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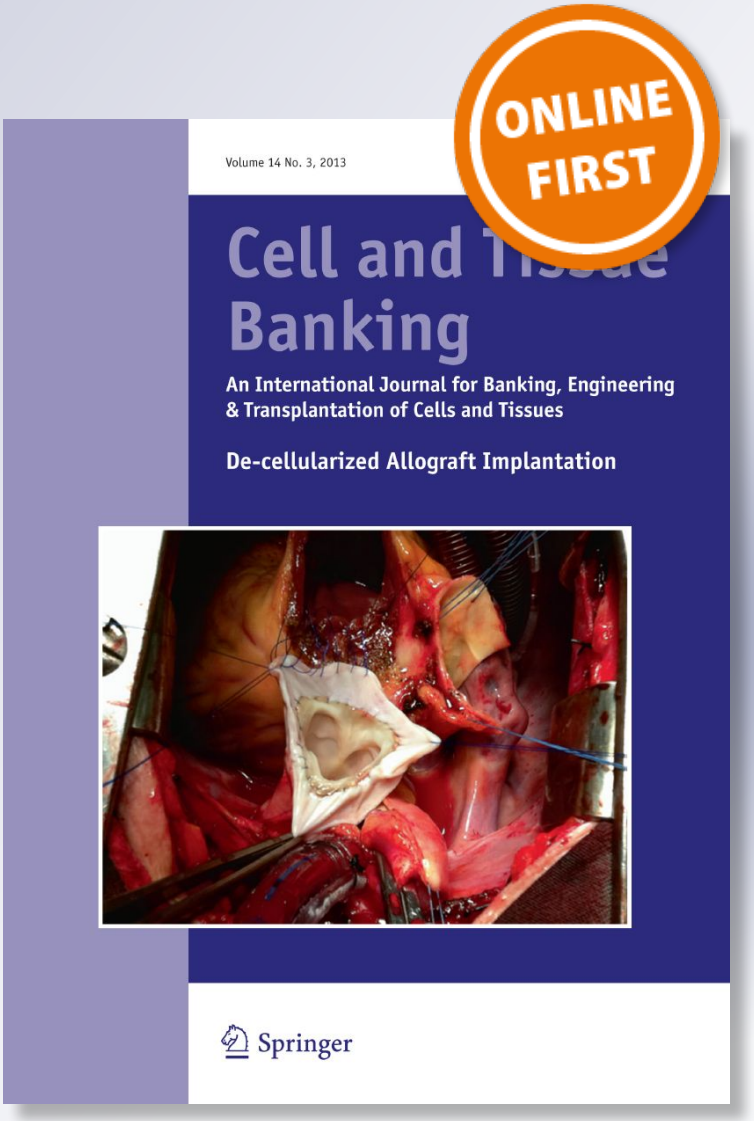

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Abstract Common terminal sterilization methods are known to alter the natural structure and properties of soft tissues. One approach to providing safe grafts with preserved biological properties is the combination of a validated chemical sterilization process followed by an aseptic packaging process. This combination of processes is an accepted method for production of sterile healthcare products as described in ANSI/AAMI ST67:2011. This article describes the validation of the peracetic acid and ethanol-based (PAAE) chemical sterilization process for allograft dermal tissues at the Musculoskeletal Transplant Foundation (MTF, Edison, NJ). The sterilization capability of the PAAE solution used during routine production of aseptically processed dermal tissue forms was determined based on requirements of relevant ISO standards, ISO 14161:2009 and ISO 14937:2009. The resistance of spores of *Bacillus subtilis*, *Clostridium sporogenes*, *Mycobacterium terrae*, *Pseudomonas aeruginosa*, *Enterococcus faecium*, *and Staphylococcus aureus* to the chemical sterilization process employed by MTF was determined. Using a worst-case scenario testing strategy, the *D* value was calculated for the most resistant microorganism, *Bacillus*. The 12*D* time parameter determined the minimum time required to achieve a SAL of 10^{-6}. Microbiological performance qualification demonstrated a complete kill of 10^6 spores at just a quarter of the full cycle time. The validation demonstrated that the PAAE sterilization process is robust, achieves sterilization of allograft dermal tissue to a SAL 10^{-6}, and that in combination with aseptic processing secures the microbiological safety of allograft dermal tissue while avoiding structural and biochemical tissue damage previously observed with other sterilization methods such as ionizing irradiation.

Keywords Aseptic processing · Human dermal tissue · Allograft · Chemical sterilization · SAL · *D* value

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Introduction

Allograft dermal tissues have a long history of clinical use for various applications including breast reconstruction, abdominal wall repair, and extremity surgery (Macadam and Lennox 2012). These tissues

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as Human Cellular and Tissue-Based Products (HCT/Ps) are regulated by both the US Food and Drug Administration (FDA) and the American Association of Tissue Banks (AATB). Central to the regulations on HCT/Ps, is the prevention of contamination and transmission of communicable diseases. For tissue processors, a demonstration that the operational environment and allograft processing steps are capable of assuring defined levels of microbiological control is paramount to ensuring safety of allograft dermal tissue. One component of tissue safety is the sterility of the allograft, where the term 'sterility' describes the state of being free from viable microorganisms. Validation of a process that results in a predefined sterility assurance level (SAL) is the approach of choice for demonstrating microbiological safety.

Although the FDA does not require a specific SAL for allograft dermal tissues, the standard expectation within the medical device industry is a SAL of 10^{-6}, where terminal sterilization (sterilization of product in the final packaging) is most commonly used (FDA 2011; Srun et al. 2012). Typical terminal sterilization methods, such as ethylene oxide or ionizing irradiation, alter allograft tissue properties resulting in poor clinical outcomes (Aspenberg et al. 1990; Tejwani et al. 2015). Therefore, sensitive biologic-based healthcare products, like allograft dermal tissue, pose a challenge in balancing sterility assurance and implant quality attributes (Matuska and McFetridge 2015; Mrazova et al. 2016). Recently, ANSI/AAMI ST67:2011 has recognized a risk-based approach for selecting alternative SALs for sterilization-sensitive products, including a SAL of 10^{-3} for tissue-based implants, as well as other methodologies for achieving sterility besides terminal sterilization. One approach within the ANSI/AAMI ST67 document describes a process that combines chemical sterilization and aseptic processing to obtain a sterile product.

The purpose of the studies described here was to demonstrate the sterilization capability of a patented process (Truncale et al. 2010) using a proprietary peracetic acid plus ethanol (PAAE) solution used for processing of allograft dermal tissues at the Musculoskeletal Transplant Foundation (MTF), while demonstrating minimal structural effect to the sterilized tissue. The study design for establishing the PAAE sterilization of allograft dermal tissue to a SAL 10^{-6} uses methods described in ISO 14937:2009. Microbial inactivation kinetics of the process are described, along with the validation of the full-scale process using a reduced exposure time or "overkill approach" as per ISO 14161:2009.

