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Original Article

Effects of Short-Duration Ethanol Dehydration on Mechanical Properties of Porcine Pericardium

Tomoya Inoue, MD,[1] Keiichi Kanda, MD, PhD,[1] Masashi Yamanami, MD, PhD,[1] Daisuke Kami, PhD,[2] Satoshi Gojo, MD, PhD,[2] and Hitoshi Yaku, MD, PhD[1]

Purpose: Autologous pericardium is an ideal material for cardiovascular reconstruction including pulmonary artery plasty. Despite the fact that dehydration by ethanol has been used to improve its surgical handling, the effects of the ethanol on mechanical properties of the pericardium have not been previously investigated. The effects of short-duration ethanol dehydration on the mechanical properties of porcine pericardium were evaluated.
Methods: Porcine pericardia (n = 3) were separated into three groups: the raw group with no treatments (RAW), the group immersed in 70% ethanol for 10 min (ET group), and the group immersed in 0.6% glutaraldehyde for 10 min (GA). We measured five parameters of mechanical properties as specified in ISO 7198.
Results: ET treatment improved surgical handling as well as GA treatment. There were no significant differences in burst pressure (*P* = 0.639), suture retention strength (*P* = 0.529), ultimate tensile strength (UTS; *P* = 0.486), or Young's modulus (*P* = 0.408). Only the ultimate strain of the GA group was significantly higher among the three groups (RAW: 33.34% ± 2.02%, ET: 37.48% ± 1.84%, GA: 44.74% ± 2.87%; *P* = 0.046).
Conclusions: Short-duration ethanol dehydration did not compromise its mechanical properties while maintaining its surgical handling improvements.

Keywords: porcine pericardium, ethanol treatment, glutaraldehyde treatment, mechanical property, surgical handling

[1]*Department of Cardiovascular Surgery Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Kyoto, Japan*
[2]*Regenerative Medicine, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Kyoto, Japan*

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Corresponding author: Keiichi Kanda, MD, PhD. Department of Cardiovascular Surgery, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, 465 Kajiicho, Kamigyo-ku, Kyoto, Kyoto 602-8566, Japan
Email: kei@koto.kpu-m.ac.jp

Introduction

Various materials, including homografts, xenografts, and artificial vascular grafts, have been developed as substitutes for cardiovascular reconstruction. Unfortunately, many of them frequently induce calcifications, severe intractable infections, immunological reactions, pseudoaneurysm formation, or stenosis because of their inability to adapt to patient growth in children. Therefore, autologous vessel wall or pericardium has been the primary choice, especially for children because it does not induce immunological reactions. Currently, autologous pericardium is frequently used for aortic valvuloplasty, even in adults.[1)] Ideally, autologous pericardium should be used in its raw condition. However, because raw pericardia are sometimes difficult to handle, they are commonly subjected to chemical pretreatments, including crosslinking by glutaraldehyde (GA).[1] Despite the fact that it is not yet commonly used, another possible option is dehydration by ethanol (ET).[2] A previous study showed that GA crosslinking induced changes in the mechanical properties of the pericardia.[3] Nevertheless, the effects of the ET dehydration on mechanical properties of the pericardium have not yet been measured. Therefore, the objective of this study was to determine the effects of short-duration ET dehydration on the mechanical properties of porcine pericardium from the practical viewpoint of cardiovascular surgeons.

Materials and Methods

Preparation and chemical treatment of porcine pericardium samples

The experimental procedures and study protocol (no. M30-31) were approved by the Animal Experiment Ethics Committee of the Kyoto Prefectural University of Medicine (Kyoto, Japan).

Porcine pericardia (animal age: 6 months, body weight [BW]: 110–120 kg, n = 3) were purchased from Tokyo Shibaura Zouki Co., Ltd (Tokyo, Japan). The pericardial specimens were frozen immediately after harvest and were kept at –20°C condition during delivery. All the samples were stored in –20°C before experiments.

