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Isolation of adipose derived stem cells

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Introduction

Adipose-derived stem cells (ASCs) are multipotent mesenchymal stromal cells with tri-lineage differentiation potential, ability of self-migration to injured tissue, fewer ethical controversies, and lower risk of rejection [1,2]. The critical element in the regenerative properties of fat grafting depends on ASC richness [3]. Separation of ASCs from adipose tissue requires an isolation protocol and should be considered in terms of quality, safety and efficacy. There are no high-quality comparative studies of ASCs isolation, so no guidelines or recommendations about methods of isolation are available. Inconsistent results may be obtained depending on the separation protocols in the experimental studies due to absence of standard separation method.

The most commonly used enzyme for ASCs isolation is collagenase. Collagenase digests the triple helix of peptide bonds in the collagen of adipose tissue. The use of collagenase which is originated from bacteria is expensive, and a time-consuming procedure [4]. Collagenase digestion to obtain ASCs is inevitably accompanied by destroying the contact between cells and this technique is regarded as a substantial manipulation [5]. According, ASCs should be mechanically isolated without using enzymes according to current European legislation [5]. The use of proteolytic enzymes (t collagenase, trypsin-EDTA solution, dispase) may adversely affect cell viability, senescence, multipotency and cell surface antigen expression. Therefore, clinical applications need to be reconsidered [6]. Additionally, collagenase preparations have also been shown to activate human complement, which could induce a local inflammatory reaction [7].

Isolation methods that do not use enzymes have also been developed. However, the non-enzymatic isolation needs large volume of lipoaspirate and have lower efficiency in cell recovery compared with enzymatic isolation because of low viability rate of ASCs [8].

Classical enzymatic protocol

The protocol regarded by Zuk et al. is still regarded as the most widely used method with some modifications for ASC isolation [9]. We will describe the isolation process of ASCs step by step.

Washing

Harvested lipoaspirate is washed with sterile 1% equal volumes of phosphate-buffered saline (PBS) until golden in color [9,10].

Digestion

Digestion with 0.075% or 0.01% collagenase/PBS solution, at a ratio of 1 ml of enzyme solution to 1 cm^3 of lipoaspirate can degrade the tight junctions and the extracellular matrix components [9,10]. This mixture is incubated at 37°C with intermittent shaking until it becomes cloudy for 30 min. After enzymatic treatment, stromal vascular fraction (SVF) can be obtained from lipoaspirate. SVF is a heterogeneous cell population composed of endothelial cells, pericytes, monocytes, macrophages, lymphocytes, red blood cells, progenitor cell and ASCs [1].

Neutralization

Neutralization of enzyme activity is then performed by adding Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS. [9]. 1% antibiotic-antimycotic solution could be added in DMEM. [11]. The sample is centrifuged at 1200 g for 10 min or 350 g for 3 min to obtain a high-density SVF pellet [9,11].

RBC lysis

To lyse red blood cells, the pellet is then suspended in NH4Cl and incubated for 10 min at room temperature This step can be omitted [10,11].

Filtering, and seeding for cell culture

The SVF is collected by centrifugation, filtered through a 100 μm mesh and incubated overnight at 37 °C and 5% CO2 in DMEM, 10% FBS, and 1% antibiotic/antimycotic solution [9]. The procedure could be performed in following orders. Passing through a strainer with a 100-μm pore diameter, centrifuging for 3 min at 350 g, suspending of the cell pellets in DMEM and seeding in culture dishes [11].

In study of Li et al. [12], the effects of NH4Cl and hypotonic NaCl solution on ASCs were compared when erythrocyte was removed. 0.3% NaCl solution was more safe, convenient, and cost-effective in ASC purification than NH4Cl.

The spontaneous stratification at 20 min instead of the centrifugation at 400 g for isolation of SVF is a method to guarantee highly viability and differentiation potential of ASCs [13].

Raposio et al [14] shortened the time for ASC isolation using enzymes by methods of Ready-to-Use ASCs Pellet. Whole processes are composed of centrifugation (remove the oil and serous fractions), enzymatic digestion (ASCs are released by from the surrounding connective tissue scaffold) a double series of washing and centrifugation.

Non-enzymatic methods

The isolation of ASCs using non-enzymatic methods have been described by several groups [4,10,15].

Bianchi et al used disrupted adipose tissue mechanically in a closed system to reduce the size of lipoaspirates [15]. Shah et al. [4]

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confirmed ASCs isolated by lipoaspirate washing protocols on the base of fluorescence activated cell sorting analysis possessed a distinct and potentially favorable immunophenotype relative to ASCs via the collagenase digestion protocols. However, these techniques are time-consuming compared to classic enzymatic isolation of ASCs.

Busser et al. [16] suggested a novel one-step, non-enzymatic methods to isolate ASCs from lipoaspirate. This new method is easier, safer, faster, and less expensive compared to the collagenase methods. Cells obtained with this method had a better long-term hematopoietic support in comparison with those with classic, enzymatic methods.

Innovative isolation technique

Enriching lipoaspirate with SVF cells obtained after enzymatic isolation could improve the long-term volume persistence of soft tissue augmentation [17]. As point of care device at operation room, three devices used for isolation of SVF were compared [18]. The authors found that the lipoaspirates enriched with enzymatic digested SVF cells had a higher number of cells more than with non-enriched lipoaspirate or enriched with a mechanical method, while stemness marker expression showed no difference. Besides, the cells isolated from the lipoaspirates enriched with enzymatic methods showed a significant colony-forming efficiency. The ASCs extracted from enriched lipoaspirate grew and differentiated faster with respect to non-enriched lipoaspirate or mechanical enrichment procedures [18].

Zhu et al. [19] reported innovative technology based on washing with filtration within a closed system supplied higher tissue viability and less contaminants to lipograft. Tremolada et al [20] found non-enzymatic fat harvest with minimal manipulation through soft mechanical action was characterized with intact SVF cells, unaltered the exosome content, and tissue healing effects of the micro-fragmented tissue.

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