Regenerative Therapy 26 (2024) 2–8

Contents lists available at ScienceDirect

Regenerative Therapy

journal homepage: http://www.elsevier.com/locate/reth

Original Article

High pressure pasteurization: Simultaneous native tissue decellularization and sterilization

Akihisa Otaka [a], Takashi Yamamoto [b], Tetsuji Yamaoka [a, *]

[a] Department of Biomedical Engineering, National Cerebral and Cardiovascular Center Research Institute, 6-1 Kishibe-Shimmachi, Suita, Osaka, 564-8565, Japan
[b] Research & Development, JMS Co. Ltd, 12-17 Kako-machi, Naka-ku, Hiroshima, Japan

ARTICLE INFO

Article history:
Received 20 October 2023
Received in revised form
21 December 2023
Accepted 25 January 2024

Keywords:
Biomaterials
Xenografts
Decellularization
High hydrostatic pressure
Sterilization

ABSTRACT

Introduction: Terminal sterilization is important for the clinical applicability of decellularized xenografts. High hydrostatic pressurization (HHP) process is a potential strategy for decellularization and decontamination of xenografts; however, its disinfection efficiency remains poorly elucidated. This study investigated the disinfection efficacy of the HHP process at physiologically relevant 36 °C against difficult-to-kill spore-forming bacteria.

Methods: *Bacillus atrophaeus* and *Geobacillus stearothermophilus* were suspended in a pressurization medium with or without antibiotic agents and pressurized under two different HHP procedures: repeated and sustained pressurization.

Results: The sustained pressurizing conditions, exploited for the conventional tissue decellularization, did not effectively eliminate the bacteria; however, repeated pressurization greatly increased the disinfection effect. Moreover, the antibiotic-containing pressurization medium further increased the disinfection efficiency to the level required for sterilization.

Conclusions: The optimized high hydrostatic pressurization can be used to sterilize biological tissues during the decellularization process and is a promising strategy for manufacturing tissue-derived healthcare products.

1. Introduction

Due to an extreme shortage of human organ donors, reconstructive transplantation surgery is limited. Thus, tissue engineering, specifically developing grafts using decellularization, has gained considerable interest [1–3]. Several decellularized xenotransplantation products have been produced from the dermis [4], bones [5], heart valves [6], and blood vessels [7]. More than 50 xenotransplantation products have been commercially available for clinical applications [2]. However, tissue-derived biomaterials are associated with several disadvantages, including the potential risk of pathogenic infections [8,9].

Terminal sterilization is often used to minimize the risk of microbial contamination; however, each sterilization method is associated with method-specific application restrictions. Currently, there are no effective methods for decontaminating tissue-derived grafts. Autoclave or heat sterilization is not appropriate for the sterilization of tissue-derived products because heat results in protein denaturation as well as biochemical and biomaterial deterioration. Ethylene oxide (EtO) or radiation negatively impacts cell adhesion to the matrix protein [10], mechanical properties of osseous tissue [11], and growth factors required for osteoinductivity [12]. These drawbacks have narrowed the choice of materials used in healthcare products and prevented various tissue-derived biomaterials from entering the market.

Pasteurization, a mild heat (up to 70 °C) treatment of food products, has been used to extend shelf life and preserve vitamin and flavor compounds in vegetables, fruit juice, or milk. High hydrostatic pressure (HHP)-based pasteurization offers improved disinfection efficiency, even at lower temperatures, due to destruction of lipid bilayer of bacteria, fungi, and viruses [13].

* Corresponding author.
E-mail addresses: akihisa.otaka@ncvc.go.jp (A. Otaka), t-yamamoto@jms.cc (T. Yamamoto), yamtet@ncvc.go.jp (T. Yamaoka).

Peer review under responsibility of the Japanese Society for Regenerative Medicine.

https://doi.org/10.1016/j.reth.2024.01.012

Optimization of process conditions (pressure, temperature, and duration) can further eliminate bacteria with high efficiency [14–16]. In recent decades, several attempts have been made to disinfect natural or synthetic biomaterials (bone graft [14] or calcium phosphate ceramic [15]) via HHP.

We have shown that HHP treatment (200–1000 MPa) can eliminate immunogenic cells in cartilage [17], skin [18,19], and blood vessel [7,20,21] and produce decellularized xenografts. Collectively, HHP has the potential to decellularize and sterilize graft tissues simultaneously; however, its disinfection efficiency has been poorly elucidated. One concern is that spore-forming ubiquitous bacteria found in epithelial tissues (skin, gastrointestinal epithelium, and mucous membrane) can resist high pressure ranging from 400 to 690 MPa [22–24]. These previous studies proposed various improvements to achieve a higher disinfection efficiency by changing the pressurization buffer, temperature, and repeated pressurization. In this study, we aimed to evaluate the disinfection efficiency of conventional HHP-decellularizing by exposing several spore-forming bacteria to 1000 MPa pressure for 10 min. Additionally, we treated spore-forming bacteria with modified HHP treatments under modified pressurization media. In order to enhance the disinfection efficiency, two pressurization schemes were compared to determine whether extended duration (60 min-HHP) or repeated pressurization (six cycles of 10 min-HHP) improved disinfection efficiency. Finally, porcine tracheal tissue was processed in the optimized HHP protocol to check its decellularization efficiency.

