Decellularized Adipose Tissue-Derived Extracellular Matrix as a Bioactive Coating Material

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Introduction: Adipose tissue is often discarded after plastic and reconstructive surgery in the form of intact fat tissue or lipoaspirates. However, adipose tissue has also been identified as a rich source of human extracellular matrix (ECM) [1]. The native ECM has been utilized as scaffolding material for tissue engineering due to their advantages in biocompatibility and their biological properties matching *in vivo*. However, the current methods developed for decellularization either involve the use of detergents such as sodium dodecyl sulphate, or tedious and lengthy processes with enzymatic digesting [2]. Even after repeat rinsing, it is still relatively difficult to completely remove all these toxic chemicals, thus limiting its applications. Therefore, it is necessary to develop alternative methods that are able to decellularize the adipose tissue without the use of any harsh chemicals whilst requiring a shorter processing period. As such, the aim of this study is to investigate the efficacy of the supercritical CO_2 method for decellularization of lipoaspirates and to utilize the ECM as a bioactive surface coating material.

Methods: Freshly excised lipoaspirate samples were obtained from patients at Tan Tock Seng Hospital, Singapore following procedures established by NHG Domain Specific Review Board (DSRB 2012/00071). Briefly, lipoaspirate was rinsed with distilled (DI) water until all the blood components were completely removed. Subsequently, the lipoaspirate was left stirring in ethanol solution for 20 min. The ethanol solution was changed and the procedure was repeated once again. The resultant tissue was then loaded into the reaction vessel of the set-up with pure ethanol as modifier. Liquid carbon dioxide was compressed with a pressurizing pump and made to flow into the reaction vessel until the desired pressure (180 bar) was achieved. The temperature of the reaction vessel was maintained at 37^0C and the decellularized adipose tissue ECM was collected after 3 h. Confirmation of decellularization was carried out using H&E staining, Oil Red O staining, Picogreen® assay and scanning electron microscope (SEM). Finally, various types of cells (i.e. immortalized human keratinocyte (HaCaT cells), human monocytic leukemia cells (THP-1 cells), human umbilical vein endothelial cells (HUVECs), and adipose tissue-derived mesenchymal stem cells (MSCs)) were cultured on the ECM coated tissue culture plate (TCP) to evaluate the cellular behaviour.

Results: The supercritical CO_2 decellularization method was successfully used to obtain the decellularized ECM. H&E and Oil Red O staining results showed no evidence of visible nuclear material and lipids in the ECM respectively. The amount of dsDNA left in the dry ECM was 43.51±3.96 ng/mg, which is safe to be used for clinical applications [3]. SEM analysis further confirmed the removal of both cells and lipids from the remaining fibrous ECM material. In addition, immunostaining results showed that key structural proteins such as collagen type I and laminin remained intact in the ECM, together with other biological factors such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF). To our knowledge, the concentration of these biological factors is much higher than current existing methods. Various types of matricellular proteins that have implications in wound healing and angiogenesis, were also found intact in the adipose tissue-derived ECM for the first time using our alternative method (Figure 1a). FTIR and contact angle results showed that successful coating of ECM was done on the tissue culture plate (TCP) surface. The ECM-coated surface promoted significant proliferation of HaCaT cells, HUVECs and MSCs. In addition, the HaCaT cell migration results showed that the ECM coating would be able to improve wound healing (Figure 1b). The THP-1 cell culture results showed no immunogenicity for the ECM-coated surface.

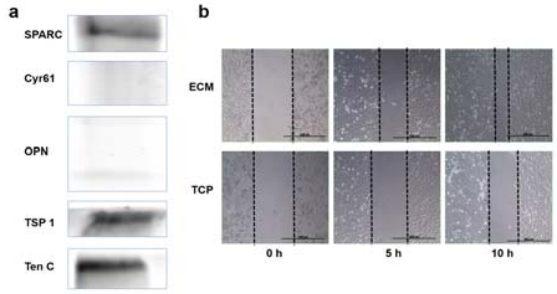

Figure 1. Key matricellular proteins were retained in ECM material: (a) western blot of SPARC, Cry61, OPN, thrombospondin 1 (TSP 1) and tenascin C (Ten C) in the ECM, (b) HaCaT cell migration test on ECM- coated TCP and normal TCP (black dotted lines demarcate the scratch gap at the time of wounding)

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Conclusion: An environmental-friendly method was developed to obtain the ECM from lipoaspirate. The ECM was used as a novel coating material, which showed to improved soft tissue regeneration, angiogenesis and wound healing. Further explorations on the use as a surface coating material onto other commercially available products to improve the performance can be studied. Overall, this study shows that the decellularized adipose tissue ECM can be used as bioactive material for tissue engineering and clinical applications.

References:

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