Acta Biomaterialia 33 (2016) 78–87

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

The effect of terminal sterilization on the material properties and in vivo remodeling of a porcine dermal biologic scaffold

Christopher L. Dearth [a,b,c,1], Timothy J. Keane [a,d,1], Christopher A. Carruthers [a,d], Janet E. Reing [a], Luai Huleihel [a], Christian A. Ranallo [a], Elizabeth W. Kollar [a], Stephen F. Badylak [a,b,d,*]

[a] McGowan Institute for Regenerative Medicine, Pittsburgh, PA 15219, United States
[b] Department of Surgery, University of Pittsburgh, Pittsburgh, PA 15219, United States
[c] DoD-VA Extremity Trauma & Amputation Center of Excellence, Walter Reed National Military Medical Center, Bethesda, MD 20889, United States
[d] Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA 15213, United States

ARTICLE INFO

Article history:
Received 28 September 2015
Received in revised form 21 January 2016
Accepted 26 January 2016
Available online 27 January 2016

Keywords:
Extracelluar matrix
Terminal sterilization

ABSTRACT

Biologic scaffolds composed of extracellular matrix are commonly used in a variety of surgical procedures. The Food and Drug Administration typically regulates biologic scaffolds as medical devices, thus requiring terminal sterilization prior to clinical use. However, to date, no consensus exists for the most effective yet minimally destructive sterilization protocol for biologic scaffold materials. The objective of the present study was to characterize the effect of ethylene oxide, gamma irradiation and electron beam (e-beam) irradiation on the material properties and the elicited in vivo remodeling response of a porcine dermal biologic scaffold. Outcome measures included biochemical, structural, and mechanical properties as well as cytocompatibility in vitro. In vivo evaluation utilized a rodent model to examine the host response to the materials following 7, 14, and 35 days. The host response to each experimental group was determined by quantitative histologic methods and by immunolabeling for macrophage polarization (M1/M2). In vitro results show that increasing irradiation dosage resulted in a dose dependent decrease in mechanical properties compared to untreated controls. Ethylene oxide-treated porcine dermal ECM resulted in decreased DNA content, extractable total protein, and bFGF content compared to untreated controls. All ETO treated, gamma irradiated, and e-beam irradiated samples had similar cytocompatibility scores in vitro. However, in vivo results showed that increasing dosages of e-beam and gamma irradiation elicited an increased rate of degradation of the biologic scaffold material following 35 days.

Statement of Significance

The FDA typically regulates biologic scaffolds derived from mammalian tissues as medical devices, thus requiring terminal sterilization prior to clinical use. However, there is little data and no consensus for the most effective yet minimally destructive sterilization protocol for such materials. The present study characterized the effect of common sterilization methods: ethylene oxide, gamma irradiation and electron beam irradiation on the material properties and the elicited in vivo remodeling response of a porcine dermal biologic scaffold. The results of the study will aid in the meaningful selection of sterilization methods for biologic scaffold materials.

1. Introduction

Biologic scaffolds composed of extracellular matrix (ECM) are commonly used in a variety of surgical applications to reinforce soft tissue, particularly in the abdominal wall and pelvic floor and in reconstructive breast surgery [1]. ECM scaffolds are produced by decellularization of source mammalian tissues and organs, including small intestine, urinary bladder, and dermis,

* Corresponding author at: University of Pittsburgh, McGowan Institute for Regenerative Medicine, 450 Technology Drive, Suite 300, Pittsburgh, PA 15219, United States.
E-mail address: badylaks@upmc.edu (S.F. Badylak).
[1] Co-first authors; authors contributed equally.

http://dx.doi.org/10.1016/j.actbio.2016.01.038
1742-7061/

among others. Over the last decade, a wide array of manufacturing protocols have been described for ECM scaffold materials, each of which vary widely in their use of chemical, enzymatic, and/or physical methods of decellularization. While it is inevitable that all processing methods used to prepare biologic scaffold materials will adversely affect the mechanical, biochemical, and cell signaling properties of the resulting ECM to some degree, the preferred methods will mitigate these effects as much as possible. Preservation of native ECM composition and ultrastructure in biologic scaffolds has been shown to facilitate beneficial constructive remodeling outcomes [2]. Specifically, the host response to biologic scaffold materials has been shown to be directly related to the efficacy of decellularization [3]. These finding have led to the proposal of a standard criteria for defining effective decellularization [2]. While much progress has been made to this end, relatively little has been studied regarding preferred methods of terminal sterilization.

The Food and Drug Administration (FDA) regulates biologic scaffolds derived from xenogeneic source tissue, including materials derived from decellularized porcine dermis, as medical devices; thus requiring these products to be terminally sterilized prior to clinical use. Common methods of terminal sterilization include electron beam (e-beam) and gamma (γ) irradiation and ethylene oxide [4]. While it is known that each method exerts its sterilizing effect by modifying the structure or function of the critical components (e.g. proteins and nucleic acids) of target microorganisms and has directed guidelines regarding bacterial load (e.g., ISO/DIS 11135-1, ISO/DIS 11137-3), each method also has the potential to alter material properties of the ECM, including mechanics, susceptibility to degradation, biocompatibility, and ultimately the elicited in vivo remodeling response [5]. In contrast to the aforementioned decellularization criteria, currently no consensus exists for the most effective yet minimally destructive sterilization protocol for any biologic scaffold material. The objective of the present study was to characterize the effect of several types and doses of terminal sterilization on the material properties and elicited in vivo remodeling response of a biologic scaffold material derived from porcine dermis.

