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Adipose-derived stem cells: Comparison between two methods of isolation for clinical applications

Edoardo Raposio [a, b], Francesco Simonacci [a, b, *], Rosario E. Perrotta [c]

[a] Department of Medicine and Surgery, Plastic Surgery Division, University of Parma, Parma, Italy
[b] Cutaneous, Mininvasive, Regenerative and Plastic Surgery Unit, Parma University Hospital, Parma, Italy
[c] Department of Medical and Surgery Specialties, Section of Plastic Surgery, University of Catania, Catania, Italy

HIGHLIGHTS

- Adipose-derived stem cells (ASCs) are effective mesenchymal stem cell population with enormous potential.
- In this study we compared two methods of adipose-derived stem cells (ASCs) isolation.
- Gathered data showed a greater amount of isolated ASCs by the ME procedure as compared to the MC one.

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ABSTRACT

Background: Adipose-derived stem cells are recognized as being an effective mesenchymal stem cell population with enormous potential in different fields of regenerative medicine and stem cell therapy. Although there is unanimous agreement on the harvesting procedure for adipose tissue, there are various protocols for adipose-derived stem cell isolation. The aim of this study was compare two methods of adipose-derived stem cells (ASCs) isolation, one based on a mechanical + enzymatic (ME) procedure and the other one exclusively mechanical (MC), in order to determine which one was superior to the other in accordance with current European and US legislation.

Methods: We reported step by step the two different methods ASCs isolation by comparing them. The ME procedure included the use of a centrifuge, an incubator and collagenase digestion solution (Collagenase NB 6 GMP Grade 17458; Serva GmbH, Heidelberg, Germany). The MC procedure was performed by vibrating shaker and centrifuge, both placed in a laminar airflow bench.

Results: With the ME procedure, a mean of 9.06×10^5 ASCs (range, 8.4 to 9.72×10^5; SD $\pm 6.6 \times 10^5$) was collected, corresponding to 25.9% of the total number of harvested cells. With the MC procedure, a mean of 5×10^5 ASCs (range: 4.0 to 6.0×10^5; SD, $\pm 1 \times 10^5$) was collected, corresponding to 5% of the total number of harvested cells.

Conclusion: Based on data collected, from the same amount of lipoaspirate the ME procedure allowed to isolate a greater number of ASCs (25,9%) compared to the MC one (5%).

1. Introduction

Fat is an active and dynamic tissue composed of several different cell types, including adipocytes, fibroblasts, smooth muscle cells, endothelial cells, and adipogenic progenitor cells called pre-adipocytes [1–4]. The evidence that MSCs (mesenchymal stem cells) could be isolated from adipose tissue has resulted in the shared idea that subcutaneous adipose tissue can be regarded as the ideal source of MSCs and as a viable alternative to bone marrow [5]. Indeed, subcutaneous adipose deposits are accessible, abundant, and can be collected in large quantities, thus providing a potential adult stem cell reservoir for each individual. Adipocytes constitute almost 90% of adipose tissue volume and nearly 65% of the total cell number [6]. Stem cells isolated from lipoaspirates have demonstrated a broad in vitro adipogenic, chondrogenic, osteogenic, and myogenic lineage commitment [7,8] as well as differentiation into pancreatic cells, hepatocytes, and neurogenic cells [9–11]. ASCs (adipose-derived

* Corresponding author. Department of Medicine and Surgery, Plastic Surgery Division, Parma University and Maggiore Hospital, Via Gramsci 14, 43126, Parma, Italy.
E-mail address: francescosimonacci@hotmail.it (F. Simonacci).

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stem cells) are a plastic-adherent, multipotent stem cell population, which display a similar differentiation potential to other MSCs, and the ability to differentiate into cells of several lineages from all three germinal layers [12]. Cytometric analysis of adipose-derived stem cells (ASCs) has shown that these cells do not express CD31 and CD45, but do express CD34, CD73, CD105, and the mesenchymal stem cell marker CD90 [13,14]. ASCs have a differentiation potential similar to that of other mesenchymal stem cells as well as a higher yield upon isolation and a greater proliferative rate in culture than bone marrow–derived stem cells [15–17]. Treatment with ASCs has proven to be crucial for the regeneration of tissue through the chemotactic, paracrine, and immunomodulatory activities and their in situ differentiation [18–21]. Owing to these potentials and because they can be easily harvested in great amounts with minimal donor-site morbidity, ASCs are particularly promising for regenerative therapies [15,17,22]. A variety of tissues and organs engineered using ASCs have been described [23]. In vitro studies rapidly progressed to in vivo experiments, where ASCs were tested with or without appropriate scaffolds in order to assess their capability to effectively regenerate and repair tissues or organs [23]. The potential of ASCs to self-renew and regenerate tissue has great implications in wound healing and skin restoration [24]. As reported in literature the fat grafting containing adult ASCs is a highly effective therapeutic approach for the treatment of degenerative, chronic lesions induced as late effects of oncologic radiation treatments [25], the correction of secondary contour defects after breast reconstruction [26–30], release of painful scar contractures [31], and treatment of burn scars [32]. ASC therapeutic effects in cranial bone, articular chondrocytes, cardiac wall regeneration, functional repair after myocardial infarction and functional improvement after stroke have all been investigated [33–37]. Some of the earliest uses of ASCs in wound healing were in the treatment of chronic fistulae in Crohn's disease with the successful healing of rectovaginal fistula [23]. Although there is unanimous agreement on the harvesting procedure for adipose tissue, it is noteworthy though that there is not a standardized protocol to isolate ASCs for clinical application, which led to an inconstancy in literature [38–40]. Hence, there is a need of a standardized method for clinical purposes, which optimize and unify process schedule and isolation procedure, as well as the whole tissue manipulation [13]. In this study we compared two methods of adipose-derived stem cells (ASCs) isolation, one based on a mechanical + enzymatic (ME) procedure and the other one exclusively mechanical (MC).