2. Methods

2.1. General pressurization procedure

We investigated the effects of HHP on two bacterial species, *Bacillus atrophaeus* (*B. atrophaeus*) and *Geobacillus stearothermophilus* (*G. stearothermophilus*), which are proven indicators of dry heat and high-pressure steam sterilization, respectively. *B. atrophaeus* and *G. stearothermophilus* were obtained from Mesa Laboratories, Inc. (Bozeman, MT). The bacterial species (1×10^6 spores/mL) were separately suspended in a pressurization medium. The suspension (1 mL) was aliquoted into the bulb of disposable polyethylene dropper pipettes and sealed using a heat sealer (Fig. 1 and Fig. S1). The aliquot capsules were disinfected using glutaraldehyde (2% Sterisol Solution, TOYO Pharmaceutical Co., Ltd., Osaka, Japan), rinsed, and placed in the chamber of an isostatic pressurization machine (Dr. Chef; Kobelco, Hyogo, Japan). The chamber pressure was increased to 1000 MPa at 65.3 MPa/min, maintained for 10 or 60 min, and decreased to atmospheric pressure at 65.3 MPa/min (Fig. 2A). The chamber temperature was maintained at 36 °C. Next, the suspension was transferred into a conical tube containing 4 mL of culture medium (Trypcase Soy broth, bioMérieux S.A., Marcy-l'Etoile, France) or onto an agarose culture plate (1.5% agar with culture medium). *B. atrophaeus* and *G. stearothermophilus* were incubated at 37 °C and 55 °C, respectively.

2.2. Effect of pressurization medium

Spore-forming bacteria were suspended in 1 mL of pressurization medium, composed of pure water or saline with 10% w/v ethanol, urea, or dimethyl sulfoxide. Sodium hypochlorite (generated by NDX-1500PLB (OSG Corporation Co., Ltd., Osaka, Japan)) and acidic electrolyzed water (generated by ROX-10WB (Hoshizaki Inc., Aichi, Japan)) was also used as the pressurization media. After one round of pressurization for 10 min, each bacterial suspension was transferred into 4 mL of culture medium and incubated for up to 1 week, as described above (General pressurization procedure). Subsequently, cell concentration was measured by optical density (OD) measurement at 490 nm using a Colourwave CO7500 Colorimeter (BioChrom Ltd., Cambridge, UK).

2.3. Pressure optimization

Spore-forming bacterial water suspensions were processed using two different HHP procedures: repeated and sustained pressurization. In the repeated process, pressurization was repeated up to six times. The pressure was increased to 1000 MPa, maintained for 10 min, and decreased to atmospheric pressure in each cycle. During the sustained process, the pressure was increased to 1000 MPa, maintained for 60 min, and decreased to atmospheric pressure. For liquid culture, the processed suspension was mixed with 4 mL of culture medium and incubated. The culture medium OD was measured at 490 nm for 7 days. For gel plate culture, the processed medium was transferred to a Luria-Bertani (LB) agar plate and incubated. Following incubation, the plaques were counted to calculate colony-forming units (CFUs).

2.4. Combined effect of pressurization and antibiotic agent

Bacterial cells were suspended in sterile penicillin-streptomycin (Sigma-Aldrich, St. Louis, MO)-containing pure water. Following the 6 time repeated pressurization process, the cell suspension was transferred to 4 mL of culture medium and incubated for two months. The culture medium OD was measured at 490 nm every 2–3 days. As a control, the same culture experiments were conducted on spore-forming bacteria treated in either HHP- or antibiotic-deficient conditions.

2.5. Decellularization of trachea

Porcine trachea was obtained from Tokyo Shibaura Zoki KK (Tokyo, Japan) and decellularized using HHP, as reported previously [25,26]. Tracheal tissues were cut into 2×2 cm^2 and packed in plastic bags with 1% penicillin-streptomycin (100 U/mL penicillin

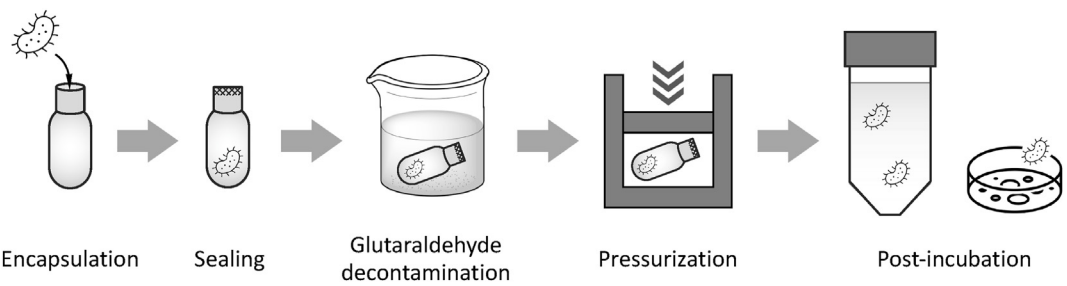

Fig. 1. High hydrostatic pressure treatment of spore-forming bacteria. Bacterial cells were encapsulated in a flexible bag and pressurized at 1000 MPa. Bacterial survival was evaluated using liquid or plate incubation.

