Current Trends in Biomedical Engineering & Biosciences
ISSN: 2572-1151

Juniper Publishers
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Opinion
Volume 2 Issue 4 - March 2017
DOI: 10.19080/CTBEB.2017.02.555595

Curr Trends Biomedical Eng & Biosci

Decellularized Extracellular Matrix

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Submission: March 02, 2017; **Published:** March 27, 2017

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Abstract

It has been demonstrated that extracellular matrix (ECM) has a critical role in promoting the cell proliferation, cell growth and repair of organs after damage. A decellularization process has been developed to maintain the intact structure of the ECM, while efficiently eliminating the cellular components that lead to minimization the immune-related adverse effects. We have successfully developed a decellularization protocol using porcine adipose tissues. The DNA and cellular contents was not found in the decellularized ECM and in situ structure was greatly preserved.

What is the Extracellular Matrix

Extracellular matrix (ECM) is one of the fundamental structures that maintain the cells in tissues. It is a gel-like material of proteins and polysaccharides which have a unique composition depending on the types of tissues [1,2,5]. ECMs are commonly used in the tissue engineering field for the reconstruction of organs and surgical applications. It provides excellent microenvironment to enhance the cell proliferation, migration and differentiation [2].

Decellularization process

Decellularization protocols have been used to extract ECM from intact adipose tissue. It is the best choice for eliminating xenogeneic epitopes from naïve porcine tissues while maintaining their ECM composition including collagen, glycosaminoglycan (GAGs), fibronectin, and elastin. The main goal of the decellularization process is to efficiently remove all xenogeneic cellular antigens, which can cause adverse effects such as inflammatory response or immune-mediated rejection of the host tissues [1,3,4].

Description of the process

Porcine adipose tissues were purchased from local market in El Paso and were kept frozen at-80°C until use. Tissues were taken out from the freezer and thawed overnight and ground to make into a pasty mixture. The tissue was then placed in a beaker containing ultrapure water for 2 days. After that, 0.5M NaCl and 1M NaCl solution were prepared to immerse the tissue for 4 hours in each, respectively. Next, tissues were decellularized by being sequentially washed with ultrapure water, 0.25% trypsin, triton X-100, 1-propanol, and ultrapure water. The final ECM product was shown to be a white, clean and fibrous structure after lyophilization. It was either used immediately for further experiments, or otherwise stored in a sterile phosphate-buffered saline solution at 4 °C until use.

Evaluating the decellularization efficiency

Methods have been well established to evaluate the decellularized ECM [1-8]. Fluorescence-based analysis has mostly been used to confirm the residual DNA contents. In addition to them, decellularized samples of ECM were analyzed histologically to view the structure of the decellularized ECM in situ, and compared to intact porcine adipose tissues. The DNA content of the decellularized ECM was negligible (1.28±0.3ng/mg dry ECM) and the residual cellular components were stained by 4,6-diamino-2-phenylindole and hematoxylin and eosin. After decellularization, the structure of ECM seemed similar compared to the naïve adipose tissues and nuclei were not detected. Furthermore, common types of collagen in adipose tissues such as collagen I, II, III, and IV were strongly detected and showed to have sustained their structure.

Conclusion

This study reports the efficient process of porcine adipose tissue-derived extracellular matrix. While it is still required to demonstrate *in vitro* and *in vivo* biocompatibility of the decellularized ECM for further applications, this method could

provide with xenogeneic biomaterials and might have a chance to be applied in regenerative medicine.

Acknowledgment

This research was supported by the National Research Council of Science & Technology (NST) grant by the Korea government (MSIP) (CRC-15-03-KIMM). Thanks to Dr. Thomas Boland for allowing the use of biomedical laboratory to conduct research.

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DOI: 10.19080/CTBEB.2017.02.555595

How to cite this article: SooHwa J, Monica M, Rahulkumar B, Kyung-Hwan J, Nam-Soo Kim. Decellularized Extracellular Matrix. Curr Trends Biomedical Eng & Biosci. 2017; 2(4): 555595. DOI: 10.19080/CTBEB.2017.02.555595.