Terminal Sterilization of Biological Tissue Matrix Using Supercritical CO_2

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Statement of Purpose: The commonly used sterilization methods, e.g. e-beam irradiation, cause scissions in collagen matrix and thus are detrimental to biological materials. The present study introduces a method that uses supercritical CO_2 in combination with peracetic acid sterilant (SC-CO_2-PAA) to terminally sterilize biological materials. Porcine acellular dermal matrix used in this study is known to provide a three-dimensional scaffold for cell infiltration and tissue regeneration, and has been used in a variety of clinical applications including abdominal wall repair and breast reconstruction. The effects of two sterilization methods, SC-CO_2-PAA and e-beam irradiation, on porcine acellular dermal matrix were evaluated and compared in this study.

Methods: Sample Preparation and Sterilization The porcine acellular dermal matrix samples (size: 5x8cm, 1mm thick) were divided into three groups: A. Control. The samples in the control group were not subjected to any sterilization treatment. B. SC-CO_2-PAA group. The samples in this group were subjected to SC-CO_2-PAA treatment in SC-CO_2 chamber (NovaSterilis, NY) for 1.5 hours, which was 3 times longer than the run time required to achieve an industrial sterility assurance level (10^{-6}) required for medical device [1]. C. E-beam. The samples in this group were subjected to e-beam irradiation of 20kGy (±2kGy). After sterilization, the samples were stored under ambient condition for up to 6 months.

In Vitro Characterization The samples' thermal stability, susceptibility to enzyme digestion, and mechanical characteristics were evaluated using differential scanning calorimetry (DSC Q2000, TA Instruments, New Castle, DE), collagenase digestion assay, and Instron Testing System 5865 (Instron Corp, Canton, MA) at 0, 1, 3 and 6-month storage time points.

In Vivo Evaluation The in vivo performance, such as cell repopulation and re-vascularization, of the acellular dermal matrix terminally sterilized with SC-CO_2-PAA was evaluated using mouse subcutaneous implantation model. One piece of sample of approximately 0.5x1.0cm in size was implanted to a subcutaneous site in the dorsal lateral region of the back of the animal. Samples were explanted at 28 days. The biopsies were fixed in 10% neutral buffered formalin, processed for histology, followed by staining with H&E for evaluation.

Results: At time 0 and up to 6-month ambient storage, the samples in the SC-CO_2-PAA group showed collagen denaturation onset and peak temperatures comparable to controls, whereas the e-beam group exhibited onset and peak temperatures about 3°C lower than the control group (Table 1). A decrease in onset and peak temperatures of the samples in the e-beam group indicated scissions in the collagen matrix caused by irradiation. The unchanged onset temperature in SC-CO_2-PAA suggested no significant scission effect from the SC-CO_2-PAA treatment. The tensile testing showed that no statistically significant differences in maximum load, maximum stress and elasticity were evident among treatment groups. The collagenase digestion assay showed that both treatment groups had comparable susceptibility to collagenase digestion with the control group. The mouse subcutaneous implantation of the samples sterilized with SC-CO_2-PAA revealed that the acellular matrix yielded significant mesenchymal cell migration into the tissue matrix, including the presence of some cells within the center of the implant samples by 28 days of implantation (Fig.1). Revascularization was also evident in the implanted tissues. The PAA residue in samples from SC-CO_2-PAA group was analyzed using PAA test strips (EMD Chemicals). It was found that the PAA residue was near the detection limit (≤5ppm) at time 0, and dropped below the detection limit (<5ppm) after 1-month ambient storage.

Table 1 Thermal Stability and Mechanical Evaluation at 6-Month Ambient Storage

Treatment	Onset T (°C)	Max Stress (MPa)
Control	59.3 ± 0.5	13 ± 3
SC-CO_2-PAA	59.4 ± 0.4	16 ± 2
E-beam	56.1 ± 0.4*	11 ± 2

* $p < 0.05$ vs. control group.

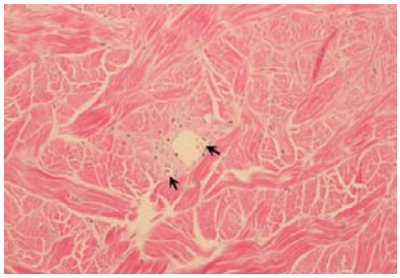

Figure 1. H&E stain of explant of acellular dermal matrix treated by SC-CO_2. Cells (arrows) were shown in the matrix.

Conclusions: E-beam irradiation of 20kGy causes scissions in collagen matrix and thus significantly reduces the onset and peak protein denaturation temperature of porcine acellular dermal matrix. In contrast to e-beam irradiation, SC-CO_2-PAA sterilization shows little effect on the thermal stability of porcine acellular dermal matrix. The acellular matrix terminally sterilized using SC-CO_2-PAA permits cell repopulation and revascularization *in vivo*. In addition, the SC-CO_2-PAA sterilized acellular dermal matrix is stable for at least 6-months under ambient storage condition. Our study has shown that SC-CO_2-PAA sterilization is a promising terminal sterilization method for biological materials.

References: [1] Qiu Q et al. J Biomed Mater Res B Appl Biomater. 2009; 91(2):572-8.