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Original Article

75% ethanol disinfection preserves biocompatibility and mechanical properties of swim bladder for cardiovascular applications

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Abstract: Objectives: To evaluate the effects of 75% ethanol on the disinfection efficacy, biocompatibility, extracellular matrix (ECM) integrity, and mechanical properties of swim bladders from *Hypophthalmichthys nobilis*, a promising biomaterial for cardiovascular applications. Methods: Swim bladders were immersed in 75% ethanol for 30, 60, or 120 minutes, followed by phosphate-buffered saline (PBS) rinsing. Disinfection efficacy was assessed using bacterial cultures; cytotoxicity and blood compatibility were evaluated using CCK-8 assays and hemolysis tests. The decellularization efficiency, ECM integrity, and mechanical properties were analyzed through histological staining, DNA quantification, and uniaxial tensile test. Results: 75% ethanol effectively disinfected the swim bladder with minimal cytotoxicity and good hemocompatibility. Histological staining demonstrated preservation of collagen fibers, elastin, and glycosaminoglycans (GAGs), indicating minimal impact on the ECM structure. The uniaxial tensile test revealed that ethanol immersion caused tissue dehydration and increased stiffness, but these effects were reversible after PBS rinsing, with mechanical properties returning to baseline. DNA content and tissue thickness normalized following PBS washing, suggesting that ethanol did not impair decellularization. Conclusions: 75% ethanol is an effective disinfectant for swim bladder biomaterials, preserving biocompatibility, ECM structure, and mechanical properties after rinsing. These findings support the use of swim bladder-derived scaffolds in cardiovascular tissue engineering applications.

Keywords: Swim bladder, ethanol, extracellular matrix, hemocompatibility, mechanical properties

Introduction

Disinfection is a crucial step in preparing biological materials for implants, such as artificial blood vessels and heart valves, to prevent post-operative infections [1]. Although 75% ethanol cannot eliminate bacterial spores, it remains widely used as a disinfectant due to its effectiveness against bacterial propagules, fungi, and most viruses [2].

Despite its common use, the effects of ethanol disinfection on the biocompatibility and integrity of the extracellular matrix (ECM) in biological materials are still not fully understood. Biocompatibility is crucial as it influences the interaction between biomaterials with living tissues and bodily fluids. The ECM provides a scaffold that helps maintain tissue shape and integrity. It is composed of various proteins and glycoproteins that facilitate cell adhesion and regulate biochemical signals, which in turn affects cell behavior. Preserving ECM integrity is vital to ensure that biomaterials possess the necessary mechanical properties to withstand the stresses encountered in the body [3]. Furthermore, the ECM supports essential processes such as cell migration, proliferation, and differentiation, which are critical for tissue regeneration and repair [4, 5]. Therefore, investigating the impact of ethanol on the ECM is important for advancing biomaterials used in tissue engineering and regenerative medicine.

Swim bladder, a tissue primarily composed of collagen I [6, 7], glycosaminoglycans (GAGs) [6,

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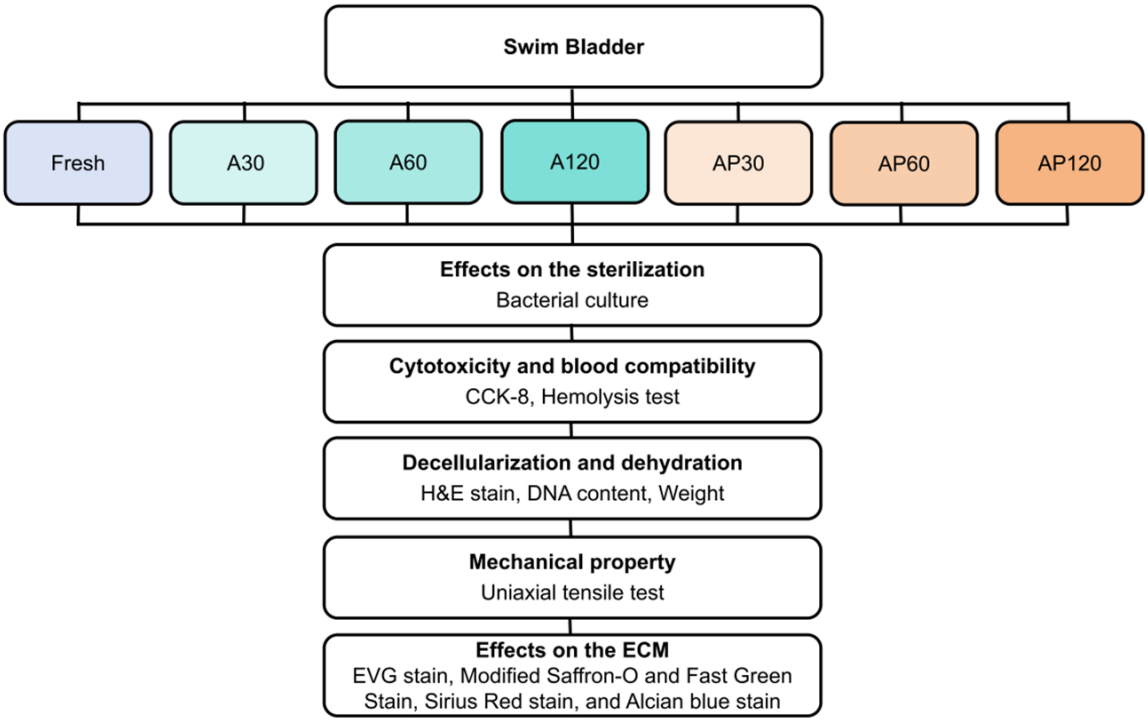

Figure 1. Flow chart of the study. CCK-8, cell counting kit-8; ECM, extracellular matrix; H&E stain, hematoxylin and eosin stain.