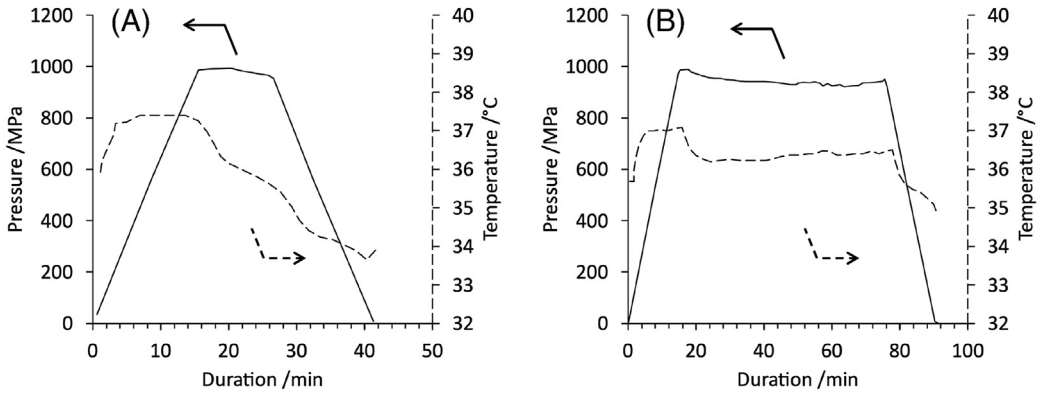

Fig. 2. Pressure and temperature in pressurization cycle. (A) 10 min- and (B) 60 min-high hydrostatic pressure.

and 100 μg/mL streptomycin; Sigma-Aldrich, St. Louis, MO) saline solution, sealed using a heat sealer. The bags were placed in the isostatic pressurization machine and pressurized once or 6 times, as described above (General pressurization procedure). The tissue was rinsed in a DNace buffer containing 40,000 U/L DNase I (Roche Diagnostics GmbH, Mannheim, Germany) and 20 mM $MgCl_2$ in saline for 2 weeks and in 2 mM ethylenediaminetetraacetic acid (EDTA·2Na) saline solution for subsequent 2 weeks. The specimens were stained with hematoxylin and eosin (HE).

2.6. Statistical analysis

All statistical comparisons were performed using Welch's ANOVA test, followed by Games-Howell post-hoc test ($\alpha = 0.05$).

3. Results

3.1. Effect of pressurization medium

Bacillus atrophaeus and *G. stearothermophilus* were pressurized in different pressurization media and subsequently incubated in culture medium. It takes 20 or 28 h for one individual *B. atrophaeus* or *G. stearothermophilus* to make a culture medium OD (490 nm) of 0.1, respectively (Fig. S2). Therefore, bacterial survival can be detected by the OD value of 2 days post-incubation. In the presence of 10% urea or dimethyl sulfoxide, the culture medium became translucent after 1 day of incubation, indicating that the HHP process and denaturing agents did not eliminate the spore-forming bacteria (Table 1 and Fig. S3). Similarly, in the presence of 10% ethanol, the culture medium became translucent 2 days following incubation, indicating that ethanol could not eliminate the bacteria either. Sodium hypochlorite or acidic electrolyzed water inhibited bacterial growth 7 days post-incubation (Table 1). Notably, even in the absence of HHP, these media displayed high disinfection efficiencies (data not shown), and therefore are promising decontamination agents before xenograft decellularization processes.

Table 1
Effect of pressurization buffer. Appearance of the post-incubation medium (C: clear; T: translucent) of *Bacillus atrophaeus* pressurized in saline or water containing ethanol (EtOH), urea, dimethyl sulfoxide (DMSO), or sodium hypochlorite or acidic electrolyzed water (NaClO).

	10% EtOH		10% Urea		10% DMSO		Saline	Water	NaClO
	Saline	Water	Saline	Water	Saline	Water			
Day 1	C	C	T	T	T	T	T	T	C
Day 2	T	T	T	T	T	T	T	T	C/T
Day 3	T	T	T	T	T	T	T	T	C/T
Day 7	T	T	T	T	T	T	T	T	C/T

3.2. Pressure optimization

We compared the disinfection efficiency of different HHP procedures: repeated 10 min-HHP pressurization (Fig. 2A) and continuous 60 min-HHP pressurization (Fig. 2B). An aqueous suspension (1×10^6 cells) was pressurized using a modified procedure and spread on a culture plate for the colony-forming assay. Fig. 3 depicts the log reductions in *B. atrophaeus* and *G. stearothermophilus* after each HHP treatment. *B. atrophaeus* and *G. stearothermophilus* survival decreased with the 10 min-HHP cycle number. The evaluation of OD also confirmed that multiple pressurization treatments effectively eliminated spore-forming bacteria, as the turbidity of the medium decreases with increased HHP cycle number (Table S1). The continuous 60 min-HHP pressurization process revealed a disinfection efficiency equivalent to that of one 10 min-HHP and lesser efficiency than the repeated HHPs. Thus, compared to pressurization duration, pressure fluctuation had a greater impact on disinfection efficiency. However, the repeated pressurization process failed to achieve more than 6 log reduction of spore-forming bacteria.

3.3. Combined effect of pressurization and antibiotic agent

The effects of penicillin and streptomycin concentration on the culture media containing 1×10^6 spore-forming bacteria was investigated. The media transparency persisted longer as the antibiotics increased (Fig. S4). In the following experiment, we chose the concentration of 1 U/mL penicillin and 1 μg/mL streptomycin, where the antibiotic did not eliminate the bacteria completely, and the medium became translucent by day 23 of incubation.

