SFE536: Decellularize Tissue and Organs Using Supercritical CO2 / Ethanol

Introduction

Tissue engineering uses extracellular matrix scaffolds (ECM) in many clinical applications for reconstruction of tissues and organs. Xenogenetic biomaterials are a common source for human tissue engineering because of the severe shortage of human tissues and organs. Standard methods for decellularization often damage the 3D architecture and alter the structural integrity of the ECM scaffold. Supercritical CO2 can be used to obtain intact ECM scaffold architecture from numerous animal organs and tissues without using harmful and toxic chemicals.

Equipment

Applied Separations Supercritical Extraction Equipment including the Helix, SFE 2, SFE 4, or Basic model.

Materials

Liquid CO2 cylinder

95% ethanol

Phosphate buffered saline solution sodium hydroxide (0.1–1 N)

Method

(see references for detailed procedures)

Preparation & slicing of tissues.
Remove the fat bed and extraneous tissue carefully. Rinse the tissues and organs using phosphate-buffered saline and freeze at -20 ◦C. Slice the frozen tissues and organs using a meat slicer to a thickness (1–5 mm). Place the frozen slices in a muslin bag.

Production of decellularized tissue & organ scaffolds
Add a specified amount of 75% to 95% ethanol into the extraction vessel and then place the tissue and organ slices onto a tissue holder and place the holder inside the scCO2 vessel system. Decellularize the samples in the Applied Separations scCO2 system set to operate at 30–50◦C and 200–

Applied Separations

930 Hamilton Street
Allentown, PA 18101
610-770-0900
www.appliedseparations.com

350 bar for 40–90 min. After supercritical CO2 processing, slowly depressurize and remove the samples. Neutralize the samples using sodium hydroxide (0.1–1 N) to produce decellularized ECM scaffolds. Next, freeze dry the ECM scaffolds and cut to appropriate sizes to fit in the culture plates. The freeze-dried decellularized ECM scaffolds may also be subjected to freeze-milling to make a powder form of the scaffold with a different size sieve, Pack the powder vials and sterilized by irradiation (25 kGy)

Conclusion

Conclusion

Supercritical CO2 and ethanol are used to decellularize a multitude of natural tissue and organs. The method is simple to use and effective in producing intact scaffolds for tissue engineering and regenerative medicine. The method has successfully produced intact scaffolds from liver, brain, kidney, pancreas, artery, heart, skin, bone, cartilage, and corneal tissues. These natural ECM scaffolds produced by the scCO2 process retain the ECM biological signals necessary for stem cell migration, adhesion, proliferation and possibly differentiation for the reconstruction of specific tissues and organs.

References

Hsieh, Dar-Jen, et al. "Protocols for the preparation and characterization of decellularized tissue and organ scaffolds for tissue engineering." *BioTechniques* 70.2 (2021): 107-115.

CESUR, Nevra Pelin, Volkan YALMAN, and Melisa TÜRKOĞLU LAÇİN. "Decellularization of cow aorta via supercritical CO2." *Journal of Applied Biological Sciences* 14.3 (2020): 268-280.

Gafarova, Elvira R., et al. "Evaluation of supercritical CO2-assisted protocols in a model of ovine aortic root decellularization." *Molecules* 25.17 (2020): 3923.

You, Ling, et al. "In vivo immunogenicity of bovine bone removed by a novel decellularization protocol based on supercritical carbon dioxide." *Artificial cells, nanomedicine, and biotechnology* 46.sup2 (2018): 334-344.

Ling, You, et al. "Improved the biocompatibility of cancellous bone with compound physicochemical decellularization process." *Regenerative biomaterials* 7.5 (2020): 443-451.

Chou, Ping-Ruey, et al. "Supercritical carbon dioxide-decellularized porcine acellular dermal matrix combined with autologous adipose-derived stem cells: its role in accelerated diabetic wound healing." *International Journal of Medical Sciences* 17.3 (2020): 354.

Chen, Meng-Yen, et al. "Supercritical carbon dioxide decellularized xenograft-3d cad/cam carved bone matrix personalized for human bone defect repair." *Genes* 13.5 (2022): 755.

Chiu, Yen-Lung, et al. "Efficacy of Supercritical Fluid Decellularized Porcine Acellular Dermal Matrix in the Post-Repair of Full-Thickness Abdominal Wall Defects in the Rabbit Hernia Model." *Processes* 10.12 (2022): 2588.

Hsieh, Dar-Jen, and Periasamy Srinivasan. "Protocols for accelerated production and purification of collagen scaffold and atelocollagen from animal tissues." *BioTechniques* 69.3 (2020): 220-225.

Amirazad, Halimeh, Mehdi Dadashpour, and Nosratollah Zarghami. "Application of decellularized bone matrix as a bioscaffold in bone tissue engineering." *Journal of biological engineering* 16.1 (2022): 1-18.

Applied Separations

930 Hamilton Street
Allentown, PA 18101
610-770-0900
www.appliedseparations.com