8], and elastin [6], is a promising biomaterial for cardiovascular applications. Some studies [6, 7, 9] have shown that swim bladders offer better biocompatibility than bovine pericardium, traditionally utilized in this field. Notably, the swim bladder demonstrates enhanced anti-calcification properties, which are critical for preventing long-term implant failure. Moreover, the swim bladder has shown great potential for endothelialization, an important factor for graft and implant integration into the vascular system. These features highlight the suitability of swim bladders for various applications, including heart valve replacements and vascular grafts. Furthermore, compared to materials like porcine pericardium, the swim bladder is free of religious sensitivity, making it a promising candidate for clinical application [6, 7, 9].

Given the limited understanding of how ethanol disinfection affects the biomaterial properties, this study aims to evaluate the effects of 75% ethanol on the disinfection efficacy, cytotoxicity, blood compatibility, decellularization, ECM integrity, and mechanical properties of swim bladders. The findings of this study will provide a foundation for optimizing the preparation protocols of swim bladder-derived scaffolds in cardiovascular tissue engineering and support the use of ethanol as a disinfection protocol in tissue engineering and regenerative medicine.

Materials and methods

Sample collection and preparation

Swim bladders were obtained from approximately 1.5 kg of *Hypophthalmichthys nobilis* purchased from a seafood market in Xinghua, Jiangsu. After slaughter, the swim bladders were transported to the laboratory in ice boxes. Upon arrival, mucous membranes and residual blood were meticulously removed. The swim bladders were thoroughly washed with sterile phosphate-buffered saline (PBS; pH 7.2-7.4; Solarbio Life Sciences, Beijing, China) containing 10% penicillin-streptomycin solution (Gibco, Grand Island, NY, USA) to minimize bacterial contamination.

Experimental groups

The swim bladders were divided into seven groups based on the treatment protocols (**Figure 1**):

Fresh Group (control): Untreated swim bladders. A30 Group: Immersed in 75% ethanol (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) for 30 min. A60 Group: Immersed in 75% ethanol for 60 min. A120 Group: Immersed in 75% ethanol for 120 min. AP30 Group: Immersed in 75% ethanol for 120 min and then rinsed with PBS for 30 min. AP60 Group: Immersed in 75% ethanol for 120 min and then rinsed with PBS for 60 min. AP120 Group: Immersed in 75% ethanol for 120 min and then rinsed with PBS for 120 min.

Assessment of disinfection efficacy

To evaluate the disinfecting efficacy of 75% ethanol, swim bladders from each group were incubated in Dulbecco's Modified Eagle Medium (DMEM; tissue surface area to volume ratio of 6 cm^2/mL; DMEM basic 1x, Gibco, Grand Island, NY, USA) at 37°C for 72 h. The incubation medium was then filtered to obtain extracts. A 500 µL aliquot of each extract was inoculated onto Colombia Blood Agar plates (Kmj, Shanghai, China) and Tryptic Soy Agar (TSA) plates (Hopebio, Qingdao, China). The extracts were evenly spread using sterile spreaders, the plates were incubated at 37°C, and bacterial colony growth was monitored daily for 72 h.

Hemolysis test

Anticoagulant blood was collected and centrifuged at 2500 rpm for 5 min to isolate red blood cells (RBC). A 2% RBC suspension was prepared in PBS. The swim bladders from each group were then placed in an Eppendorf tube containing 1 mL of RBC suspension and incubated at 37°C for 3 hours. The supernatant was collected after centrifugation (2500 rpm, 5 minutes). The absorbance (OD_{SW}) at 545 nm was determined using an Epoch microplate spectrophotometer (Bio Tek, Winooski, VT, USA). RBCs were diluted with deionized water or PBS to achieve a concentration of 2%, and the OD values were recorded as the positive control (OD_{PC}) and negative control (OD_{NC}), respectively.

Hemolytic rate (%) = $(OD_{Sw}-OD_{NC})/(OD_{PC}-OD_{NC}) \times 100\%$

Cytotoxicity analysis

Cytotoxicity of swim bladder extracts was evaluated using a Cell Counting Kit-8 (CCK-8) assay with mouse L929 fibroblast cells. Swim bladder samples were incubated in DMEM (6 cm^2 tissue per 1 mL DMEM) at 37°C for 72 h to produce the extracts. L929 cells were seeded into 96-well plates at a density of 3,500 cells per well and cultured for 48 h. The medium was then replaced with 100 µL of swim bladder extract, and cells were incubated for an additional 24 or 48 h. Following incubation, 10 µL of CCK-8 reagent (Dojindo Laboratories, Kumamoto, Japan) was added to each well, and the plates were incubated for 4 h. The absorbance was measured at 450 nm (OD_{SB}). Cell viability was calculated using the following formula:

Cell viability (%) = $(OD_{SB}-OD_B)/(OD_{PC}-OD_B) \times 100\%$

Where OD_B is the absorbance of the blank (medium with CCK-8, no cells), and OD_{PC} is the absorbance of the positive control (cells with medium and CCK-8).

Evaluation of decellularization effectiveness

Histological analyses were performed to assess decellularization. Samples were fixed in formalin, dehydrated, embedded in paraffin, and sectioned at 5 µm thickness. The sections were stained with Hematoxylin and Eosin (H&E) following standard protocols. The presence or absence of cellular nuclei was evaluated using a light microscope.

