Introduction: Hydrogels derived from extracellular matrix (ECM) have been shown to mediate inflammation and promote a constructive tissue remodeling response. However, well-accepted terminal sterilization methods (i.e., gamma irradiation, electron beam irradiation, ethylene oxide (EtO) exposure) inhibit ECM hydrogel formation, which is typically achieved upon incubation at physiologic conditions. It is important to identify a sterilization method that is conducive to formation of ECM hydrogels prior to clinical translation. Supercritical CO₂ (scCO₂) sterilization, a relatively novel method of sterilization, deactivates bacteria when CO₂ is held above critical temperature and pressure was tested in addition to common sterilization techniques. Preliminary work with porcine urinary bladder matrix (UBM) indicated that exposure to ethylene oxide and 30-kGy irradiation have detrimental effects on ECM hydrogel formation, and that scCO₂ sterilization of lyophilized pre-gel is the only tested sterilizing method that permits gel formation. The objective of the present study was to characterize the viscoelastic and biologic properties of scCO₂ sterilized lyophilized ECM hydrogels derived from various tissues.

Materials and Methods: Eight porcine tissues were decellularized for ECM hydrogel production: urinary bladder (UBM), small intestine (SIS), liver (liECM), bone (boECM), esophagus (eECM), dermis (dECM), pericardium muscle (carECM), and colon (coECM). Following decellularization, all tissues were digested with 1 mg/mL pepsin in 0.01N HCl at an ECM concentration of 10 mg/mL for 48 hours to produce pre-gel, after which they were lyophilized and underwent scCO₂ sterilization or 30-kGy gamma irradiation. Dried samples were then reconstituted in deionized water at 4°C overnight. After pH neutralization and salt balancing, rheological testing was performed with an AR 2000 rheometer to determine the storage and loss moduli (G’ and G” respectively) of the hydrogel. Rheologic properties were recorded while raising temperature from 10°C to 37°C over the course of 1 minute then retaining these conditions for 1 hour. Biologic activity of the sterilized ECM scaffolds was assessed via macrophage cytokine secretion with enzyme-linked immunoabsorbent assays (ELISAs) and perivascular stem cell migration with Boyden Chamber assays. All samples were run in triplicate (n = 3).

Results and Discussion: The effect of sterilization on gel stiffness was evaluated by comparison of maximum G’ values (Figure 1). ECM exposed to high dose (30 kGy) gamma irradiation did not form a gel, all displaying liquid-like storage moduli. CoECM, dECM, carECM, and eECM sample controls did not gel consistently and were excluded from the study. Interestingly, rheological properties of ECM scaffolds were not affected by scCO₂ sterilization with the scCO₂-sterilized ECM hydrogels displaying equivalent stiffness values to non-sterilized control samples (n=2). Similarly, there was no difference in biologic properties of sterilized samples, including perivascular stem cell migration (n = 2) and macrophage secretion of tumor necrosis factor (n = 3), between sterilized samples and the non-sterilized control samples.

Conclusions: Sterilization with scCO₂ permits the formation of a hydrogel comparable to non-sterilized controls. The sterilized hydrogel exhibits chemotactic properties towards stem cells and elicits macrophage cytokine secretion that is equivalent to samples that did not undergo terminal sterilization. Together, these data suggest that ECM form and method of sterilization is critically important hydrogel formation but sterilization does not appear to impact the biologic properties of ECM scaffolds.