After natural thawing at room temperature, superficial fat was surgically removed, and the samples were then placed on a cutting board with natural tension. Each pericardium was cut into circles with diameters of 12 mm (n = 9) and strips with widths of 5 mm and lengths of 30 mm (n = 27) in random directions.

The samples were randomly divided into three groups: a group was maintained in saline with no treatment (RAW group), one was immersed in 70% ET for 10 min (ET group), and another was immersed in 0.6% GA for 10 min (GA group) at room temperature. After each treatment, they were rinsed in saline three times for 3 min to wash out the remaining chemicals. To maintain wet conditions, all the samples were stored in saline at room temperature before examination.

We measured five parameters of mechanical properties: burst pressure, suture retention strength, ultimate tensile strength (UTS), Young's modulus, and ultimate strain.

Burst pressure measurements

Pericardia trimmed to form round membranes with diameters of 12 mm (n = 3 for each treatment) were mounted in a sheet-sample folder (**Fig. 1a**) with a 6-mm hole for pressure loading and maintained wet in saline. A custom pressure measurement circuit (**Fig. 1b**) consisting of a pressurizing inflation device (Indeflator 1000186; Abbott Vascular, CA, Redwood City, USA), a sheet-sample folder, a waterproof digital pressure sensor (PSE560-01; SMC, Tokyo, Japan), and an analogue data logger (U3-HV; LabJack Corporation, Lakewood, CO, USA) was connected via USB to a computer for data sampling, with a rate of 100 Hz. The pressure was loaded at a rate of 600–800 mmHg/sec until failure. Only the samples in which rupture occurred at a location away from the edge of the folder holes were selected (**Fig. 1c**).

Suture retention strength test

Suture retention strength tests were conducted as specified in ISO 7198.[4] The bottom end of the pericardium strip 15 mm in length (n = 6 for each treatment) was clamped using a test-piece grasping device with 5-mm bite (the length of the remaining free pericardium is 10 mm). A single 2-mm bite of a 5-0 Prolene suture with BB-1 needle (Ethicon Inc., Somerville, NJ, USA) was placed at the top end of the pericardium (**Fig. 1d**) and pulled up at a constant speed of 100 mm/min. The force curve was recorded using a uniaxial tensile tester (EZ-SX 100N; Shimadzu, Kyoto, Japan) until failure. The maximal value before failure was defined as suture retention strength. The tissue samples were placed in a specially designed water chamber (**Fig. 1e**) filled with saline that maintained the test-pieces in a liquid environment.

Uniaxial tensile test

Both ends of the pericardium strips (30 mm in length, n = 6 for each treatment) were clamped with test-piece grasping devices (**Fig. 1f**). The length between grasping devices was pre-set at 10 mm. All the actual minimal widths of the strips were measured using digital Vernier calipers to convert the value to 5-mm equivalents. The upper test-piece grasping device was then pulled up at a rate of 100 mm/min. The force curve was recorded until tissue failure in wet conditions using the same apparatus used for the suture retention testing. It was verified that failure occurred in the middle of the tissue away from the test-piece grasping devices. From the recorded force curve, three mechanical parameters were determined: UTS, which is the maximal tension divided by the