DNA content in the swim bladder samples was quantified using the TIANamp Genomic DNA Kit (Tiangen Biotech Co., Ltd., Beijing, China) according to the manufacturer's instructions. The DNA concentration was measured spectrophotometrically, and the results were expressed as nanograms of DNA per milligram of tissue.

Assessment of dehydration effect

Swim bladder samples (n=8 per group) were trimmed to 2 cm × 1 cm, gently blotted to remove surface moisture, and weighed to obtain fresh weights. The samples were then immersed in 75% ethanol for 30 min (A30), 60 min (A60), and 120 min (A120). Weights were recorded after each ethanol immersion period to assess dehydration. Subsequently, samples immersed in ethanol for 120 min were rinsed with PBS for 30 min (AP30), 60 min (AP60), and 120 min (AP120), and their weights were measured to evaluate rehydration.

Mechanical property analysis

The mechanical properties of the swim bladder samples were assessed using uniaxial tensile testing. The thickness of the swim bladders was measured using a micrometer (IP64, SanLiang, Japan). The tissues were cut into 2.0 × 1.0 cm strips along the direction of the fiber bundle. A tensile testing machine (Zwick, Germany) was used to stretch the tissue axially at a rate of 5 mm/min (n=6 for each group). The ultimate tensile strength, elongation at break, and elastic modulus were determined based on the stress-strain curve.

ECM integrity

Histological staining was performed on paraffin-embedded sections to evaluate the integrity of ECM components. Collagen fibers were visualized using Elastica van Gieson (EVG) staining (Solarbio LIFE SCIENCES, Beijing, China), modified saffron-O and fast green staining (Solarbio LIFE SCIENCES, Beijing, China), and Sirius red staining (Biosharp Life Sciences, Beijing, China). Elastic fibers were identified using EVG staining (Solarbio LIFE SCIENCES, Beijing, China) and modified saffron-O and fast green staining (Solarbio LIFE SCIENCES, Beijing, China). GAGs were stained using modified saffron-O and fast green staining (Solarbio LIFE SCIENCES, Beijing, China), and Alcian blue staining (GENMED SCIENTIFICS INC., U.S.A). Images were captured using an optical microscope (Olympus, Germany).

Statistical analysis

All quantitative data were presented as mean ± standard deviation (SD) for normally distributed variables, or as medians with interquartile ranges (IQR) for non-normally distributed variables. Statistical analyses were performed using SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA). Analysis of variance (ANOVA) was employed to compare means across multiple groups. A one-way ANOVA was performed when variances were equal, followed by post hoc comparisons using the Bonferroni or Scheffé method if significant differences were found. When variances were unequal, the Brown-Forsythe test was utilized, with post hoc comparisons using Tamhane's T2 method. A repeated measurement ANOVA was conducted to compare the weights of swim bladders after ethanol disinfection at different durations. The Shapiro-Wilk test was performed first to assess the normality of the data. For normally distributed data, repeated-measures ANOVA was applied, with Mauchly's test assessing the sphericity assumption. If the sphericity assumption was met, results were interpreted accordingly; if violated, the Greenhouse-Geisser correction was applied. When significant differences were found, pairwise comparisons between the fresh group and other groups were conducted using the Bonferroni method. $P<0.05$ was considered statistically significant. Graphs were generated using GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA) and Origin 2021 (OriginLab Corporation, Northampton, MA, USA).

Results

Disinfection efficacy

The bacterial culture results are shown in **Figure 2**. In the fresh group, bacterial colonies appeared on both Colombia Blood Agar and TSA plates within the first day of incubation. The number of colonies increased over the following two days. In contrast, no bacterial colony was observed in any of the groups treated with 75% ethanol in 72 hours of incubation. These findings suggest that 30-minute incubation with 75% ethanol has a good disinfection effect on the swim bladder tissue.

Hemolysis test and cytotoxicity analysis

The hemolysis test results are presented in **Figure 3A** and **3B**. All experimental groups exhibited hemolysis rates below 5%, meeting the ISO 10993-4:2002 standard for blood compatibility. These results suggest that swim bladder material possesses excellent hemocompatibility, and treatment with 75% ethanol does not adversely affect this property.

Cell viability was assessed using the CCK-8 assay (**Figure 3C**). The fresh group displayed lower cell viability, with values of 40.14% on day 1 and 2.56% on day 2. In contrast, all ethanol-treated groups (A30, A60, A120, AP30, AP60, and AP120) demonstrated cell viability exceeding 70%, which was considered non-cytotoxic according to the ISO standards. These results indicate that immersion in 75% ethanol effectively improved cell viability without notable