Materials and methods

Microorganism selection and preparation

Test microorganisms were selected to represent various microbial types: those with known resistance to chemical disinfectants/sterilants and other sterilization processes, as well as those found naturally on dermal tissue. To determine those that were most common on dermal tissue, microorganisms found in the transport solution of incoming dermal tissue donors over the course of 1 year were classified; see Fig. 1.

In an effort to represent the most frequently occurring microorganisms found in dermal tissue transport solution, and also include a broad spectrum of different types of bacteria (Gram negative, Gram positive, spore-formers), the following microorganisms were selected for use in the studies reported here: *Bacillus subtilis* subsp. *spizizenii or Bacillus atrophaeus* (ATCC® 6633 or ATCC® 9372), *Clostridium sporogenes* (ATCC® 11437), *Mycobacterium terrae* (ATCC® 15755), *Pseudomonas aeruginosa* (ATCC® 9027), *Staphylococcus aureus* (ATCC® 6538), and *Enterococcus faecium* (ATCC® 700221).

Microorganisms were purchased commercially and cultured per conditions described in Table 1. Microorganism solutions used for inoculation were standardized by photometry to 1×10^8 colony-forming units (CFU)/ml, and diluted in sterile phosphate-buffered water to yield a population of $\sim$1.0 to 3.0×10^7 CFU/ml. For the Neutralization Validation, the organisms were diluted to yield a final population of $\leq$100 CFU.

Study design and validation strategy

The study design was based on the guidance/requirements concerning sterilizing agent characterization (microbicidal effectiveness, ME) and microbiological performance qualification (MPQ) detailed in ISO 14937:2009 and ISO 14161:2009 (Annex F). The ME study demonstrates the lethal action of the PAAE

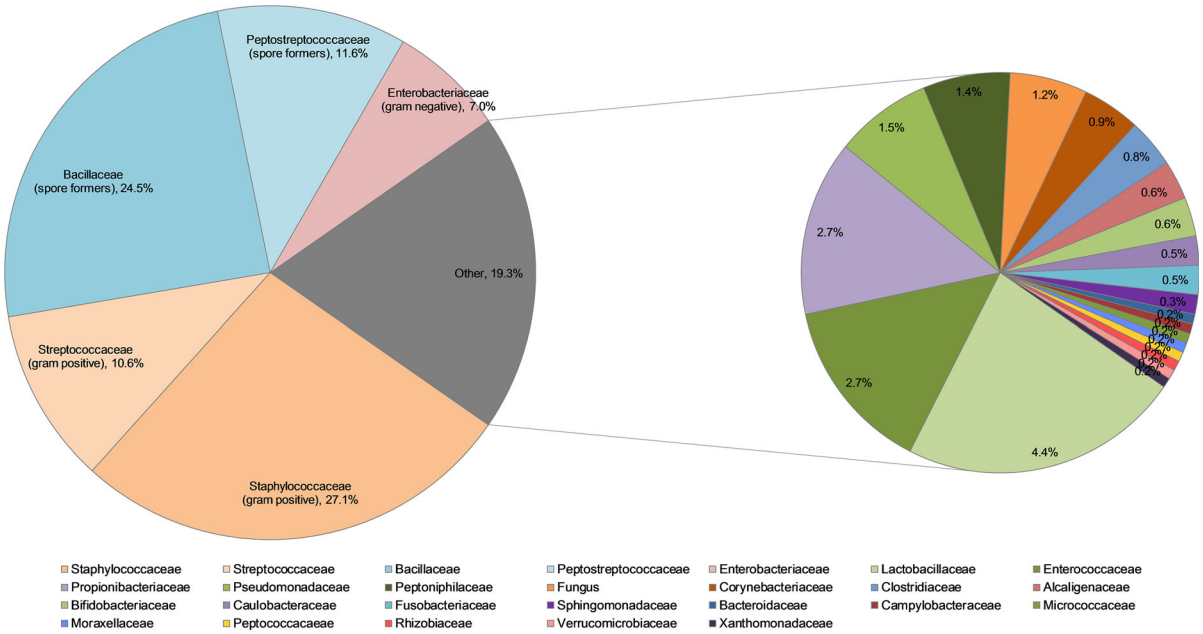

Fig. 1 Distribution of microbial families found in unprocessed dermal tissues (in dermal transport solution). Distribution shows that the majority (~80%) are comprised of bacteria residing in five families representing spore formers, gram positive, and gram negative bacteria. The most frequently isolated species were found within the *Bacillaceae* and *Staphylococcaceae* families. The legend lists bacterial families from highest to lowest percentage of occurrence (*left* to *right*, and *down*)

Table 1 Microorganism incubation and culture conditions

Microorganism	Culture medium	Incubation conditions
Bacillus subtilis subsp., *spizizenii*	Soybean casein digest broth	2–3 days, 35–39 °C
Bacillus atrophaeus	Soybean casein digest broth	2–3 days, 35–39 °C
Mycobacterium terrae	Middlebrook 7H11 agar	7–14 days, 35–39 °C, CO_2 conditions
Clostridium sporogenes	Reinforced clostridial agar	2–3 days, 35–39 °C, anaerobic conditions
Staphylococcus aureus	Soybean casein digest broth	2–3 days, 35–39 °C
Pseudomonas aeruginosa	Soybean casein digest broth	2–3 days, 35–39 °C
Enterococcus faecium	Soybean casein digest broth	2–3 days, 35–39 °C

solution against a representative range of microorganisms and defines the microbial inactivation kinetics and *D* value. The MPQ part of the study demonstrates that, on application of the sterilization process, the specified requirement for sterility will be met—a SAL of 10^{-6} or better.

For both the ME and MPQ studies, "worst case" test conditions were used for selected process parameters. The PAAE solution was formulated at a lower concentration than used routinely. Table 2 describes the test parameters and rationale for their selection.

All studies were conducted by a third-party laboratory facility (Wuxi Apptec, Marietta, GA).