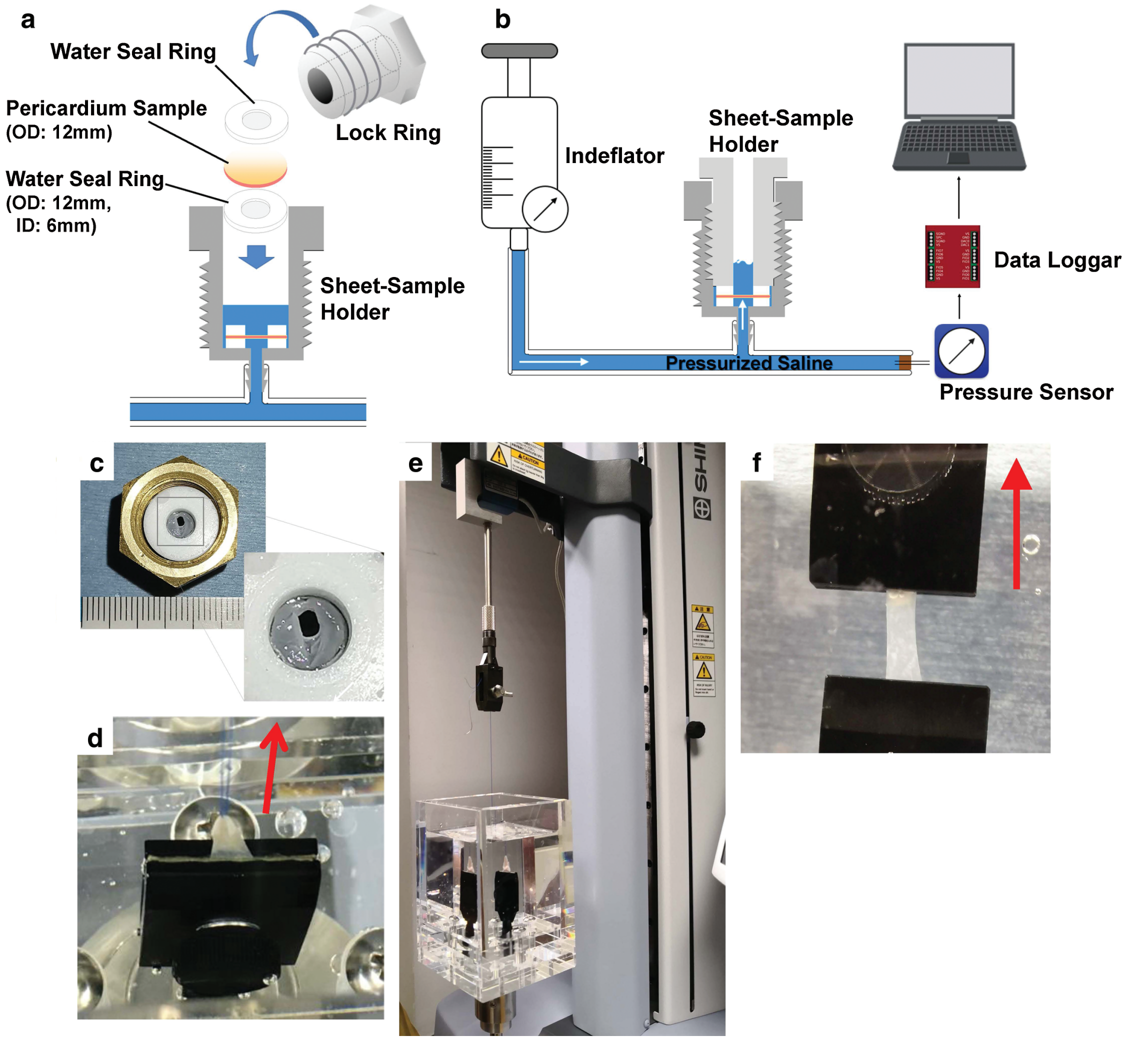

Fig. 1 Schematic drawings of a sheet-sample holder (**a**) and entire circuit appearance (**b**) for burst pressure measurement. (**c**) Example of a ruptured sample. (**d**) Sample of a suture retention strength test held in saline using the test-piece grasping device. A 5-0 Prolene suture was placed at the top end of the pericardium. (**e**) Entire appearance of the system including the specially designed water chamber for the suture retention strength test. (**f**) Strip of pericardium held by two grasping devices.

cross-sectional area of the tissue during the recording (MPa = N/mm^2); the Young's modulus, which was obtained from the maximal slope of the stress–strain curve (MPa); and ultimate strain, which was obtained from the elongated length at the maximal tension divided by the initial length (%).

Statistical Analyses

Results are expressed as mean ± standard error (SE). The statistical significances of the differences were evaluated by analysis of variance using SPSS Statistics version 24 (IBM Corp., Armonk, NY, USA), with P <0.05 considered significant.