The spore-forming bacteria were subjected to the six cycle-pressurization in the presence of 1 U/mL penicillin and 1 μg/mL streptomycin and subsequently incubated in the culture medium. The OD value remained low for >20 days of post-incubation (HHP+/AB+ in Fig. 4A and B) in this condition; however, in the absence of antibiotics (HHP+/AB-) or HHP (HHP-/AB+), the OD value increased by day 2 or day 20, respectively. For longer period, only HHP+/AB+ condition maintained the clarity of culture medium for >100 days. Collectively, the results indicate that antibiotic agents enhanced disinfection efficiency of HHP to achieve more than 6 log reduction of spore-forming bacteria.

3.4. Decellularization of trachea

The porcine trachea was subjected to two different pressurization methods: the conventional (10 min-HHP once) and the modified protocol (six cycles of 10 min-HHP). HE stain of decellularized trachea revealed that all cells in the tracheal epithelium were enucleated in both conditions (Fig. 5). The decellularized

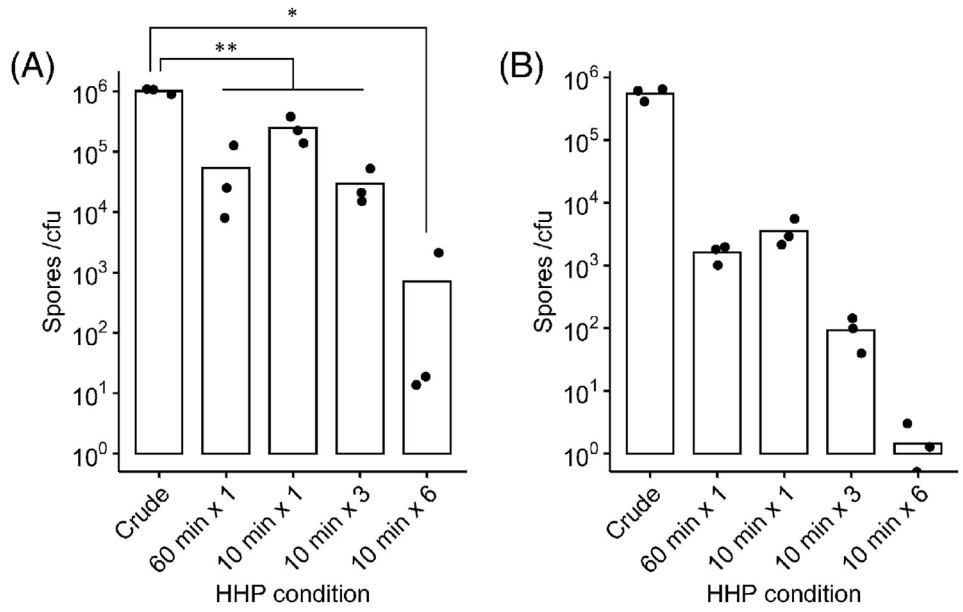

Fig. 3. Survival of (A) *Bacillus atrophaeus* and (B) *Geobacillus stearothermophilus* after pressurization treatment. Survival rates of each strain were tested after being subjected to different pressurization protocols. Colony forming units were measured and presented in logarithmic levels. Statistical significance was observed in both strains under Welch's ANOVA test. Only pairwise significance under Games-Howell post-hoc test was indicated with asterisks (*: $p < 0.05$; **: $p < 0.01$; $N = 3$).