Preparation of dermal tissue

For the ME and MPQ studies, 2 mm thick sections (3 cm × 5 cm dimensions) were recovered from four dermal tissue donors. The sections were cleaned and decellularized with NaCl and Triton-X 100 solutions, and then shipped frozen to the third-party laboratory. The dermal tissue sections were stored at −70 °C until time of use and thawed prior to inoculation. For the

Table 2 Test parameter selection summary

Parameter	Routine condition	ME kinetics study	Microbial performance qualification	Rationale
Material	Decellularized dermal tissue up to 2 mm thick	2 mm full thickness decellularized dermal tissue	2 mm full thickness decellularized dermal tissue	Use of thick-cut full thickness tissue is a challenge to solution permeation as it is denser by virtue of thickness and including the dermal–epidermal junction and superficial region of dermis
Temperature	Ambient temperature, 20–25 °C	Ambient temperature, 20–25 °C	Ambient temperature, 20–25 °C	Routine production takes place in ambient temperature
Sterilant age (days)	<72 h	>72 h, <96h	>144 h	Peracetic acid is known to decrease in potency overtime. PAA is not used
Tissue to volume ratio (cm^2/ml)	0.3 cm^2/ml	0.3 cm^2/ml	0.62 cm^2/ml	Maximizing tissue to volume ratio increases amount of organic load seen by sterilant
Exposure time (mins)	120 min (full cycle)	Various (between 1–10 min)	¼ cycle—30 min ½ cycle—60 min	Various time points in ME to determine kill kinetics. MPQ performed at both ¼ and ½ time to determine robustness
Agitation speed (rpm)	90 rpm	65 rpm	90 rpm	Agitation speed affects penetration and access of sterilant to tissue
Vacuum (in Hg)	22 in Hg	None	22 in Hg	Vacuum facilitates solution penetration through tissue by applying pressure gradient

MPQ study, an additional $\sim$5000 cm^2 of dermal tissue was obtained.

Preparation of the PAAE solution

The solution for the ME study was prepared from stock 35% peracetic acid (PAA) (Peroxychem, Philadelphia, PA) with 95% specially denatured alcohol (SDA-3C, Pharmco-AAPER, Shelbyville, KY) in USP purified water, to a final v/v concentration of peracetic acid at the lower limit of that used in routine processing of allograft dermal tissue. The solution was aged between 72 and 96 h prior to use ("aged" solution).

The solution for the MPQ study was the same formulation used in routine production but was "aged" for 144 h prior to use, a condition well beyond that specified in routine production (72 h).

Neutralization validation

Appropriate neutralization of the PAAE solution was verified prior to the ME and MPQ studies in accordance with the requirements of USP <1227>, "Validation of Microbial Recovery from Pharmacopeial Articles". Three sections of dermal tissue were placed in the PAAE solution for 15 s at a ratio of 0.3 cm^2 of tissue per ml of solution. The sections were then placed in Dey Engley medium with catalase and sonicated for 5 min followed by hand shaking for 1 min. Aliquots of the Dey Engley medium were removed and added to 100 ml of Fluid D (USP, 0.1% peptone, 0.1% polysorbate 80) and membrane filtered. During the final rinse, Fluid D was inoculated with <100 CFU of the test microorganism and membrane filtered. Membrane filters were then incubated under the conditions required for the particular test microorganism (Table 1) and counted.

Validity of the neutralization procedure was verified by comparing the microorganism recovery to positive controls in accordance with USP <1227>.

ME study methodology

Four dermal tissue sections from each of four separate donors were used per microorganism. The dermal-facing side of thawed dermal tissue allograft sections were individually inoculated with $\geq 10^6$ CFU of one of

each of the test microorganisms, incubated for 15 min at 4 °C to allow time for adherence to the tissue/drying of the inoculum, and exposed to the aged PAAE solution for 2 min. Tissue was exposed at a ratio of 0.3 cm^2 of tissue per ml of PAAE solution with worst-case test parameters of shaking at 65 rpm shaking and no application of vacuum at an average temperature of 23 °C (20–25 °C range) as described in Table 2. After exposure, the dermal tissue sections were neutralized and the extraction solution membrane filtered as previously described. Membrane filters were then placed on solidified agar with growth medium as described in Table 1, incubated and assayed for surviving CFUs. The microorganism demonstrating the highest CFU count of survivors (the least $\log_{10}$ reduction from initial concentration) after the 2-min exposure was selected for the inactivation kinetics study.

For the kinetics study, at least five exposure periods including time 0 (unexposed) and an estimated 4-$\log_{10}$ reduction time point (as recommended by ISO 14161) were utilized to generate an inactivation rate curve. Time periods tested were 1, 2, 2.5, 3, 6, and 10 min. After exposure, the dermal tissue sections were treated as above and assayed for surviving CFU.

The geometric mean of the surviving CFU count for each of the four dermal tissue sections (each section from a different donor) per exposure period was calculated using the equation:

$$\sqrt[4]{\log_{10}(\text{section1}) \times \log_{10}(\text{section2}) \times \log_{10}(\text{section3}) \times \log_{10}(\text{section4})} = \text{mean CFU per time point}$$

The slope of the best-fit rectilinear curve (m), D value, and regression coefficient of determination (r^2) for the most resistant organism, were calculated using mean CFU per time point and the survivor curve methodology described in ISO 14161:2009.

The D value or time required to reduce viable count by one $\log_{10}$ or 90% was calculated using the equation:

$$D = -1\left(\frac{1}{m}\right)$$

To determine validity of the regression, standardized residuals were analyzed for normality using the Anderson–Darling normality test ($p < 0.05$ was considered significant).

The time required for the process to achieve a SAL of 10^{-6} is $12 \times D$ (*12D*).

MPQ study methodology

Since the routine PAAE exposure time for allograft dermal tissues was 120 min, the MPQ study was performed with reduced extent of treatment times of 30 and 60 min using full-scale production type equipment used for routine processing. Fifteen dermal tissue sections were inoculated with $\geq 10^6$ CFU of the most resistant microorganism; they were folded in half and interleaved within 5000 cm^2 of dermal tissue in a stainless steel canister. A plastic tie wrap was used to hold the folded halves together.

The canister was sealed closed and an 8000-ml aliquot of 144-h aged PAAE solution added followed by evacuation to 22 inHg. The canister was continuously agitated at an average temperature 23 °C for the desired exposure time. Test parameters are further described in Table 2.

A positive control consisting of inoculated unexposed dermal tissue was assayed for CFU for both the 30- and 60-min exposure tests.

In a parallel study intended to demonstrate sustained potency of the PAAE solution, 144-h aged PAAE solution was run using full-scale production type equipment and conditions with $\sim$5000 cm^2 dermal tissue for 120 min. The tissue was removed from the canister. The solution was then inoculated to yield at least 10^6 CFU/100 ml of *B. atrophaeus* spores, and run for a second time for a time course of 15, 30, 45, and 60 min. At each time point, three aliquots of 100 ml were assayed for surviving CFU.

Examination of dermal tissue structure and biomechanical properties

Histological analysis and tensile strength testing were performed to examine the effect on properties of dermal tissue before and after processing with the routine PAAE sterilization process. Unprocessed and

PAAE-sterilized dermal tissue sections were fixed in 10% formalin, embedded in paraffin, sectioned, and stained using hematoxylin and eosin, and for Collagen I, III, elastin, vitronectin, and glycosaminoglycans (GAGs via Alcian Blue/Periodic Acid Schiff (PAS) staining) using standard histological/immunohistochemical protocols by a third party laboratory Premier Laboratories, LLC. (Boulder, CO). These were imaged at a 20× magnification.