Results

Macroscopic changes in the pericardial tissues before and after chemical modification

In the surgical handling tests, three cardiovascular surgeons evaluated the ease of confirming, trimming, and suturing the pericardial edge by cutting out the treated pericardia. Objective comparison of surgical handling among various types of chemical treatments is difficult. Nevertheless, macroscopically, the RAW group had difficulty in maintaining the flat wall edge shape with bending, rolling, and shrinkage (**Fig. 2a**). The ET (**Fig. 2b**) and GA groups (**Fig. 2c**) kept their

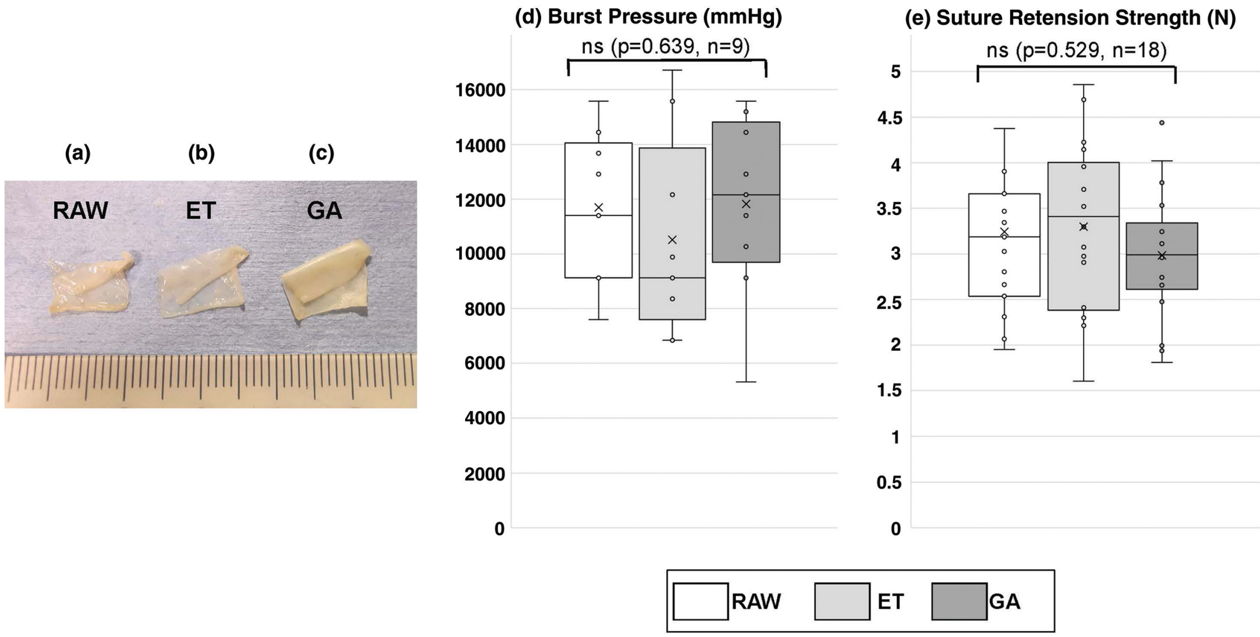

Fig. 2 Macroscopic appearance of the porcine pericardia (**a**) before (RAW) and after (**b**) ET and (**c**) GA treatment and results of the (**d**) burst pressure and (**e**) suture retention strength tests of the three groups. ET: ethanol; GA: glutaraldehyde; ns: no statistical differences; X: mean value.

flat edge shape well. They were very easy to size, cut, and suture. The handling improvement effect was considered to be appropriate in both chemical treatments.

Burst pressure measurements

There were no significant differences in burst pressure among the three groups (RAW: 11696 ± 899.64 mmHg, ET: 10513 ± 1095.0 mmHg, GA: 11822 ± 1092.3 mmHg; $P = 0.639$). The minimal burst pressure of the RAW, ET, and GA groups were 7600, 6840, and 5320 mmHg, respectively, all of which were high enough to serve as cardiovascular substitutes (**Fig. 2d**).