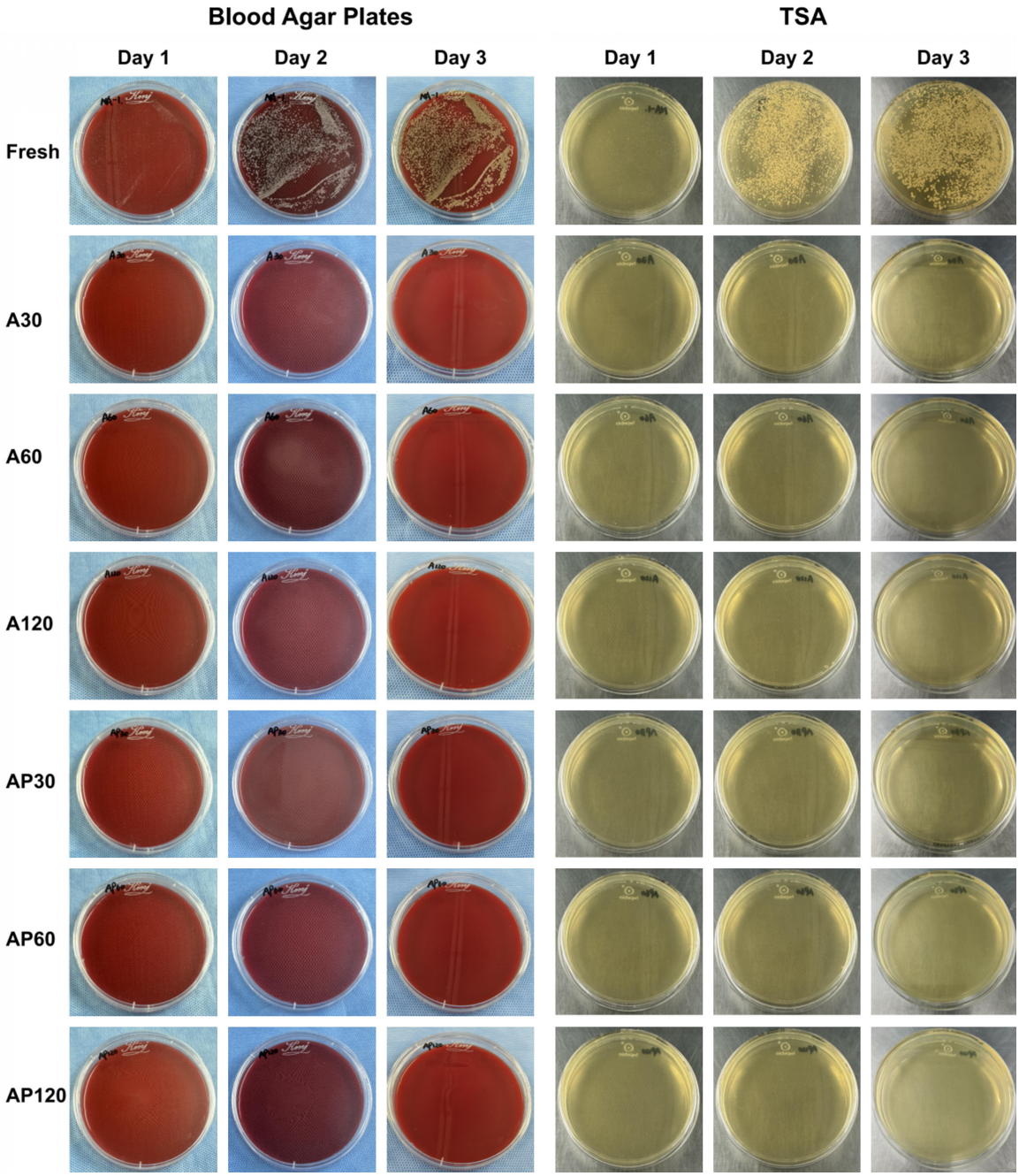

Figure 2. The results of bacterial culture. In the fresh group, bacterial colonies appeared on both Colombia Blood Agar and TSA plates within the first day of incubation. No bacterial colony was observed in any groups treated with 75% ethanol in 72 hours of incubation. TSA, Tryptic Soy Agar.

cytotoxicity, even with prolonged exposure and without immediate PBS rinsing.

Decellularization effectiveness and tissue dehydration

After immersion in 75% ethanol, the decellularization effect on the swim bladder was not evident. H&E staining revealed no noticeable changes in the nuclei of any ethanol-soaked group compared to the fresh group (**Figure 4A-G**).

The DNA quantitative test (**Figure 4H**) revealed that the fresh group had a DNA concentration of 288.8±32.67 ng/mg. In contrast, after soak-