Tensile strength (MPa) of allograft dermal tissue was determined by cutting dermal tissue sections using ASTM D638 Type V dies and pulling each section in tension at a rate of 50.8 mm/min until failure, using a MTS 858 Mini-Bionix tensile testing machine (Eden Prairie, MN). Tensile strength (MPa) was calculated by dividing peak load (N) with the crossectional area (cm^2) of the narrow width of the Type V shaped dermal tissue section. Data from at least 13 donors per group was analyzed in Minitab 17.0 for differences using a student's *t* test with two-tailed $\alpha = 0.05$.

Results

Neutralization validation

Results of the neutralization validation testing met the acceptance criteria of greater than 70% recovery for all microorganisms tested. The neutralizer was non-toxic to the microorganisms.

ME study

All initial inoculum counts were above 10^6 CFU. The most resistant microorganism based on comparison of survivor CFU counts after 2 min of exposure was *B. subtilis* followed by *M. terrae* and *P. aeruginosa* (Fig. 2).

A total of seven exposure times were tested for *B. subtilis* spores including time zero (no exposure to PAAE solution). The time for a 4-log reduction in survivor count was ~6 min. An inactivation curve with regression fit for *B. subtilis* spores was generated and is depicted in Fig. 3a. The slope of the linear regression was −0.43 with an r^2 of 0.89, meeting requirements for line fit per the ISO 14161:2009 standard (Annex F) and demonstrating linear inactivation kinetics as required for validating a sterilization process.

The calculated *D* value for *B. subtilis* spores was ~2.3 min with a 12*D* (SAL 10^{-6}) time of ~28 min. Results of Anderson–Darling normality test confirmed the normality of the residuals indicating appropriateness of the regression model ($p = 0.711$); see Fig. 3b.

MPQ study

The inoculum count was $\geq 10^6$ CFU of *B. atrophaeus* spores. No CFU were recovered for either the 30- or 60-min exposures under the worst-case test parameters (Table 2). These results demonstrate the attainment of

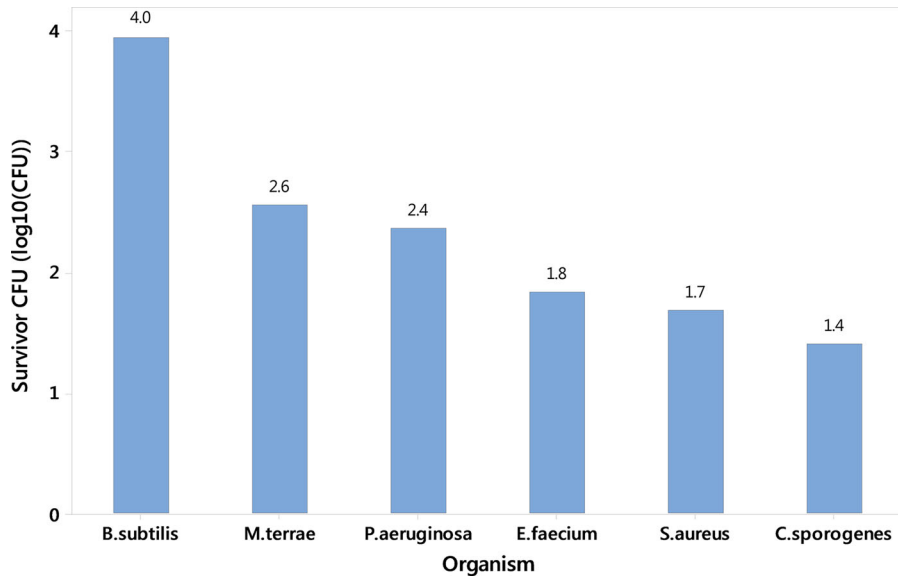

Fig. 2 Microbial resistance comparison: survivor CFU (log_{10}(CFU)) of test microorganisms at 2-min exposure. Figure shows that *B. subtilis* is most resistant to PAAE

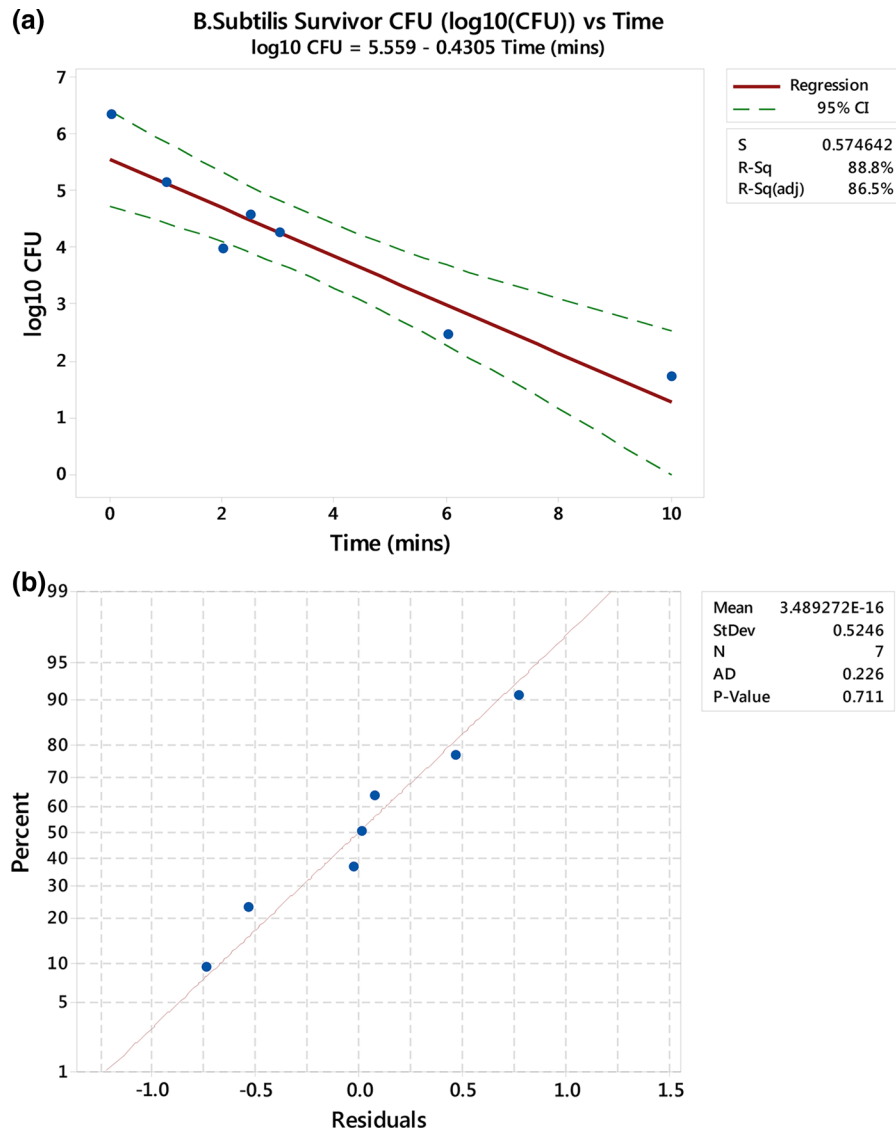

Fig. 3 **a** *B. subtilis* Survivor CFU ($\log_{10}$(CFU)) versus time (min) with linear fit and 95% CI (data presented as mean of four measurements per time point). **b** Normal probability plot of *B. subtilis* regression residuals demonstrates the goodness-of-fit of the linear regression model

a SAL better than 10^{-6} after the 120-min PAAE exposure used in routine processing.