2. Methods

2.1. Experimental design

The effect of terminal sterilization on a porcine dermal biologic scaffold was examined using in vitro and in vivo test systems. Porcine dermal ECM scaffold materials were exposed to one of three terminal sterilization methods – ETO, γ-irradiation, and e-beam irradiation. The γ-irradiation and e-beam irradiation groups were further subdivided into three dosage levels – 10, 25, and 40 kGy. All of the doses of sterilization evaluated within the current study are generally accepted to meet bioburden reduction criteria (i.e., a 6 log reduction). Quantification of bioburden reduction was not determined in the present study.

Two non-sterilized control groups (non-sterilized porcine dermal ECM and non-sterilized intact porcine dermis) were also evaluated. All materials were evaluated for biochemical (DNA, sulfated glycosaminoglycan, & bFGF content), structural (scanning electron microscopy), and mechanical (thickness, porosity index, ball burst, suture retention) properties as well as cytocompatibility with human microvascular endothelial cells (HMEC-1). In the in vivo experiments, a rodent 1.5 cm × 1.5 cm bilateral partial thickness abdominal wall defect model was used to examine the host response to the materials following 7, 14, or 35 days (n = 4/group/-time point). The host response to each experimental group was determined by quantitative histologic methods and by immunolabeling for macrophage polarization (M1/M2) within explanted specimens.

2.2. Preparation and sterilization of dermal ECM scaffolds

Porcine full thickness skin from the dorsolateral flank of market weight pigs was harvested and processed immediately after euthanasia as previously described [6]. All full thickness skin sheets were cut into 35-cm × 50-cm rectangles. All samples were then mechanically delaminated to remove subcutaneous fat, excess connective tissue and the epidermis. While the mechanical delamination step is inherently variable, the allowed tolerances for these devices result in very small amounts of variability. Thus, the samples all started at the same thickness (i.e., they are not significantly different). The harvested sheets of porcine dermis were immediately frozen at −80 °C. Porcine dermis sheets designated to be treated with decellularization protocols were removed from the freezer and cut into sections measuring 3–7 cm × 3–7 cm. Dermis sections were decellularized as described previously [6]. Briefly, dermis was treated on a vortex shaker at 300 RPM at room temperature in the following solutions: 0.25% trypsin for 6 h, 1×; deionized water, 15 min, 3×; 70% ethanol, 10–12 h, 1×; 3% H_2O_2, 15 min, 1×, deionized water, 15 min, 2×; 1% Triton X-100 in 0.26% EDTA/0.69% Tris, 6 h, 1× and then overnight, 1×; deionized water, 15 min, 3×; 0.1% peracetic acid/4% ethanol, 2 h, 1×; PBS, 15 min, 2×; and finally deionized water, 15 min, 2×. Following decellularization, all dermal ECM sheets were lyophilized. Lyophilized dermal sheets were sterilized with ETO gas (16 h cycle at 50 °C in a Series 3plus EOGas Sterilizer, Anderson Sterilizers, Inc., Haw River, NC), γ-10 kGy, γ-25 kGy, γ-45 kGy, e-beam 10 kGy, e-beam 25 kGy, or e-beam 45 kGy.

2.3. Assessment of cellular content

Decellularization efficacy of dermis samples was assessed by three previously published criteria: (1) the absence of visible nuclear material on hematoxylin and eosin (H&E) stained and 40,6-diamidino-2-phenylindole (DAPI) stained sections; (2) a Quant-iT Pico-Green assay (Invitrogen, Carlsbad, CA) for quantification of double-stranded DNA; and (3) evaluation of a 2% agarose gel to determine the size of remaining DNA fragments [2].

2.3.1. Measuring DNA content

Scaffolds were digested in 0.6% proteinase K solution for at least 24 h at 50 °C, until no visible tissue remained. Phenol/chloroform/isoamyl alcohol was added, and samples were centrifuged at 10,000g for 10 min at 4 °C. The top aqueous phase containing the DNA was transferred into a new tube. Sodium acetate and ethanol was added to each sample, and the solution was mixed and placed at −80 °C overnight. While still frozen, the samples were centrifuged at 4 °C for 10 min at 10,000g. The supernatant was discarded, and all residual alcohol was removed. The pellet was suspended in TE (10 mM Tris/1 mM EDTA) buffer. Double stranded DNA was quantified using Quant-iT PicoGreen Reagent (Invitrogen Corp., Carlsbad, CA) according to the manufacturer's instructions. The dsDNA assay was performed twice (n = 2) with two technical replicates per assay.

2.3.2. DNA fragmentation analysis

To determine the fragment size of remnant DNA, equal concentrations of extracted DNA from each sample were separated on a 2% agarose gel containing 0.5% ethidium bromide and visualized with ultraviolet transillumination using a reference 100-bp ladder (New England BioLabs, Ipswich, MA).