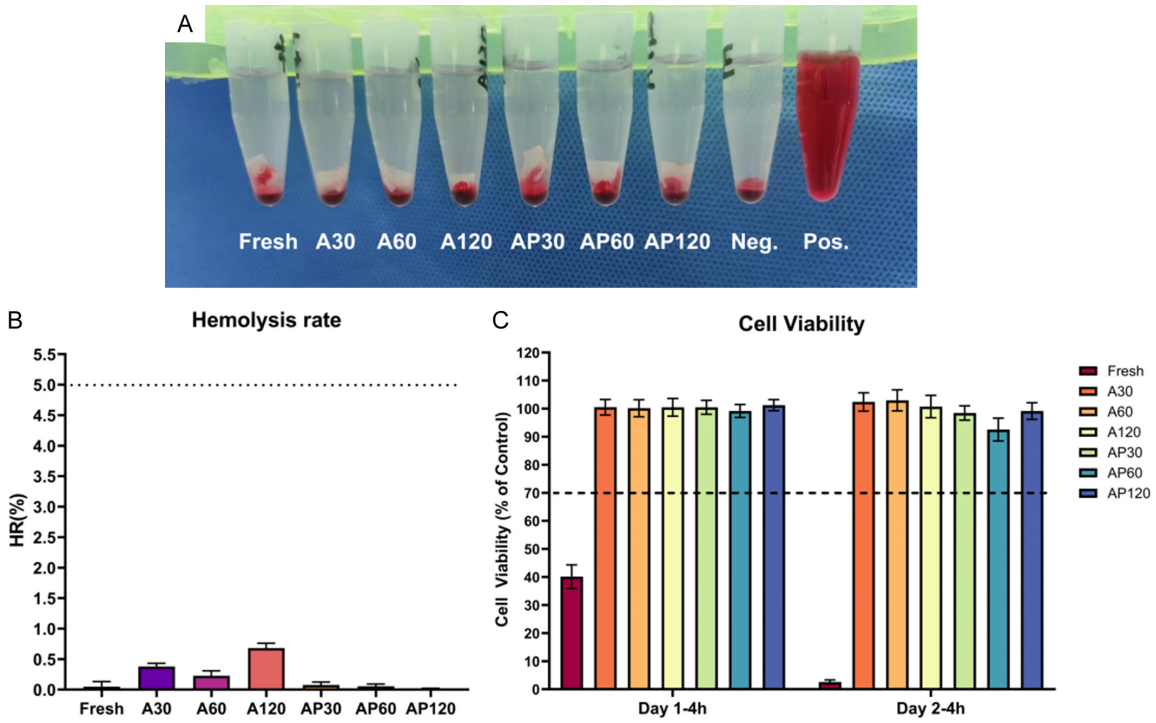

Figure 3. The results of hemolytic test in vitro and cytotoxicity analysis. A, B. Hemolytic test and hemolysis rate; C. CCK-8 for cytotoxicity analysis.

ing in 75% ethanol, the DNA concentrations increased to 995.6±63.74 ng/mg, 1254.0±68.64 ng/mg, and 632.8±37.21 ng/mg at 30 min, 60 min, and 120 min, respectively. These values were significantly higher than those observed in fresh tissue (P<0.001). After washing with PBS, the DNA content gradually returned to baseline. The DNA content was measured as 320.5±21.30 ng/mg after a 30-minute PBS wash, which remained significantly higher than that of the fresh group (P<0.001). However, the AP60 and AP120 groups showed DNA contents of 288.8±17.23 ng/mg and 267.0±37.71 ng/mg, which were not significantly different from the fresh group (P=1.000 and 0.215, respectively).

The swim bladder weight (**Figure 4I**) was 42.21±11.24 mg in the fresh group, 25.34±6.87 mg in the A30 group, 24.01±6.23 mg in the A60 group, 22.46±5.73 mg in the A120 group, 36.64±9.38 mg in the AP30 group, 39.26±10.16 mg in the AP60 group, and 41.21±9.65 mg in the A120 group. The weight was significantly lower in the A30 (P<0.001), A60 (P=0.001), and A120 (P=0.001) groups compared to the fresh group. Following immersion in PBS, no significant differences in weight were observed between the AP60, AP120 groups and the fresh group (P=0.133 and 1.000, respectively).

Mechanical properties

The mechanical properties of the swim bladders are summarized in **Table 1**.

Ethanol immersion significantly reduced tissue thickness (**Figure 5A**). The swim bladder tissues of the A60 and A120 groups were thinner than those in the fresh group (P=0.010 and 0.002, respectively). After rinsing with PBS, tissue thickness partially recovered, with no significant difference from the fresh group.

The elastic modulus (**Figure 5B**) increased after ethanol immersion, with the A60 group showing a significantly higher elastic modulus than the fresh and AP120 groups (P=0.004 and 0.017, respectively). After PBS rinsing, the elastic modulus values decreased and approached those of the fresh tissue, indicating a reversible temporary stiffening effect. There were no significant differences in elongation at break (**Figure 5C**) among the groups (P=0.510).