Additionally, there were no surviving CFU of *B. atrophaeus* spores at all time points for the PAAE solution that was inoculated after having been exposed to ~5000 cm^2 dermal tissue for the 120 min routine PAAE exposure time. The study demonstrates continued sterilization efficacy of the PAAE solution even after the full cycle time. The neutralization validation supporting this portion of the study using membrane filtered fresh PAAE solution (without tissue) also met acceptance criteria.

Examination of dermal tissue structure and biomechanical properties

Histological analysis showed that extracellular matrix components Collagen I, III, vitronectin, elastin, and glycosaminoglycans (GAGs) are retained in dermal tissues processed with PAAE (Fig. 4). H&E staining showed that collagen structure was maintained in dermal tissues after PAAE-sterilization indicating minimal effects to the tissue microstructure (Fig. 4).

Average tensile strength (MPa) of PAAE-sterilized dermal tissues was higher compared with unprocessed

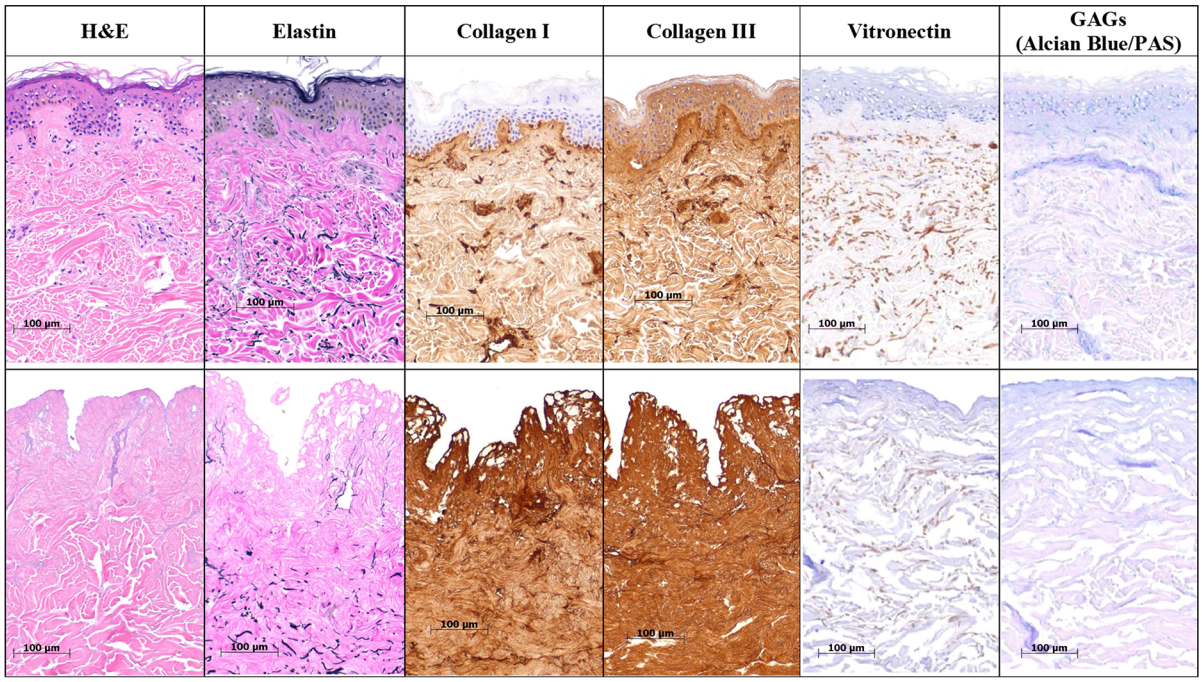

Fig. 4 Representative micrographs of H&E and IHC staining of unprocessed and PAAE sterilized dermal tissue. Histological analysis shows dermal tissue structure remains intact and retains key matrix proteins after processing. All images at 20× magnification. *Top row*: unprocessed dermis. *Bottom row*: dermis after PAAE

dermal tissues, 15.4 ± 5.0 versus 11.2 ± 4.9 MPa respectively ($p < 0.001$) (Fig. 5).

Discussion

Sterilization of allograft dermal tissues by PAAE

Peracetic acid itself is a potent sterilant with fungicidal, bactericidal, sporicidal, and virucidal properties, and has long been utilized for processing of collagenous tissues (Dearth et al. 2016; Hodde and Hiles 2002; Lomas et al. 2003; Scheffler et al. 2007). A recent review conducted by Johnston et al. (2016) concluded that PAA sterilization is comparable in efficacy to low-temperature gamma irradiation at 25 kGy in reduction of contamination rates in allograft dermal tissues. PAA concentrations as low as 0.1% have been reported for allograft dermal tissue processing (Huang et al. 2004; Lomas et al. 2003). Due to its benign breakdown components and minimal effects to structural integrity, peracetic acid is advantageous over other chemical sterilants such as glutaraldehyde or ethylene oxide that may leave toxic residues or alter tissue structure (Huang et al. 2004; Lomas et al. 2003; Matuska and McFetridge 2015). As such, it has been commonly used in various FDA-cleared chemical sterilization and high level disinfectant formulations such as Acecide®-C (Olympus Medical/Best Sanitizers, Inc.) and Nu-Cidex™ (Johnson and Johnson/ASP Ltd.) as well as the primary active component in commercially available sterilization systems such as the Nova2200™ system (NovaSterilis) and the SYSTEM 1E® (Steris) (Malchesky 2001; Wehmeyer et al. 2015).