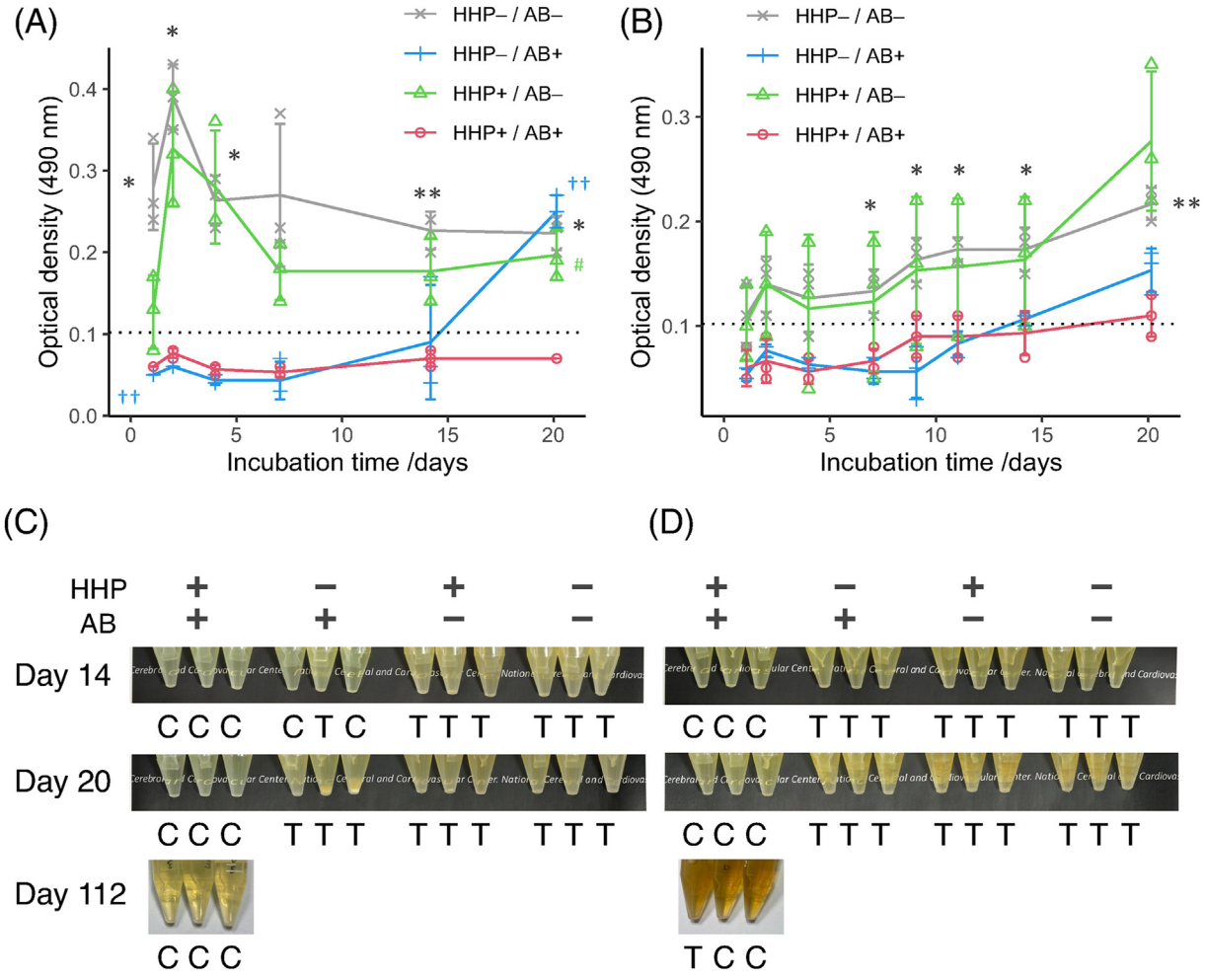

Fig. 4. Combined effect of repeated pressurization (HHP) and antibiotic agent (AB). Mean optical density (OD) at 490 nm of post-incubation medium with HHP-treated *Bacillus atrophaeus* (A) and *Geobacillus stearothermophilus* (B). Horizontal broken line indicates OD of native culture medium. Games-Howell post-hoc test assessed statistical differences in OD at each time point. Significance compared with HHP+/AB+ is denoted by * for HHP−/AB−, † for HHP−/AB+, and # for HHP+/AB− (*, #: $p < 0.05$; **, ††: $p < 0.01$; $N = 3$; mean ± SD). Transparency of post-incubation medium containing *Bacillus atrophaeus* (C) and *Geobacillus stearothermophilus* (D) was maintained for >100 days in HHP+/AB+ group. C: clear; T: translucent.