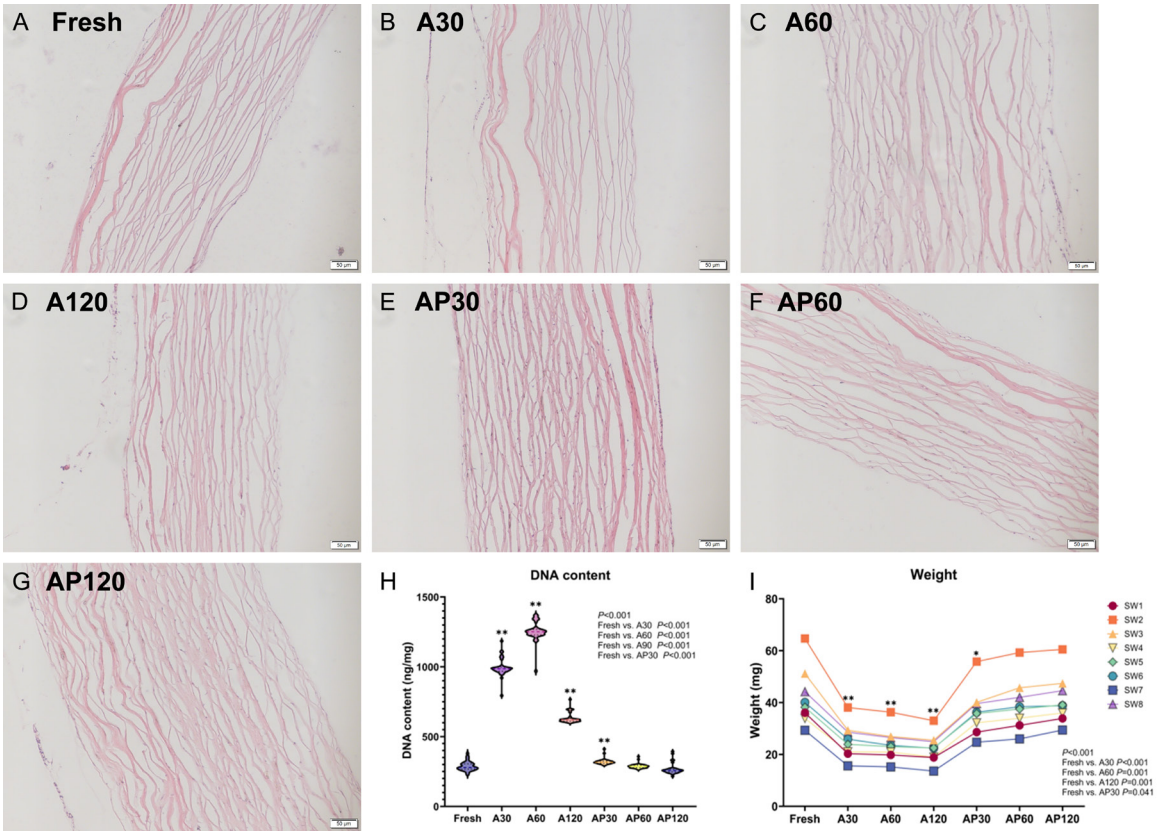

Figure 4. Changes in H&E staining (20×), DNA quantification, and tissue weight. H&E staining: (A) Fresh, (B) A30, (C) A60, (D) A120, (E) AP30, (F) AP60, (G) AP120, (H) Quantitative DNA testing, (I) Weight changes. Bar=50 μm. *; $P<0.05$, **; $P<0.01$ compared with the fresh group.

Table 1. The mechanical properties of each group

	Thickness (mm)	Elastic modulus (MPa)	Elongation at break (%)	Tensile strength (MPa)
Fresh	0.210±0.020	8.15±1.69	40.23±5.11	1.64±0.49
A30	0.181±0.019	11.14±3.71	41.62±2.95	2.32±0.65
A60	0.164±0.008	14.22±3.32	40.80±5.02	2.85±0.58
A120	0.157±0.017	12.04±1.53	43.02±6.01	2.59±0.29
AP30	0.179±0.029	10.04±2.50	41.97±4.64	2.06±0.40
AP60	0.179±0.024	10.71±0.99	37.20±4.73	2.01±0.42
AP120	0.178±0.021	8.90±2.69	41.38±4.54	1.86±0.57

Ethanol immersion enhanced the ultimate tensile strength of the tissue (**Figure 5D**). The A60 group exhibited significantly higher tensile strength than the fresh and AP120 groups (P=0.004 and 0.033, respectively). Additionally, the A120 group had higher tensile strength than the fresh group (P=0.048). These improvements suggest that 75% ethanol temporarily increases the tissue resistance to tensile forces. The stress-strain curves for all the groups are presented in **Figure 5E**. Overall, 75% ethanol caused a temporary increase in tissue stiffness and tensile strength, and these changes were partially reversible after PBS rinsing, with mechanical properties approaching those of fresh tissue.

ECM integrity

Histological staining for collagen fibers, elastin fibers, and glycosaminoglycans demonstrated that the structural integrity of the ECM was well preserved across all groups (**Figure 6**). No significant degradation or alteration of ECM com-

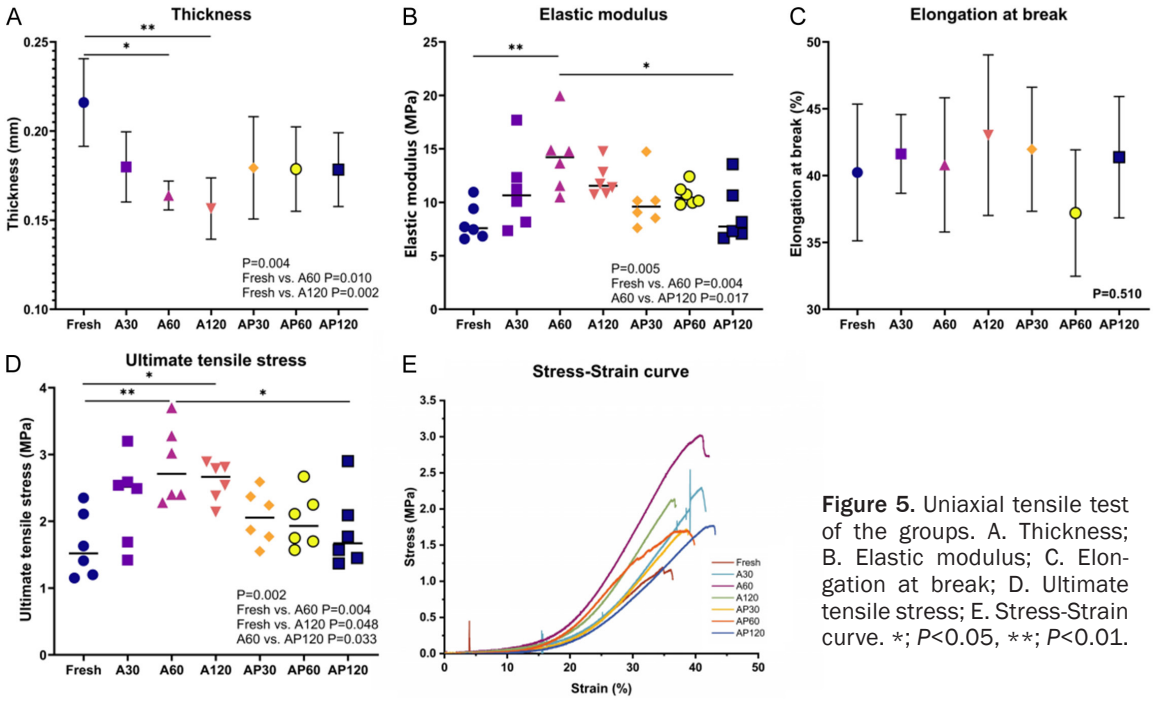

Figure 5. Uniaxial tensile test of the groups. A. Thickness; B. Elastic modulus; C. Elongation at break; D. Ultimate tensile stress; E. Stress-Strain curve. *; $P<0.05$, **; $P<0.01$.

ponents was observed following treatment with 75% ethanol and subsequent PBS rinsing. These findings suggest that ethanol disinfection does not adversely affect the ECM structure of swim bladder tissues.