The successful use of the peracetic acid and ethanol combination as a sterilant of bone and soft tissue implants has been reported in the literature (Hodde and Hiles 2002; Pruss et al. 2003; Scheffler et al. 2005). Since 1984, Leaper et al. showed that the microbicidal efficacy of the peracetic acid is enhanced by increasing ethanol concentrations (Leaper 1984). In recent years, Nerandzic et al. showed first that sporicidal activity of ethanol is enhanced when acidified, and second, that dilute peracetic acid in combination with ethanol results in superior sporicidal activity compared with acidified ethanol alone or dilute PAA alone at 10 min (Nerandzic et al. 2015, 2016). Conducting the PAAE

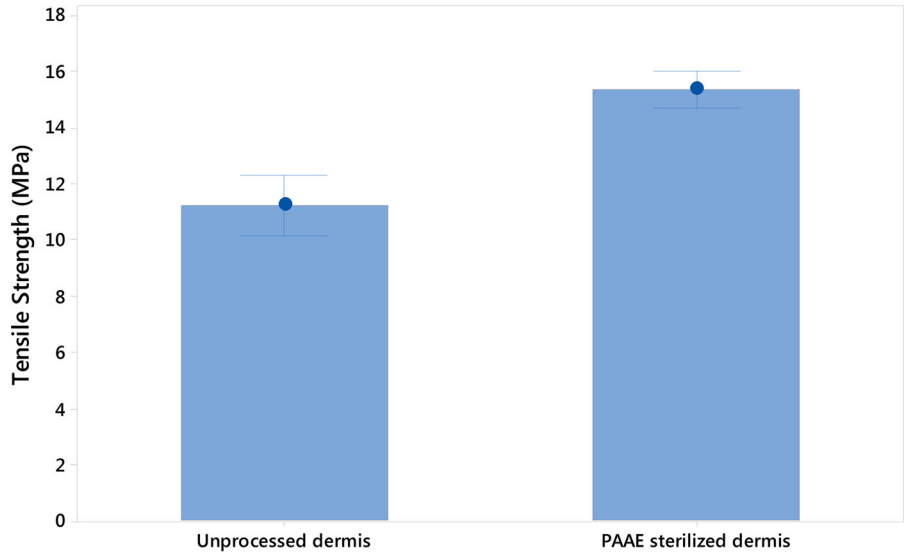

Fig. 5 Tensile strength (MPa) comparison of unprocessed and PAAE sterilized dermal tissue. Data is mean ± 95% confidence intervals of 13 and 23 donors per group respectively

sterilization process under vacuum improves penetration throughout the allograft dermal tissue (Leaper 1984; Malchesky 2001; Mills and Wironen 2002).

Validating and demonstrating robustness of a sterilization process requires, among other items, a specific knowledge of the sterilizing agent, critical process parameters (concentration, temperature, etc.), microbicidal effectiveness, and demonstration through an MPQ that the process is reproducible (ANSI/AAMI/ISO14937 2009/(R)2013). This study aimed to demonstrate that the process was capable of achieving a SAL 10^{-6} or better using a set of worst-case test conditions.

The results of the ME testing were as expected showing that *Bacillus* spores have the highest resistance to the action of the PAAE solution, and that a SAL 10^{-6} or 12*D* is expected in ~28 min (*D* value of 2.3 min) via the demonstration of linear inactivation kinetics.

In the MPQ study, a set of worst case conditions were used: 2 mm full-thickness dermal tissue sections, "aged" PAAE solution well beyond its expiry time (Malchesky 2001; Shetty et al. 1999), and a dermal tissue load in the sterilization vessel greater than two-times that of routine processing. Under these conditions, no surviving CFU of *Bacillus* spores were found after 30 min of exposure of the inoculated dermal tissue sections to the PAAE solution demonstrating greater than six spore log reductions or a 6*D* or better process at a quarter of the full cycle. Routine processing conditions clearly result in at least 24*D*, or a SAL well below (better than) 10^{-6}.

The studies reported here did not include yeast or mold species. These were excluded from the study based on known less resistance compared to bacterial spores (Lensing and Oei 1985; Shetty et al. 1999). Published studies suggest that yeast or mold species might be closest in resistance to the non-spore forming bacteria used in this study (Malchesky 2001; McDonnell and Russell 1999).

Sterilization within an aseptic process

Ensuring microbiological safety of an allograft while maintaining its integrity and quality for clinical performance can be a challenge for tissue-based healthcare products. While terminal sterilization is often used, the effects of the sterilizing agent and/or process on tissue properties and efficacy must be considered and evaluated.

It is well known that terminal sterilization methods like ionizing irradiation can alter the macro- and micro-structure of allograft soft tissue and other methods such as ethylene oxide may leave toxic residues (Delgado et al. 2014; Matuska and McFetridge 2015; Mrazova et al. 2016; Rooney et al. 2008). It has also been shown that such tissue alterations can affect clinical performance (Aspenberg et al. 1990;

Tejwani et al. 2015). Recently, it has been shown that cell–matrix interactions and cell behavior are altered when allograft dermal tissues are terminally sterilized via irradiation as compared with being aseptically processed and sterilized with PAAE using the parameters validated within this study (Dasgupta et al. 2016; Nilsen et al. 2016). The detrimental effects of irradiation on polymeric structures, and tissue-based healthcare products, are so well understood, that efforts towards irradiation process modifications such as use of low temperature, nitrogen, or low oxygen concentration are continually being pursued (Al Kayal et al. 2015; Hamer et al. 1999; Medel et al. 2009; Rooney et al. 2008). These modifications can lessen radiation effects in some situations but might not offer complete protection for the tissue (Gouk et al. 2008).

MTF utilizes the PAAE sterilization process for all allograft dermal tissues including the brands FlexHD® Structural and FlexHD® Pliable for plastic and reconstructive applications, AlloPatch HD for sports medicine applications (rotator cuff repair) and AlloPatch® Pliable for wound care applications. Structural dermal tissues comprise the papillary and reticular dermis while pliable dermal tissues refer to those comprised only of the deeper and less dense reticular dermal layer. The biological and biomechanical properties of reticular dermal tissues sterilized using the PAAE process were published recently (Dasgupta et al. 2016; Nilsen et al. 2016). These two reports characterize the presence of relevant structural matrix proteins including Collagen I, III, and elastin, and demonstrate similar matrix stability compared to unprocessed reticular dermis via in vitro enzymatic degradation studies. Additional histological and biomechanical testing results presented within this manuscript show that structural dermal tissues also retain key matrix proteins after being processed with PAAE: Collagen I, III, elastin, vitronectin, and GAGs. These matrix proteins provide structural support for cell infiltration and influence cell attachment, motility, and proliferation –among many other biological functions (Dasgupta et al. 2016; Grinnell et al. 1992). H&E micrographs showed similar intact collagen structure in the papillary and reticular areas of unprocessed and processed dermis. Post-PAAE results show that tensile strength is increased compared to the native state, which was expected based on prior reports on collagen-based grafts treated with peracetic acid (Delgado et al. 2014; Freytes et al. 2004). In this case, the increased tensile strength (rather than the opposite) is favorable for these allograft dermal tissues (and therefore, a tolerable processing effect) since they can be used in load bearing applications such as abdominal wall repair, where these grafts have been shown to perform well clinically (Bochicchio et al. 2013).