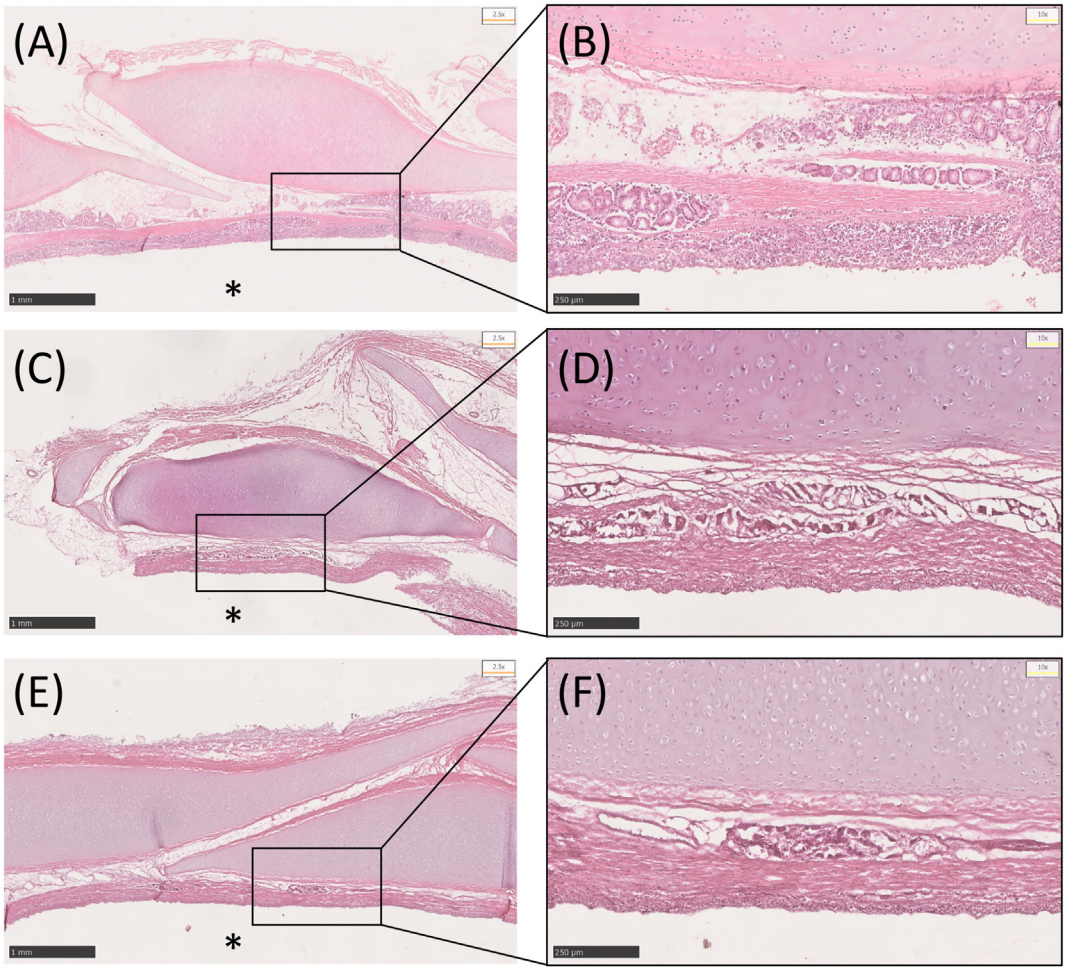

Fig. 5. HE stains of porcine trachea after HHP decellularization. Sagittal section of native trachea (A and B), trachea pressurized with 10 min-HHP once (C and D), and 6 times (E and F). Histological structure of tracheal wall was preserved in both HHP conditions. Asterisks indicate tracheal lumen. Bar: 1 mm (A, C, E). Tracheal epithelia were enucleated after pressurization. Bar: 250 μm (B, D, F).

tracheal wall showed no histological difference between the two conditions and was consistent with the previous histology reported in Ref. [25]. The modified protocol is applicable for trachea decellularization.

4. Discussion

Disinfection efficiency of spore-forming bacteria during HHP-based decellularization remains to be elucidated. This study showed that several spore-forming bacteria resisted conventional HHP process used in xenograft decellularization [7,21]. Therefore, we aimed to improve the disinfection efficiency of the process by increasing pressurization time (six cycles of 10 min-HHP). In addition, antibiotic agents in the decellularization medium further improved the efficiency. Sterilization of healthcare products requires a more thorough microorganism elimination than food products, such as fruit juice, jams, tofu, ham, and shellfish [23]. The concept of the sterility assurance level (SAL) is derived from kinetic studies on microbial inactivation, that is, the probability of viable microorganisms present on or inside a product unit after sterilization [27]. A SAL value of 10^{-6} is recommended for healthcare products, assuring that less than one out of a million contaminants survive on or inside the product following sterilization, as determined in ISO 13004:2022 or 11135:2014. HHP in the presence of antibiotics eliminated approximately 1×10^6 spore-forming bacteria. The disinfection efficiency can be improved further by combining other sterilization methods, for instance, rinsing graft tissues with sodium hypochlorite or acidic electrolyzed water before HHP decellularization. HHP-based decellularization in the presence of antibiotic agents will be a beneficial platform for simultaneous decellularization and sterilization of tissue-derived healthcare products.

In HHP pasteurization, several microbial suppression mechanisms have been proposed. The pressurization results in ascospore germination (waking bacteria up with spore-coat displacement), and the subsequent pressurization are intended to inactivate germinated ascospores [23,28]. Pressure fluctuation also induces the release of molecular components from the coat and core of *Bacillus subtilis* spores, leading to structural imperfections and thermal intolerance [29]. High pressure transiently disrupts the permeability of the *Escherichia coli* outer membrane for water-soluble agents [30]. Penicillin and streptomycin inhibit wall peptidoglycan synthesis by binding proteins inside the spore coat, such as penicillin-binding proteins and ribosomes. Collectively, repeated HHP processes may disrupt the coating structure, improve permeability, and enhance antibiotic integration, consequently eliminating the spore-forming bacteria.

HHP-based sterilization offers several benefits: (1) it does not damage the covalent bonds in biomolecules, (2) it can be performed in the presence of water, (3) the effect reaches the inner part of the

object, and (4) there is minimal energy loss and water pollution. Thanks to these features, HHP will be applicable to sterilize various healthcare products, such as decellularized xenografts, hydrogels, or functional biomolecules (peptide or antibody drugs). Currently, decellularized tissues are sterilized using chemicals, radiation, and ethylene oxide [2]. Chemical or radiation sterilization, such as gamma- or electron beam radiation induces polypeptide scission or molecular crosslinking, leading to the alterations in biomechanical and biomaterial properties of the final products [10,27]. EtO sterilization is limited by EtO gas penetrability; only a porous lyophilized products can be sterilized using this method. Moreover, EtO emissions from some commercial sterilizers contribute to elevated cancer risk for people living in nearby communities [31]. The U.S. Food and Drug Administration (FDA) has encouraged the development of novel sterilization methods to replace EtO. Overall, HHP treatment can overcome the disadvantages of conventional sterilization.

Several limitations must be addressed in the future study. First, further protocol optimization will be needed according to each specific application. As a preliminary assessment, this study revealed that the modified protocol has as high disinfection efficiency as the level of sterilization. Second, the effect of repeated pressurization on decellularized tissue was not fully elucidated. It is already known that the pressurization at 1000 MPa has minimal impact on the matrix and physical characteristics of graft tissue [21]; however, the impact of the repeated pressurization on the physical and regeneration properties of decellularized grafts remains for future experiments. The third concern is the remaining antibiotic agents, which might have increased the risk of antibiotic-resistant bacteria. Optimization of the process design to eliminate agents before shipping is required. Furthermore, additives such as L-alanine [24], lysozyme, nisin, and EDTA [30], enhanced HHP disinfection efficiency. Identifying agents that do not cause antimicrobial resistance is required.