Suture retention strength test

There was no significant difference in suture retention strength among the three groups (RAW: 3.242 ± 0.261 N, ET: 3.298 ± 0.214 N, GA: 2.985 ± 0.165 N; $P = 0.529$; **Fig. 2e**).

Uniaxial tensile test

The UTS was calculated including the thickness of the specimen acquired from the light microscope measurement (0.129 ± 0.0029 mm). There were no significant differences in UTS among the three groups (RAW: 18.238 ± 1.987 MPa, ET: 19.104 ± 2.234 MPa, GA: 16.059 ± 1.233 MPa; $P = 0.486$; **Fig. 3a**) or Young's modulus (RAW: 49.64 ± 0.551 MPa, ET: 45.44 ± 7.19 MPa, GA: 39.21 ± 2.88 MPa; $P = 0.408$; **Fig. 3b**). Only the ultimate strain of the GA group was significantly higher among the three groups (RAW: 33.34% ± 2.02%, ET: 37.48% ± 1.84%, GA: 44.74% ± 2.87%; $P = 0.046$; **Fig. 3c**).

Discussion

Historically, autologous pericardia have been widely used for the reconstruction of cardiovascular tissues, including pulmonary arteries, septal defects, and valvular leaflets. Artificial vascular grafts (e.g., polyester fiber grafts, polytetrafluoroethylene) can induce intractable infections.[5] Furthermore, xenogeneic grafts were reported to form pseudoaneurysms or calcifications.[6] Therefore, especially for child patients, autologous vessel wall or pericardium is the primary choice material for cardiovascular reconstruction. Moreover, autologous pericardia have recently been used for valvular leaflet reconstruction in adult patients when avoiding prosthetic valvular replacement, such as women who wish to bear children.[1]

Because raw pericardia are often difficult to handle because of their inability to maintain their flat shape especially at the edges, several types of chemical treatments such as crosslinking by GA are applied to improve their surgical handling.[1] A previous study reported that such