2. Materials and methods

In 2016, we described the ME procedure (Fig. 1) carried out through both mechanical (centrifugation) and enzymatic (collagenase) isolation process [13,22]. The ME procedure which was specifically designed for clinical application, appeared easy, safe and fast (80 min), allowing collection of a ready-to-use ASC pellet. The operating room was set up with a centrifuge (Lipokit; Medikhan, Korea) and an incubator (Celltibator; Medikhan, Korea). After a conventional liposuction, the harvested fat tissue (100 ml) underwent a first centrifugation (1600 RPM × 6 min), obtaining about 50 ml of high quality concentrated adipose tissue, which was suddenly mixed with 50 ml collagenase digestion solution (Collagenase NB 6 GMP Grade 17458; Serva GmbH, Heidelberg, Germany), previously diluted with sterile phosphate-buffered saline (PBS) as follows: 1 g of collagenase was suspended in 10 mL PBS, and 1 mL of the obtained solution was further added with 49 mL of PBS. The solution obtained (lipoaspirate + collagenase digestion solution) was then incubated for 30 min at 37 °C in a shaker-incubator (Celltibator; Medikhan) and, after that, it was centrifuged at 200 relative centrifuge force 4 min. Subsequently, only 10 mL of SVF were left and washed 2 times, each one with 45 ml saline solution. After each washing, syringes containing SVF were positioned inside the centrifuge at 200 relative centrifuge force 4 min. The cellular pellet at the bottom of the syringe was then ready for use, vehiculated by 5 mL of saline solution. The entire process was performed in a closed- circuit system, which guaranteed sterility and the safety. Once an ASC pellet was obtained in this manner, it was injected into the skin at the wound edges, as well as at the bottom of chronic skin ulcers, to promote wound-healing processes [15]. The MC isolation process (Fig. 2) was performed by a vibrating shaker (Multi Reax; Heidolph, Schwabach, Germany) and a centrifuge (MPW 223; Johnson & Johnson Medical, New Brunswick, N.J.), both placed into a laminar air flow bench (1200 FLO; FIMS, Concorezzo, Italy) [14]. After liposuction, the harvested fat tissue (80 ml) was collected in eight 10-ml plastic test tubes, positioned in the vibrating shaker at 6000 vibrations/minute for 6 min, and immediately after, centrifuged at 1600 rpm for 6 min. Subsequently, always under the same laminar flow cabinet as above, the pellet at the bottom of each tube is collected by means of an automated pipetting system (Rota-Filler 5000; Heathrow Scientific, Nottingham, United Kingdom) and poured into a 10-ml Luer-Lok syringe. The entire isolation process lasted approximately 15 min. After the ASCs obtained were injected into the bottom and the edge of the wounds.

3. Results

In both procedure, the percentage of vitality cells was 99%. Isolated cells were characterized by flow cytometry assay, using the following markers: $CD31^-$, $CD34^+$, $CD45^-$ $CD73^+$, and $CD90^+$. With the ME procedure, a mean of 9.06×10^5 ASCs (range, 8.4 to 9.72×10^5; SD $\pm$ 6.6×10^5) was collected from 100 ml of lipoaspirate, corresponding to 25.9% of the total number of harvested cells (mean: 3.5×10^6). With the MC procedure, a mean of 5×10^5 ASCs (range: 4.0 to 6.0×10^5; SD, $\pm 1 \times 10^5$) was collected from 80 ml of lipoaspirate, corresponding to 5% of the total number of harvested cells (mean: 1×10^7). Considering 100 ml of lipoaspirate MC procedure allowed to obtain a mean of 6.25×10^5 ASCs (range: 5.0 to 7.5×10^5; SD, $\pm 1 \times 10^5$), corresponding to 5% of the total number of harvested cells (mean: 1.25×10^7).

4. Discussion

Gathered data showed a greater amount (25.9%) of isolated ASCs by the ME procedure as compared to the MC one (5%). Considering a total of 100 ml of lipoaspirate the ME procedures allowed to isolate a mean of 9.06×10^5 ASCs (range, 8.4 to 9.72×10^5; SD $\pm$ 6.6×10^5) compared to a mean of 6.25×10^5 ASCs (range: 5.0 to 7.5×10^5; SD, $\pm$ 1×10^5) by means the MC procedure (Fig. 3). This discrepancy, in our opinion, was partly justified by the greater amount of total cells yielded by the MC procedure (mean: 1.25×10^7) as compare to the ME procedure (mean: 3.5×10^6). Despite the ME procedure was superior as regards the number of ASCs isolated, the time required for the entire isolation process was greater (80 min) compared to the MC procedure (15 min). The use of collagenase as an injectable pharmaceutical is associated with the risk of serious side effects, such as cutaneous ulcers, nerve injury, tendon or ligament damage, and allergic reactions [14]. However, no study has been carried out reporting the residual collagenase activity in adipose-derived stem cell samples inoculated in humans [14]. Chang et al. [41] analyzed the toxicity of residual collagenase in mice. They reported that residual collagenase activity could not be found after only several washing steps. Moreover, there are no studies establishing fetal bovine serum (used for collagenase inactivation) security regarding its clinical use [14]. ASCs have come to be regarded as the ideal stem cell for