5. Conclusions

The efficiency of HHP-based tissue decellularization for disinfection purposes has not been fully established yet. To investigate this, spore-forming bacteria were subjected to several HHP treatments. The conventional decellularization protocol is unable to achieve the required level for sterilization, with less than 3 log reduction in spore-forming bacteria. However, subjecting the bacteria to six cycles of 10-min HHP in the presence of antibiotics effectively improved the disinfection to meet the sterilization level, demonstrating that sterilization can be achieved by optimizing the HHP treatment conditions. In this optimized condition, the porcine trachea was successfully decellularized. The modified conditions simultaneously allow for the sterilization and decellularization of tissue-derived biomaterials. The optimized pressurization condition is a promising method to decellularize and sterilize graft tissues simultaneously and will provide a novel process of xenografts decellularization.

Funding

This work was supported by the MHLW Research on Regulatory Science of Pharmaceuticals and Medical Devices Program (Grant Number JPMH 22KC5001), the Japan Agency for Medical Research and Development (AMED) (Grant Number JP22bm1123003), the Japan Science and Technology Agency (CREST) (Grant Number JPMJCR22L5), and the Japan Health Research Promotion Bureau Research Fund for Young Investigators (Grant number 2022-Y-07).

Authors' contributions

Akihisa Otaka: Conceptualization, data curation, methodology, investigation, visualization, validation, funding acquisition, and writing—original draft. Takashi Yamamoto: Conceptualization, methodology, resources, and writing—review and editing. Tetsuji Yamaoka: Conceptualization, supervision, methodology, resources, funding acquisition, and writing—review and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank Ryo Shiotani from Kyoto University for his experimental assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.reth.2024.01.012.

References

[1] Zhang X, Chen X, Hong H, Hu R, Liu J, Liu C. Decellularized extracellular matrix scaffolds: recent trends and emerging strategies in tissue engineering. Bioact Mater 2022;10:15–31. https://doi.org/10.1016/j.bioactmat.2021.09.014.
[2] Capella-Monsonís H, Zeugolis DI. Decellularized xenografts in regenerative medicine: from processing to clinical application. Xenotransplantation 2021;28:1–27. https://doi.org/10.1111/xen.12683.
[3] Chen FM, Liu X. Advancing biomaterials of human origin for tissue engineering. Prog Polym Sci 2016;53:86–168. https://doi.org/10.1016/j.progpolymsci.2015.02.004.
[4] Troy J, Karlnoski R, Downes K, Brown KS, Cruse CW, Smith DJ, et al. The use of EZ Derm® in partial-thickness burns: an institutional review of 157 patients. Eplasty 2013;13:e14.
[5] Meyer S, Floerkemeier T, Windhagen H. Histological osseointegration of Tutobone®: first results in human. Arch Orthop Trauma Surg 2008;128:539–44. https://doi.org/10.1007/s00402-007-0402-z.
[6] Rieder E, Kasimir MT, Silberhumer G, Seebacher G, Wolner E, Simon P, et al. Decellularization protocols of porcine heart valves differ importantly in efficiency of cell removal and susceptibility of the matrix to recellularization with human vascular cells. J Thorac Cardiovasc Surg 2004;127:399–405. https://doi.org/10.1016/j.jtcvs.2003.06.017.
[7] Mahara A, Somekawa S, Kobayashi N, Hirano Y, Kimura Y, Fujisato T, et al. Tissue-engineered acellular small diameter long-bypass grafts with neointima-inducing activity. Biomaterials 2015;58:54–62. https://doi.org/10.1016/j.biomaterials.2015.04.031.
[8] Fishman JA, Scobie L, Takeuchi Y. Xenotransplantation-associated infectious risk: a WHO consultation. Xenotransplantation 2012;19:72–81. https://doi.org/10.1111/j.1399-3089.2012.00693.x.
[9] Fishman JA. Prevention of infection in xenotransplantation: designated pathogen-free swine in the safety equation. Xenotransplantation 2020;27:1–6. https://doi.org/10.1111/xen.12595.
[10] Matuska AM, McFetridge PS. The effect of terminal sterilization on structural and biophysical properties of a decellularized collagen-based scaffold; Implications for stem cell adhesion. J Biomed Mater Res Part B Appl Biomater 2015;103:397–406. https://doi.org/10.1002/jbm.b.33213.
[11] Boyce T, Edwards J, Scarborough N. Allograft bone: the influence of processing on safety and performance. Orthop Clin N Am 1999;30:571–81. https://doi.org/10.1016/S0030-5898(05)70110-3.
[12] Pekkarinen T, Hietala O, Jämsä T, Jalovaara P. Gamma irradiation and ethylene oxide in the sterilization of native reindeer bone morphogenetic protein extract. Scand J Surg 2005;94:67–70. https://doi.org/10.1177/145749690509400116.
[13] Rivalain N, Roquain J, Demazeau G. Development of high hydrostatic pressure in biosciences: pressure effect on biological structures and potential applications in Biotechnologies. Biotechnol Adv 2010;28:659–72. https://doi.org/10.1016/j.biotechadv.2010.04.001.
[14] Gollwitzer H, Mittelmeier W, Brendle M, Weber P, Miethke T, Hofmann GO, et al. High hydrostatic pressure for disinfection of bone grafts and biomaterials: an experimental study. Open Orthop J 2009;3:1–7. https://doi.org/10.2174/1874325000903010001.
[15] Brouillet M, Gautier H, Mièvegeville AF, Bouler JM, Merle C, Caillon J. Inactivation of Staphylococcus aureus in calcium phosphate biomaterials via isostatic compression. J Biomed Mater Res Part B Appl Biomater 2009;91:348–53. https://doi.org/10.1002/jbm.b.31408.
[16] Kalchayanand N, Sikes A, Dunne CP, Ray B. Factors influencing death and injury of foodborne pathogens by hydrostatic pressure-pasteurization. Food Microbiol 1998;15:207–14. https://doi.org/10.1006/fmic.1997.0155.