MTF's aseptic process for allograft soft tissue involves a combination of aseptic recovery and preparation of the tissue, sterilization with a proprietary PAAE formulation in an enclosed container under vacuum, followed by aseptic packaging. In

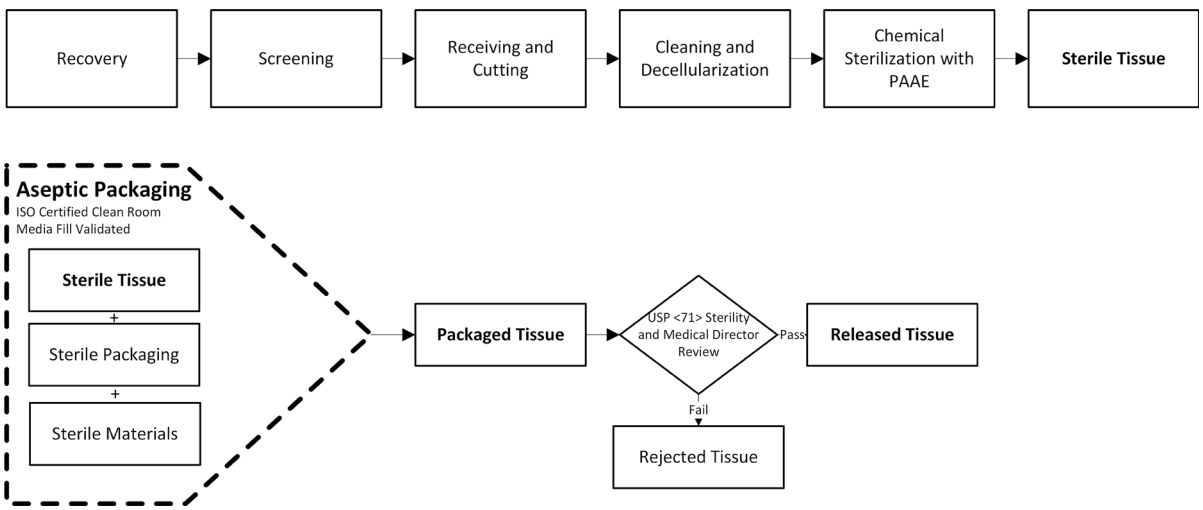

Fig. 6 High-level process flow for allograft dermal tissue processing at MTF. Flow describes first obtaining a sterile tissue through PAAE sterilization followed by aseptic packaging using sterile components and batch release via USP <71> sterility testing and Medical Director review

alignment with the requirements and guidance in current standards documents, MTF aims to maintain the post-disinfection sterility of allograft soft tissue by using a specially designed, controlled, and monitored aseptic process (AATB 2016; ANSI/AAMI/ISO13408 2011). Key aspects of the aseptic process employed at MTF are the use of sterile materials and equipment, a specially designed and microbiologically monitored processing environment, qualified personnel, and a final packaging process that is validated based on media fill validation guidelines (FDA 2004, 2012; PDA 2011). Figure 6 illustrates a high-level process flow for MTF's aseptic operation.

PAAE-sterilized allografts are transported in a sealed stainless steel container to the final packaging cleanroom room where the tissue is hydrated, cut, and packaged under ISO Class 4 conditions. Gamma irradiation or moist heat sterilization are used to sterilize all packaging instrumentation and components to a SAL of 10^{-6} or better. As a final monitoring activity, every donor batch is subject to the United States Pharmacopeia <71> sterility test and must pass with zero positive tests of sterility in order to be released for clinical use. The overall process ensures an extremely low probability of a nonsterile unit for allograft dermal tissue in the final package.

To summarize, we have validated a PAAE-based sterilization method (SAL $10^{-6)}$ and demonstrated that its combination with aseptic processing secures the microbiological safety of the allograft dermal tissue, while avoiding structural and biochemical damage previously observed with common sterilization methods like ionizing irradiation.

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Compliance with ethical standards

Conflict of interest A. Phipps, M. Ngo, E. Chnari, and J. Osborne are employees of the Musculoskeletal Transplant Foundation. J. Kowalski is a consultant for the Musculoskeletal Transplant Foundation (MTF). E. Vaynshteyn and K. Merritt have no conflict of interest to disclose.

References

AATB (2016) Guidance document—microbial process validation and surveillance program. American Association of Tissue Banks, McLean

Al Kayal T et al (2015) Evaluation of the effect of a gamma irradiated DBM-pluronic F127 composite on bone regeneration in Wistar rat. PLoS ONE 10:e0125110. doi:10.1371/journal.pone.0125110

ANSI/AAMI/ISO13408 (2011) Aseptic processing of healthcare products—part 1: general requirements. AAMI

ANSI/AAMI/ISO14937 (2009/(R)2013) Sterilization of healthcare products—general requirements for characterization of a sterilizing agent and the development, validation and routine control of a sterilization process for medical devices. Association for the Advancement of Medical Instrumentation, Arlington

Aspenberg P, Johnsson E, Thorngren KG (1990) Dose-dependent reduction of bone inductive properties by ethylene oxide. J Bone Joint Surg 72:1036–1037

Bochicchio GV, De Castro GP, Bochicchio KM, Weeks J, Rodriguez E, Scalea TM (2013) Comparison study of acellular dermal matrices in complicated hernia surgery. J Am Coll Surg 217:606–613. doi:10.1016/j.jamcollsurg.2013.04.041

Dasgupta A, Orgill D, Galiano RD, Zelen CM, Huang YC, Chnari E, Li WW (2016) A novel reticular dermal graft leverages architectural and biological properties to support wound repair. Plast Reconstr Surg Glob Open 4:e1065. doi:10.1097/GOX.0000000000001065

Dearth CL et al (2016) The effect of terminal sterilization on the material properties and in vivo remodeling of a porcine dermal biologic scaffold. Acta Biomater 33:78–87. doi:10.1016/j.actbio.2016.01.038

Delgado LM, Pandit A, Zeugolis DI (2014) Influence of sterilisation methods on collagen-based devices stability and properties. Expert Rev Med Devices 11:305–314. doi:10.1586/17434440.2014.900436

FDA (2004) Guidance for industry: sterile drug products produced by aseptic processing—current good manufacturing practice. CDER/CBER/ORA, Rockville

FDA (2011) Guidance for Industry Current Good Tissue Practice (CGTP) and additional requirements for manufacturers of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps). CBER, Rockville

