Comparative study of different types of decellularization methods for adipose tissue

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Introduction: Adipose tissue is often discarded after plastic and reconstructive surgery. However, it has previously been shown that adipose tissue is a promising stem cell-source (1), as well as a rich source of human extracellular matrix (ECM) material (2). Hence, several research groups have used combinations of enzymatic, chemical and physical agents to decellularize adipose tissue for extracting the ECM, which is essential to organ functioning (3,4). Although these methods were capable of removing the cells, lipid and DNA contents, their major drawbacks include utilization of huge amounts of reagents and long processing times (2). As such, this study focuses on the comparison of purely physical, chemical and enzymatic method of decellularization on their ability to maintain structure; remove lipid and DNA content; and preserve biochemical composition. Overall, we observed that the physical method is advantageous as compared to the other methods in complying with the aforementioned parameters.

Methods: Adipose tissue samples were procured from TTSH following procedures established by National Healthcare Group Domain Specific Review Board (DSRB 2012/00071) and weighed. Samples were then decellularized by purely enzymatic, chemical and physical method. The enzymatic method uses trypsin, DNase and RNase; chemical method uses sodium dodecyl sulphate (SDS) and Triton-X100 while the physical method uses the homogenizer. In order to ensure complete decellularization, cryosections of the different sample groups were stained using Oil Red O. This staining technique discloses the effectiveness of the decellularization protocols to remove cells and lipid. Concentration of residual DNA was estimated using picogreen assay. Scanning electron micrographs reveal the structure of the ECM post various decellularization processes. ELISA assays (for Glucoseaminoglycans (GAGs), Vascular Endothelial Growth Factor (VEGF) and basic Fibroblast Growth Factor (bFGF)) determine the degree to which these methods are superior in preserving the biochemical composition of the tissue.

Results: The ECM that resulted from physical processing (ECM-P), showed more efficient cell and lipid removal in comparison to the one extracted by the enzymatic and chemical method (ECM-E and ECM-C, respectively) (Figure 1). The DNA content after all the methods was estimated to be <50ng/g, which is the criteria for a material to be safe for implantation (Table 1). ECM-P had the highest levels of GAG, VEGF, bFGF and collagen contents (Table 2). Our data suggests that the ECM extracted by physical processing is capable of conserving the biochemical properties of the native tissue.

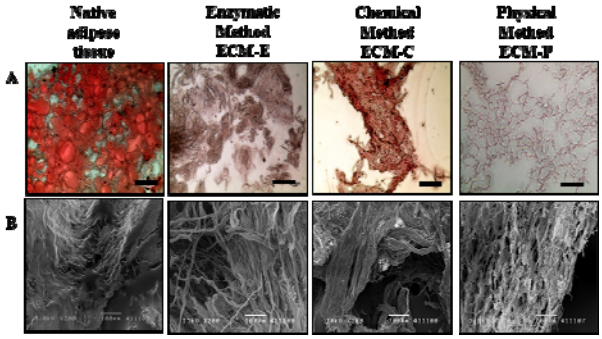

Figure 1 Characterization of different decellularization methods by a) Oil Red O staining and b) Scanning electron microscopy.

Table 1 DNA content for the different decellularization methods

Method	DNA content
Enzymatic	37.4ng/mg
Chemical	40.1ng/mg
Physical	48.6ng/mg

Table 2 Concentration of GAG, bFGF and VEGF for the different decellularization methods

Method	GAG content µg/ g	bFGF content pg/g	VEGF content pg/g
Enzymatic	17.01±5.47	0.001	0.55±0.10
Chemical	277.72±100.19	0.32±0.17	0.37±0.02
Physical	2293.3±164.9	206.32±48.16	8.16±1.03

Conclusion: The physical method for obtaining ECM from adipose tissue requires a shorter processing time, and also allows for the preservation of biochemical components of the tissue more efficiently. Hence, ongoing studies are being carried out to investigate the response of cells and tissues to the ECM-P. It is envisioned that ECM-based scaffolds or coatings could be useful for various tissue engineering applications.

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