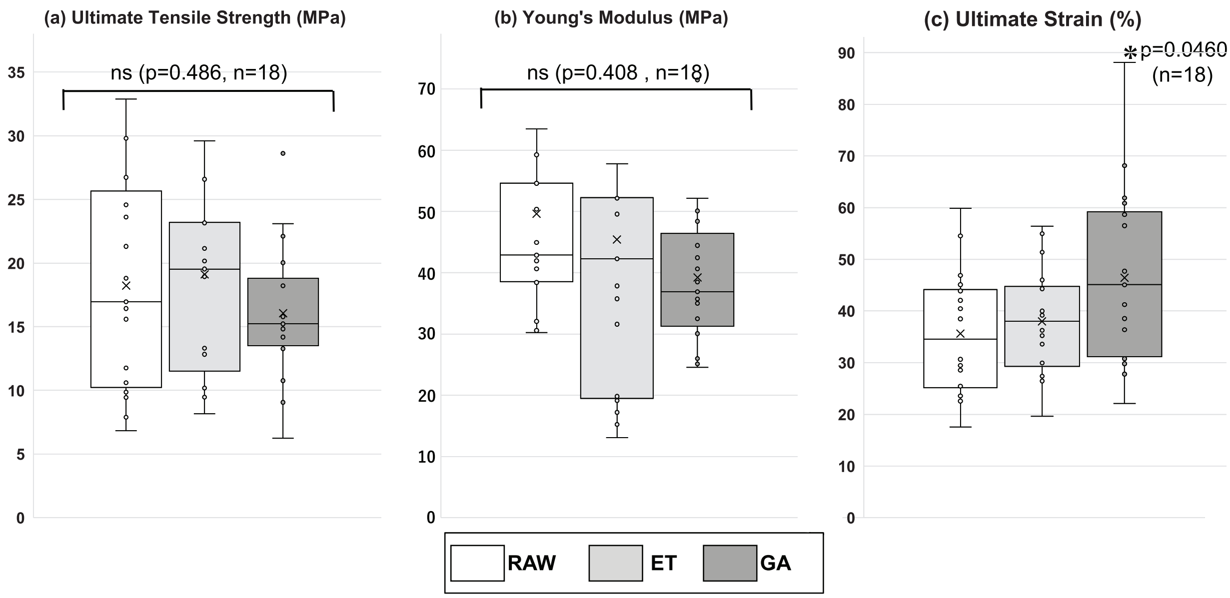

Fig. 3 Results from uniaxial tensile test: (**a**) ultimate tensile strength, (**b**) Young's modulus, and (**c**) ultimate strain of the three groups. RAW: raw group with no treatments, ET: group immersed in ethanol, GA: group immersed in glutaraldehyde, ns: no statistical differences, X: mean value. *The ultimate strain of the GA group was significantly higher than those of the RAW and ET groups.

chemical treatments induced changes in their mechanical properties.[7] Another possible option is dehydration using ET.[2] By macroscopic evaluation and actual surgical handling tests including suturing, ET treatment improved surgical handling as well as GA treatment even though it is very difficult to evaluate objectively. In the present study, we evaluated the effects of short-duration chemical treatments in terms of not only subjective surgical handling but also of objective mechanical properties. We measured various mechanical parameters according to ISO 7198, the global standard evaluation protocol of tubular vascular grafts and vascular patches,[4] including burst pressure, suture retention strength, UTS, Young's modulus, and ultimate strain. Among these five parameters, burst pressure and suture retention strength are considered most important from the practical view of cardiovascular surgeons. Nevertheless, they have been rarely evaluated in past studies of pericardia.

There were no significant differences in burst pressure among the three groups. The minimal burst pressure for all three groups was sufficiently high to serve as cardiovascular substitutes. Suture retention strength is another important parameter for cardiovascular surgeons because it directly influences the surgical procedure itself. There were no significant differences in suture retention strength among the three groups. These findings suggest that there is no concern for ET treatment regarding the strength of the pericardia in practical surgical procedures as well as for GA treatment.

We evaluated three other mechanical properties using uniaxial tensile testing. There were no significant differences in the UTS or Young's modulus. Only the ultimate strain of the GA group was significantly higher among the three groups. UTS can be converted to the theoretical burst pressure values using Laplace's relationship[8] because there were no significant differences in UTS values among the three treatments, the theoretical burst pressure was not altered by chemical treatment either. Prolonged GA treatment was reported to decrease flexibility.[3] If the Young's modulus was increased by chemical treatments, this corresponds to a decrease in compliance that might cause anastomotic problems due to the higher initial compliance mismatch of the treated pericardium as compared with that of the raw pericardium; however, concerns about this issue have decreased with short-duration chemical treatments. As a result, neither the short-duration ET treatment nor the GA treatment induced unfavorable changes in any of the five mechanical aspects. These findings suggest that adopting these chemical treatments might be an option from both the perspectives of macroscopic handling and mechanical properties. However, from the viewpoint of toxicity, GA has high retention and it is difficult to rinse thoroughly if the treatment is performed with higher concentrations or longer duration.[9] There are also clinical reports that GA toxicity lasts for long periods. Anastomotic problems due to tissue destruction by the toxicity of bovine serum albumin and glutaraldehyde glue have been reported.[10] Furthermore, it has been reported that GA-treated tissue grafts induced calcification reaction after transplantation.[6] Therefore, many surgeons currently use a lower concentration of GA with short-duration treatment.[1] Despite the fact that such GA treatment would not practically induce significant problems in terms of mechanical aspects, we currently select ET for standard pretreatment of autologous pericardium for its easier surgical handling and lower toxicity.[2] ET treatment is another traditional chemical treatment and is gradually prevailing; however, no previous studies have clearly described the differences between ET and GA treatments in terms of their effects on the mechanical properties of the pericardia. The comparison data of ET and GA treatment could be useful for the application of ET treatment. There is a need to be cautious in using ET-treated pericardium in high-pressure systems because ultimate strain % in ET-treated pericardium is lower than in GA-treated pericardium. We are currently using ET-treated pericardium only for reconstruction of the low-pressure system such as the plasty of pulmonary arteries. We will continue this series of experiments further with the aim to expand the applications.