FDA (2012) Media fills for validation of aseptic preparations for positron emission tomography (PET) drugs. CDER, Silver Spring

Freytes DO, Badylak SF, Webster TJ, Geddes LA, Rundell AE (2004) Biaxial strength of multilaminated extracellular matrix scaffolds. Biomaterials 25:2353–2361

Gouk SS, Lim TM, Teoh SH, Sun WQ (2008) Alterations of human acellular tissue matrix by gamma irradiation: histology, biomechanical property, stability, in vitro cell repopulation, and remodeling. J Biomed Mater Res Part B Appl Biomater 84:205–217. doi:10.1002/jbm.b.30862

Grinnell F, Ho CH, Wysocki A (1992) Degradation of fibronectin and vitronectin in chronic wound fluid: analysis by cell blotting, immunoblotting, and cell adhesion assays. J Invest Dermatol 98:410–416

Hamer A, Stockley I, Elson R (1999) Changes in allograft bone irradiated at different temperatures. Bone Joint J 81:342–344

Hodde J, Hiles M (2002) Virus safety of a porcine-derived medical device: evaluation of a viral inactivation method. Biotechnol Bioeng 79:211–216. doi:10.1002/bit.10281

Huang Q, Dawson RA, Pegg DE, Kearney JN, Macneil S (2004) Use of peracetic acid to sterilize human donor skin for production of acellular dermal matrices for clinical use. Wound Repair Regen 12:276–287. doi:10.1111/j.1067-1927.2004.012312.x

Johnston C et al (2016) Disinfection of human skin allografts in tissue banking: a systematic review report. Cell Tissue Bank 17:585–592 doi:10.1007/s10561-016-9569-2

Leaper S (1984) Synergistic killing of spores of *Bacillus subtilis* by peracetic acid and alcohol. Int J Food Sci Technol 19:355–360. doi:10.1111/j.1365-2621.1984.tb00359.x

Lensing HH, Oei HL (1985) Investigations on the sporicidal and fungicidal activity of disinfectants. Zentralblatt fur Bakteriologie, Mikrobiologie und Hygiene 1 Abt Originale B Hygiene 181:487–495

Lomas RJ, Cruse-Sawyer JE, Simpson C, Ingham E, Bojar R, Kearney JN (2003) Assessment of the biological properties of human split skin allografts disinfected with peracetic acid and preserved in glycerol. Burns J Int Soc Burn Injuries 29:515–525

Macadam SA, Lennox PA (2012) Acellular dermal matrices: use in reconstructive and aesthetic breast surgery. Can J Plast Surg (Journal canadien de chirurgie plastique) 20:75–89

Malchesky PS (2001) Medical application of peracetic acid. In: Block SS (ed) Disinfection, sterilization, and preservation. Lippincott, Williams, & Wilkins, Philadelphia, pp 979–998

Matuska AM, McFetridge PS (2015) 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 103:397–406. doi:10.1002/jbm.b.33213

McDonnell G, Russell AD (1999) Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev 12:147–179

Medel FJ et al (2009) Gamma inert sterilization: a solution to polyethylene oxidation? J Bone Joint Surg Am Vol 91:839–849. doi:10.2106/JBJS.H.00538

Mills CR, Wironen JF (2002) Cyclic implant perfusion cleaning and passivation process. United States Patent 6482584, November 19, 2002

Mrazova H, Koller J, Kubisova K, Fujerikova G, Klincova E, Babal P (2016) Comparison of structural changes in skin and amnion tissue grafts for transplantation induced by gamma and electron beam irradiation for sterilization. Cell Tissue Bank 17:255–260. doi:10.1007/s10561-015-9536-3

Nerandzic MM, Sunkesula VC, Setlow P, Donskey CJ (2015) Unlocking the sporicidal potential of ethanol: induced sporicidal activity of ethanol against *Clostridium difficile* and *Bacillus spores* under altered physical and chemical conditions. PLoS ONE 10:e0132805

Nerandzic MM, Setlow P, Donskey CJ (2016) A cumulative spore killing approach: synergistic sporicidal activity of dilute peracetic acid and ethanol at low pH against *Clostridium difficile* and *Bacillus subtilis* spores. In: Open forum infectious diseases, vol 1. Oxford University Press, Oxford, p ofv206

Nilsen TJ, Dasgupta A, Huang Y-C, Wilson H, Chnari E (2016) Do processing methods make a difference in acellular dermal matrix properties? Aesthet Surg J 36:sjw163

PDA (2011) PDA Technical Report No. 22 (Revised 2011), Process simulation for aseptically filled products. Parenteral Drug Association, Inc.

Pruss A et al (2003) Peracetic acid-ethanol treatment of allogeneic avital bone tissue transplants—a reliable sterilization method. Ann Transplant 8:34–42

Rooney P, Eagle M, Hogg P, Lomas R, Kearney J (2008) Sterilisation of skin allograft with gamma irradiation. Burns J Int Soc Burn Injuries 34:664–673. doi:10.1016/j.burns.2007.08.021

Scheffler SU, Scherler J, Pruss A, von Versen R, Weiler A (2005) Biomechanical comparison of human bone-patellar tendon-bone grafts after sterilization with peracetic acid ethanol. Cell Tissue Bank 6:109–115. doi:10.1007/s10561-004-6403-z

Scheffler S, Trautmann S, Smith M, Kalus U, von Versen R, Pauli G, Pruss A (2007) No influence of collagenous proteins of Achilles tendon, skin and cartilage on the virus-inactivating efficacy of peracetic acid-ethanol. Biol J Int Assoc Biol Stand 35:355–359. doi:10.1016/j.biologicals.2007.03.004

Shetty N, Srinivasan S, Holton J, Ridgway GL (1999) Evaluation of microbicidal activity of a new disinfectant: Sterilox 2500 against *Clostridium difficile* spores, *Helicobacter pylori*, vancomycin resistant Enterococcus species, *Candida albicans* and several Mycobacterium species. J Hosp Infect 41:101–105

Srun SW, Nissen BJ, Bryans TD, Bonjean M (2012) Medical device SALs and surgical site infections: a mathematical model. Biomed Instrum Technol 46:230–237. doi:10.2345/0899-8205-46.3.230

Tejwani SG, Chen J, Funahashi TT, Love R, Maletis GB (2015) Revision risk after allograft anterior cruciate ligament reconstruction. Am J Sports Med 43:2696–2705. doi:10.1177/0363546515589168

Truncale K, Cartmell JS, Syring C, Von Versen R, Ngo M (2010) Soft tissue processing. USA Patent 7,723,108

Wehmeyer JL, Natesan S, Christy RJ (2015) Development of a sterile amniotic membrane tissue graft using supercritical carbon dioxide. Tissue Eng Part C Methods 21:649–659. doi:10.1089/ten.TEC.2014.0304