularized groups showed loss of muscle cells compared to the control, consistent with previous findings using rat heart scaffolds, where MT staining revealed loss of myocardial tissue post-decellularization [32]. MT staining also highlights damage to epithelial components, further demonstrating SDS's ability to disrupt cellular integration and nuclear content [33].

Among the treatment groups, ECM damage was most evident in Group 3 (1% SDS), where collagen staining was minimal and largely restricted to regions around the cartilage plate. In contrast, Group 1 (0.1% SDS) showed the most intact ECM, followed by Group 2 (0.5% SDS). These findings align with studies showing that higher SDS concentrations correlate with greater ECM disruption. MT-stained histological images confirmed that 1% SDS-treated tissue had more ECM degradation, especially at lacuna boundaries, compared to tissue treated with lower SDS concentrations.

Quantitative analysis using ImageJ supported these findings. Group 1 had the highest ECM fraction area, indicating better ECM preservation, while Group 3 had the lowest. This suggests that although increasing SDS concentration enhances cell removal, it also compromises ECM integrity. SDS can degrade ECM proteins such as collagen, elastin, laminin, and glycosaminoglycans (GAGs), with GAGs being especially susceptible [34]. Studies show that increasing SDS concentration leads to GAG depletion, collagen loss, and damage to the basal membrane, impairing ECM function [28].

Although SDS is effective for cell removal, its impact on ECM preservation depends on both concentration and exposure time. Previous study demonstrated that even with the same SDS concentration (e.g., 0.5%), varying the decellularization duration affects collagen retention (36). SDS has also been shown to preserve ECM architecture better than enzymes like trypsin or other detergents like Triton X-100 in porcine valve tissue [35]. Moreover, 1% SDS preserved more GAGs than 1% Triton in another comparative study [24].

The ANOVA test showed a significant difference in ECM fraction area among groups and post hoc LSD tests revealed significant differences between Group 1 and control, Group 2 and control, and Group 3 and control. These results confirm that all SDS-treated groups experienced ECM loss compared to the control.

Based on auricular transparency, Group 3 demonstrated the best macroscopic result, achieving the fastest and most complete decellularization. This supports previous findings that 1% SDS is highly effective for creating scaffolds [24]. However, based on HE staining, both 0.5% and 1% SDS-treated groups showed the best microscopic outcomes in terms of reduced cell nuclei. For MT staining, Groups 1 and 2 (0.1% and 0.5% SDS) showed the most intact ECM and fraction areas closest to the control. In contrast, 1% SDS caused substantial ECM damage. Because ECM integrity is essential for cell migration, proliferation, and differentiation, ECM preservation is critical for scaffold viability in tissue engineering [36].

Scaffold quality cannot be assessed solely by visible cell count or ECM fraction area. Further analyses are needed. DNA quantification would confirm residual DNA content and assess antigenicity risk [24]. Scanning electron microscopy could evaluate ECM surface architecture [2]. Additional tests for ECM components (collagen, elastin, GAGs) would inform scaffold suitability for recellularization [2,17].

While SDS effectively removes cells, its potential to damage ECM warrants reconsideration of protocol parameters. To ensure scaffold suitability for tissue engineering, alternative or combined decellularization agents should be explored. Future research will include these tests to determine whether SDS-based auricular scaffolds are viable for clinical use in organ transplantation and tissue engineering applications.

Conclusion

Variations in SDS concentration influenced the decellularization process of mouse auricular tissue. The most effective concentration was 0.5% SDS. This conclusion is supported by several findings: auricles treated with 0.5% SDS exhibited greater transparency, effective cell removal as

indicated by fewer visible elastic cartilage cell nuclei (p=0.001), and preservation of an abundant and intact ECM, as observed microscopically and supported by fraction area analysis using ImageJ software (p=0.012), compared to the other groups.

Ethics approval

This study received ethical approval from the Health Research Ethics Committee, Faculty of Medicine and Health Sciences, Universitas Warmadewa (Approval No. 369/Unwar/FKIK/EC-KEPK/X/2023), dated October 3, 2023.

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

We hereby confirm that no artificial intelligence (AI) tools or methodologies were utilized at any stage of this study, including during data collection, analysis, visualization, or manuscript preparation. All work presented in this study was conducted manually by the authors without the assistance of AI-based tools or systems.

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References

1. World Health Organization. Birth defects surveillance: Quick reference handbook of selected congenital anomalies and infections. Geneva: World Health Organization; 2020.
2. Al Z, Wafa EI, Meyer MKR, *et al.* Tissue engineering the pinna: Comparison and characterization of human decellularized auricular biological scaffolds. ACS Appl Bio Mater 2021;4:7234-7242.
3. Jovic TH, Gibson JAG, Griffiths R, *et al.* Microtia: A data linkage study of epidemiology and implications for service delivery. Front Pediatr 2021;9:1-10.
4. Jones ES, Gibson JAG, Dobbs TD, *et al.* The Psychological, social and educational impact of prominent ears: A systematic review. J Plastic Reconstr Aesthet Surg 2020;73(12):2111-2120.
5. Andrews J, Hohman MH. Ear microtia. StatPearls: Treasure Island; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK563243/. Accessed: 12 May 2023.
6. Childs RDF, Nakao H, Isogai N, *et al.* An analytical study of neocartilage from microtia and otoplasty surgical remnants: A possible application for BMP7 in microtia development and regeneration. PLoS One 2020;15(6):1-23.
7. Shang Y, Wang G, Zhen Y, *et al.* Application of decellularization-recellularization technique in plastic and reconstructive surgery. Chin Med J 2023;136(17):2017-2027.
8. Porzionato A, Stocco E, Barbon S, *et al.* Tissue-engineered grafts from human decellularized extracellular matrices: A systematic review and future perspectives. Int J Mol Sci 2018;19(12):1-79.