Discussion

This study demonstrated that immersion in 75% ethanol effectively disinfected the swim bladder without compromising its biocompatibility, blood compatibility, decellularization effectiveness, ECM integrity, or mechanical properties.

Ethanol disinfects by denaturing proteins and disrupting microbial enzyme systems, making it a widely used disinfectant [2]. However, due to its inability to eliminate bacterial spores, ethanol is suitable for disinfection rather than sterilization. Previous studies have demonstrated the efficacy of ethanol in disinfecting various biological tissues and organs, including bovine pericardium [10], porcine radial artery [11], human umbilical veins (HUVs) [12], equine carotid arteries [13], rat abdominal aortas [14], swim bladder [6, 15], porcine bladder [16], rat bladder [17], porcine kidney [18], porcine liver [19], porcine bone [20], and equine tendon [21]. The recommended concentration for ethanol-based disinfection ranges from 70% to 80%, with exposure times varying from 20 min to 3 days [2, 6, 10-12, 15, 22, 23]. For swim bladders, some studies have used 75% ethanol for overnight immersion [6] or 80% ethanol for 4 h [15] for disinfection. Our research found that soaking swim bladders in 75% ethanol for 30 min resulted in the absence of colony growth on the culture plates, suggesting that effective disinfection of swim bladders with 75% ethanol may require at least 30 min of immersion.

Cytotoxicity and hemocompatibility tests indicated that short immersion disinfection in 75% ethanol (not exceeding 2 h) did not adversely affect the biocompatibility of swim bladders. This study found that even without subsequent washing with PBS, the cell compatibility and hemocompatibility of swim bladders immersed in 75% ethanol for 2 h could meet the ISO standards for cell compatibility and hemocompatibility (cell viability remained above 70%, and hemolysis rates were below 5%). Poornejad et al. [18] assessed the cytotoxicity of pig kidney materials after disinfection with 70% ethanol using the live/dead method and observed no evident toxicity over 3 d. Therefore, we conclude that short-term disinfection of the swim bladder using 75% ethanol is safe in terms of cytotoxicity and hemocompatibility.

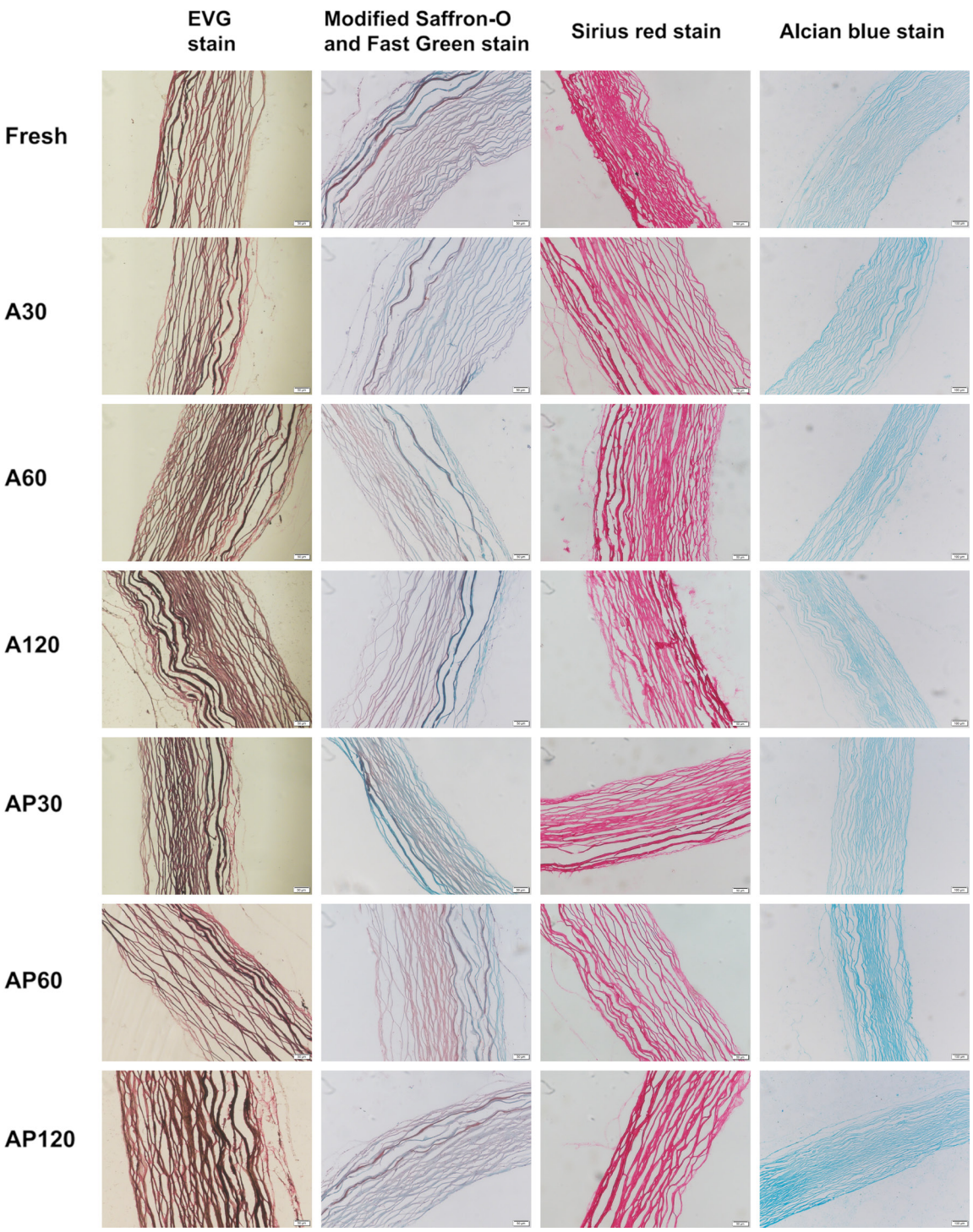

Figure 6. EVG stain (20×), Modified Saffron-O and fast Green stain (20×), Sirius red stain (20×), and Alcian blue stain of the groups (20×). Bar=50 μm. EVG, Elastica van Gieson.