Conclusions

Short-duration ET treatments of porcine pericardium did not compromise its mechanical properties while maintaining its surgical handling improvements. Proper chemical treatment could provide surgical handling improvements by maintaining the mechanical properties of the pericardium as a cardiovascular reconstruction material.

Study Limitations

There were some limitations of this study. We used porcine pericardium, which is easily available and commonly used in similar *in vitro* experiments, and those treated by GA are commonly used for bioartificial heart valvular leaflets clinically. Nevertheless, when human autologous pericardium is used clinically, its mechanical properties could be altered differently by chemical treatments. Because the results of our animal experiments might have implications for the selection of the ET treatment for human autologous pericardia used in cardiovascular surgery, a similar investigation using human pericardia is needed.

Second, although we evaluated the effects of chemical treatments from a mechanical point of view, the usefulness of the chemically treated pericardium as cardiovascular substitutes as well as its biological characteristics such as bioactivity and biocompatibility should be evaluated. Further studies are needed to investigate the effects of various chemical treatments on these aspects using animals and ongoing clinical implantations.[2] Another issue is the conditions of the ET and GA treatments. For example, the elasticity of the pericardia was altered depending on the GA treatment period.[3] Because we evaluated only one clinically relevant treatment condition each for ET and GA,[1,2] the effects of other conditions of chemical pretreatments, such as various concentrations, temperatures, and durations of ET treatments should also be evaluated to identify the ideal conditions. Finally, other pretreatments should also be evaluated; many researchers are investigating the use of decellularized pericardium,[11] whose mechanical property could be differently altered.

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Disclosure Statement

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

References

1) Ozaki S, Kawase I, Yamashita H, et al. A total of 404 cases of aortic valve reconstruction with glutaraldehyde-treated autologous pericardium. J Thorac Cardiovasc Surg 2014; **147**: 301–6.
2) Fujita S, Yamagishi M, Miyazaki T, et al. 2018. Leaflet-base-preserving truncal valve repair with ethanol-treated autologous pericardium. J Thorac Cardiovasc Surg 2018 Sep 28. ahead of print.

3) Hofferberth SC, Baird CW, Hoganson DM, et al. Mechanical properties of autologous pericardium change with fixation time: implications for valve reconstruction. Semin Thorac Cardiovasc Surg 2019; **31**: 852–4.
4) ISO 7198: 2016 Cardiovascular implants and extracorporeal systems - Vascular prostheses - Tubular vascular grafts and vascular patches.
5) Hasse B, Husmann L, Zinkernagel A, et al. Vascular graft infections. Swiss Med Wkly 2013 Jan 24; 143: w13754.
6) Jorge-Herrero E, Garcia Paez JM, Del Castillo-Olivares Ramos JL. Tissue heart valve mineralization: Review of calcification mechanisms and strategies for prevention. J Appl Biomater Biomech 2005; **3**: 67–82.
7) Yamashita H, Ozaki S, Iwasaki K, et al. Tensile strength of human pericardium treated with glutaraldehyde. Ann Thorac Cardiovasc Surg 2012; **18**: 434–7.
8) Nieponice A, Soletti L, Guan J, et al. Development of a tissue- engineered vascular graft combining a biodegradable scaffold, muscle-derived stem cells and a rotational vacuum seeding technique. Biomaterials 2008; **29**: 825–33.
9) Neethling WM1, Glancy R, Hodge AJ. Mitigation of calcification and cytotoxicity of a glutaraldehyde-preserved bovine pericardial matrix: improved biocompatibility after extended implantation in the subcutaneous rat model. J Heart Valve Dis 2010; **19**: 778–85.
10) Luk A, David TE, Butany J. Complications of Bioglue postsurgery for aortic dissections and aortic valve replacement. J Clin Pathol 2012; **65**: 1008–12.
11) Meyer M. 2019 Processing of collagen based biomaterials and the resulting materials properties. Biomed Eng Online. 2019; **18**: 24.