9. Neishabouri A, Khaboushan AS, Daghigh F, *et al.* Decellularization in tissue engineering and regenerative medicine: Evaluation, modification, and application methods. Front Bioeng Biotechnol 2022;10:1-21.
10. Jiang S, Zhuang Y, Cai M, *et al.* Decellularized extracellular matrix: A promising strategy for skin repair and regeneration. Eng Regen 2023;4(4):357-374.
11. Talaei-Khozani T, Yaghoubi A. An overview of post transplantation events of decellularized scaffolds. Transpl Immunol 2022;74:101640.
12. Mendibil U, Ruiz R, Carrion SR, *et al.* Tissue-specific decellularization methods: Rationale and strategies to achieve regenerative compounds. Int J Mol Sci 2020;21(15):1-29.
13. Rabbani M, Zakian N, Alimoradi N. Contribution of physical methods in decellularization of animal tissues. J Med Signals Sens 2021;11(1):1-11.
14. Kanda H, Oya K, Wahyudiono, *et al.* Surfactant-free decellularization of porcine auricular cartilage using liquefied dimethyl ether and DNase. Materials 2023;16(8):1-14.
15. Dewi AAAAP, Antarianto RD, Pawitan JA. Characterization of Indonesia decellularized liver cubes scaffold using scanning electron microscopy. J Biomim Biomater Biomed Eng 2021;52:38-46.
16. Damodaran RG, Vermette P. Tissue and organ decellularization in regenerative medicine. Biotechnol Prog 2018;34(6):1494-1505.
17. Dewi AAAP, Sumadewi KT, Witari PD, *et al.* Macroscopic and microscopic features of pancreatic scaffold generated by SDS-based decellularization using multiple needle injections. Intis Sains Med 2023;14(3):1028-1031.
18. Jaya PKD, Apsari PIB, Dewi PMACPN, *et al.* Effects of *Moringa oleifera* extract as an Immunomodulator of lymphocyte cells and macrophages in BALB/c mice infected with *Plasmodium berghei*. Folia Med Indones 2023;59(3):214-221.
19. Nooshin BJ, Tayebi T, Babajani A, *et al.* Effects of different perfusing routes through the portal vein, hepatic vein, and biliary duct on whole rat liver decellularization. Cell J 2023;25(1):35-44.
20. Mescher AL. Junqueira'S basic histology text and atlas. Fifteenth Edition. New York: McGraw-Hill Education; 2018.
21. Antarianto RD, Dewi AAAAP, Pragiwaksana A, *et al.* Decellularization of liver cubes using multiple site syringe injection for generating native liver scaffold: Preliminary report. Padang: AIP Conference Proceedings; 2019.
22. Narciso M, Ulldemolins A, Júnior C, *et al.* Novel decellularization method for tissue slices. Front Bioeng Biotechnol 2022;10:1-13.
23. Allu I, Sahi AK, Koppadi M, *et al.* Decellularization techniques for tissue engineering: Towards replicating native extracellular matrix architecture in liver regeneration. J Funct Biomater 2023;14(10):518.
24. Gaetani R, Aude S, Demaddalena LL, *et al.* Evaluation of different decellularization protocols on the generation of pancreas-derived hydrogels. Tissue Eng Part C Methods 2018;24(12):697-708.
25. Zhao F, Yang J, Li J, *et al.* Multiple cellular responses guarantee yeast survival in presence of the cell membrane/wall interfering agent sodium dodecyl sulfate. Biochem Biophys Res Commun 2020;527:276-282.
26. Somuncu ÖS. Decellularization concept in regenerative medicine. Adv Exp Med Biol 2020;1212:71-85.
27. Moffat D, Ye K, Jin S. Decellularization for the retention of tissue niches. J Tissue Eng 2022;13:1-29.
28. Sembiring YE, Ledyastatin RA, Nurrahmah A, *et al.* Comparative assessment of various concentration and exposure time of sodium dodecyl sulfate as decellularization agents for small-vessels vascular tissue engineering. Open Access Maced J Med Sci 2022;10(B):1-9.
29. Forouzesh F, Rabbani M, Bonakdar S. A comparison between ultrasonic bath and direct sonicator on osteochondral tissue decellularization. J Med Signals Sens 2019;9(4):227-233.
30. Bongolan T, Chow T, Whiteley J, *et al.* Optimazation of SDS-based kidney decellularization results in ECM scaffold applicable for 3D recellularization. Montreal: ISN WCN; 2021.
31. Cao C, Cao Z, Yu P, *et al.* Genome-wide identification for genes involved in sodium dodecyl sulfate toxicity in *Saccharomyces cerevisiae*. BMC Microbiol 2020;20(34):1-11.
32. Zhang X, Chen X, Hong H, *et al.* Decellularized extracellular matrix scaffolds: Recent trends and emerging strategies in tissue engineering. Bioact Mater 2022;10:15-31.
33. Duisit J, Amiel H, Wüthrich T, *et al.* Perfusion-decellularization of human ear grafts enables ECM-based scaffolds for auricular vascularized composite tissue engineering. Acta Biomater 2018;73:339-354.
34. Fernández J, Ahearne M. The impact of decellularization methods on extracellular matrix derived hydrogels. Sci Rep 2019;9(1):1-12.
35. Friedrich EE, Lanier ST, Niknam S, *et al.* Residual sodium dodecyl sulfate in decellularized muscle matrices leads to fibroblast activation in vitro and foreign body response in vivo. J Tissue Eng Regen Med 2018;12(3):1-30.
36. Cheng J, Li J, Cai Z, *et al.* Decellularization of porcine carotid arteries using low-concentration sodium dodecyl sulfate. Int J Artif Organs 2021;44(7):497-508.