Solvents, such as alcohols, are commonly used for chemical-based decellularization techniques; however, their effectiveness in achieving complete decellularization requires further improvement [24]. Based on H&E staining, soaking swim bladders in 75% ethanol for 2 h in this study did not effectively remove cell nuclei. Additionally, from 30 min of ethanol immersion, the DNA content of the swim bladder increased, likely due to ethanol-induced

dehydration, which resulted in weight loss. However, this dehydration was reversible, as both the tissue weight and DNA content returned to levels not significantly different from the fresh control group after 60 minutes of subsequent PBS immersion. Hussein et al. [23] reported similar results, finding no significant difference in the DNA content of decellularized pig liver scaffolds treated with PBS and 70% ethanol (soaked in 70% ethanol for 2 h, followed by washing with PBS for 2 h). Lumpkins et al. [25] observed the absence of nuclei in decellularized porcine intervertebral disc tissues after a 24-hour decellularization process using a combination of 25% acetone and 75% ethanol. This difference may be attributed to the longer ethanol soaking time and the concurrent use of acetone. In contrast, this study found that soaking swim bladders in 75% ethanol for 30 min resulted in significant dehydration, but the dehydration effect within 2 hours was reversible, with recovery occurring after 60 min of PBS washing. Overall, soaking swim bladders in 75% ethanol for 2 h did not produce significant decellularization effect.

The ECM is mainly composed of collagen, elastin, glycosaminoglycans, and other components, playing a critical role in biocompatibility, cell adhesion, proliferation, differentiation, mechanical properties, as well as vascular and tissue regeneration. Maintaining the integrity of the ECM structure is crucial for preserving the functionality of biomaterials and promoting cell adhesion, migration, proliferation, and differentiation on tissue-engineering scaffolds. Despite the importance of ECM integrity, research on the effects of ethanol on the ECM remains limited and somewhat controversial. Hussein et al. [23] reported a reduction in GAG and collagen levels in pig liver scaffolds after ethanol treatment. In contrast, Guimaraes et al. [26] found no significant difference in collagen content between the PBS and 70% ethanol preservation protocols for pig tracheal scaffolds. In this study, histological staining revealed that 2 h of disinfection with 75% ethanol had no significant effect on the collagen, elastin, and GAG content in the swim bladder ECM, nor did it significantly impact the ECM structure.

This study observed that 75% ethanol disinfection reversibly affected the swim bladder thickness, stiffness, and ultimate tensile strength, which were largely restored to their untreated state after PBS washing. As the ethanol soaking time increased, the swim bladder thickness decreased, and a statistically significant difference was observed between the initial thickness and that after 60 min of soaking. After soaking in 75% ethanol for 60 min, the elastic modulus and ultimate tensile strength of the swim bladder increased, followed by a slight decline. This change is likely related to ethanol-induced dehydration, as Tarnowski et al. [27] found that ethanol can reduce moisture content in collagen fibers, similar to the findings of Lumpkins et al. [25]. After ethanol disinfection, the swim bladder tissues gradually recovered its thickness, stiffness, and ultimate tensile strength during PBS washing. After 2 h of PBS washing, a significant difference in mechanical properties remained compared to 60 min of ethanol soaking; however, no significant difference was found when compared to the untreated state. Studies by Guimaraes et al. [26] and Sergeevichev et al. [28] also reported no significant effect of ethanol disinfection on the mechanical properties of the trachea and arteries. These findings suggest that the reversible effects of 75% ethanol disinfection on the swim bladder's elastic modulus and ultimate tensile strength can be fully mitigated by washing with PBS for 2 hours, maintaining mechanical properties similar to those before treatment. This also supports the use of a 2 h 75% ethanol disinfection followed by 2 h of PBS washing as an effective approach to maintaining ECM integrity in the swim bladder.

Limitations

Despite these positive outcomes, this study had several limitations. Electron microscopy was not used to examine ultrastructural changes in the tissue, which could provide deeper insights into the subtle alterations caused by ethanol. Additionally, the effects of ethanol on growth factors and cytokines within the ECM were not assessed. These factors are essential for scaffold performance in tissue engineering and should be explored in further investigations.

Conclusions

This study confirmed that immersing the swim bladder in 75% ethanol for at least 30 min can effectively disinfect the material without com-

promising its biocompatibility, hemocompatibility, ECM integrity, or mechanical properties. Although ethanol immersion may temporarily increase tissue stiffness and tensile strength due to dehydration, these changes are largely reversible after rinsing with PBS, with mechanical properties approaching those of fresh tissue. The preservation of key ECM components and the compliance with ISO standards for cell compatibility and hemocompatibility highlight the applicability of 75% ethanol disinfection for preparing swim bladder tissue as a cardiovascular biomaterial. These findings also support the broader application of 75% ethanol disinfection in tissue engineering and regenerative medicine.

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Disclosure of conflict of interest

None.

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