[17] Watanabe N, Mizuno M, Matsuda J, Nakamura N, Otabe K, Katano H, et al. Comparison of high-hydrostatic-pressure decellularized versus freeze-thawed porcine menisci. J Orthop Res 2019;37:2466–75. https://doi.org/10.1002/jor.24350.
[18] Morimoto N, Mahara A, Jinno C, Ogawa M, Kakudo N, Suzuki S, et al. The superiority of the autografts inactivated by high hydrostatic pressure to decellularized allografts in a porcine model. J Biomed Mater Res Part B Appl Biomater 2017;105:2653–61. https://doi.org/10.1002/jbm.b.33807.
[19] Jinno C, Morimoto N, Mahara A, Sakamoto M, Ogino S, Fujisato T, et al. Extracorporeal high-pressure therapy (EHPT) for malignant melanoma consisting of simultaneous tumor eradication and autologous dermal substitute preparation. Regen Ther 2020;15:187–94. https://doi.org/10.1016/j.reth.2020.09.003.
[20] Yamanaka H, Morimoto N, Yamaoka T. Decellularization of submillimeter-diameter vascular scaffolds using peracetic acid. J Artif Organs 2020;23:156–62. https://doi.org/10.1007/s10047-019-01152-0.
[21] Funamoto S, Nam K, Kimura T, Murakoshi A, Hashimoto Y, Niwaya K, et al. The use of high-hydrostatic pressure treatment to decellularize blood vessels. Biomaterials 2010;31:3590–5. https://doi.org/10.1016/j.biomaterials.2010.01.073.
[22] Moerman F. High hydrostatic pressure inactivation of vegetative microorganisms, aerobic and anaerobic spores in pork Marengo, a low acidic particulate food product. Meat Sci 2005;69:225–32. https://doi.org/10.1016/j.meatsci.2004.07.001.
[23] Pinto CA, Moreira SA, Fidalgo LG, Inácio RS, Barba FJ, Saraiva JA. Effects of high-pressure processing on fungi spores: factors affecting spore germination and inactivation and impact on ultrastructure. Compr Rev Food Sci Food Saf 2020;19:553–73. https://doi.org/10.1111/1541-4337.12534.
[24] Raso J, Góngora-Nieto MM, Barbosa-Cánovas GV, Swanson BG. Influence of several environmental factors on the initiation of germination and inactivation of Bacillus cereus by high hydrostatic pressure. Int J Food Microbiol 1998;44:125–32. https://doi.org/10.1016/s0168-1605(98)00130-5.
[25] Ohno M, Fuchimoto Y, Hsu HC, Higuchi M, Komura M, Yamaoka T, et al. Airway reconstruction using decellularized tracheal allografts in a porcine model. Pediatr Surg Int 2017;33:1065–71. https://doi.org/10.1007/s00383-017-4138-8.
[26] Ohno M, Fuchimoto Y, Higuchi M, Yamaoka T, Komura M, Umezawa A, et al. Long-term observation of airway reconstruction using decellularized tracheal allografts in micro-miniature pigs at growing stage. Regen Ther 2020;15:64–9. https://doi.org/10.1016/j.reth.2020.04.010.
[27] Dziedzic-Goclawska A, Kaminski A, Uhrynowska-Tyszkiewicz I, Stachowicz W. Irradiation as a safety procedure in tissue banking. Cell Tissue Bank 2005;6:201–19. https://doi.org/10.1007/s10561-005-0338-x.
[28] Billeaud C. High hydrostatic pressure treatment ensures the microbiologi-cal safety of human milk including bacillus cereus and preser-vation of bioactive proteins including lipase and immuno-proteins: a narrative review. Foods 2021;10. https://doi.org/10.3390/foods10061327.
[29] Akasaka K, Maeno A, Yamazaki A. Direct high-pressure NMR observation of dipicolinic acid leaking from bacterial spore: a crucial step for thermal inactivation. Biophys Chem 2017;231:10–4. https://doi.org/10.1016/j.bpc.2017.04.008.
[30] Hauben KJA, Wuytack EY, Soontjens CCF, Michiels CW. High-pressure transient sensitization of Escherlchia coli to lysozyme and nisin by disruption of outer-membrane permeability. J Food Protect 1996;59:350–5. https://doi.org/10.4315/0362-028X-59.4.350.
[31] Lynch HN, Kozal JS, Russell AJ, Thompson WJ, Divis HR, Freid RD, et al. Systematic review of the scientific evidence on ethylene oxide as a human carcinogen. Chem Biol Interact 2022;364:110031. https://doi.org/10.1016/j.cbi.2022.110031.