



Improvement of adipose tissue–derived cells by low-energy extracorporeal shock wave therapy

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Abstract

Background. Cell-based therapies with autologous adipose tissue–derived cells have shown great potential in several clinical studies in the last decades. The majority of these studies have been using the stromal vascular fraction (SVF), a heterogeneous mixture of fibroblasts, lymphocytes, monocytes/macrophages, endothelial cells, endothelial progenitor cells, pericytes and adipose-derived stromal/stem cells (ASC) among others. Although possible clinical applications of autologous adipose tissue–derived cells are manifold, they are limited by insufficient uniformity in cell identity and regenerative potency. **Methods.** In our experimental set-up, low-energy extracorporeal shock wave therapy (ESWT) was performed on freshly obtained human adipose tissue and isolated adipose tissue SVF cells aiming to equalize and enhance stem cell properties and functionality. **Results.** After ESWT on adipose tissue we could achieve higher cellular adenosine triphosphate (ATP) levels compared with ESWT on the isolated SVF as well as the control. ESWT on adipose tissue resulted in a significantly higher expression of single mesenchymal and vascular marker compared with untreated control. Analysis of SVF protein secretome revealed a significant enhancement in insulin-like growth factor (IGF)-1 and placental growth factor (PLGF) after ESWT on adipose tissue. **Discussion.** Summarizing we could show that ESWT on adipose tissue enhanced the cellular ATP content and modified the expression of single mesenchymal and vascular marker, and thus potentially provides a more regenerative cell population. Because the effectiveness of autologous cell therapy is dependent on the therapeutic potency of the patient's cells, this technology might raise the number of patients eligible for autologous cell transplantation.

Key Words: adipose tissue, adipose-derived stromal/stem cells, extracorporeal shock wave therapy, stromal vascular fraction

Introduction

Cell-based therapies with autologous adipose tissue–derived cells have shown great potential in several clinical studies in the last decades. In the field of aesthetic and reconstructive medicine an abundance of knowledge was accumulated in the last century [1] and later was extended through clinical studies in regenerative medicine and tissue engineering [2–4]. The majority of studies have been using the stromal vascular fraction (SVF), a heterogeneous mixture of fibroblasts, lymphocytes, monocytes/macrophages, endothelial cells, endothelial progenitor cells, pericytes

and adipose-derived stromal/stem cells (ASC) among others [5–10]. In clinical case studies and trials treating soft tissue defects [4,11–14], bone and cartilage defects [15–19], gastrointestinal lesions [20], immune disorders [21,22], neurological injuries [23] and cardiovascular diseases [24], SVF and ASC have already proven their regenerative potential. Although possible clinical applications of autologous adipose tissue–derived cells are manifold, they are limited by drawbacks concerning stem cell identity and potency of the isolated cell population. Different cell isolation protocols and methods but also closed automated isolation devices bring up cell populations with

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variable content of potentially therapeutic cells within the fat graft or the SVF [25]. In addition, donor variability results in a highly heterogeneous cell composition and functionality, which may reduce reproducibility and efficacy, and increase the risk for transplantation of low-potent cells into the patient. SVF cells and ASC can be characterized with a distinct surface marker profile and have the ability to differentiate at least into the mesodermal lineages. This is defined in a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) together with the International Society for Cellular Therapy (ISCT) to provide guidance for standardization between different research groups [26]. Cultivation, purification and differentiation of ASC are standard procedures for clinical trials, but it remains difficult to meet the requirements of regulatory agencies for stem cell translation into clinics. To increase therapeutic cell potency, numerous strategies have been evaluated for activation of cells or cell material such as physical stimulation using low level light therapy (LLLT) [27–29], photobiostimulation [30,31] or radio electric asymmetric conveyer [32]. Moreover, the efficiency of ASC transplants was improved by the addition of activated platelet-rich plasma (PRP) [33,34] or growth factors [35].

In this study we aimed to improve stem cell properties and reduce donor variability by mild mechanical stimulation using extracorporeal shock wave therapy (ESWT).

Extracorporeal shock waves are sonic pulses, characterized by an initial increase, reaching a positive peak of up to 100 MPa within 10 ns, followed by a negative amplitude of up to -10 MPa and a total life cycle of less than 10 μ s [36]. Biological responses are thought to be triggered by the high initial pressure, followed by a tensile force and the resulting mechanical stimulation [36]. ESWT has been applied for several decades in the clinics and has demonstrated beneficial effects on tissue regeneration in non-union fractures [37–39], ischemia-induced tissue necrosis [40] or post-traumatic necrosis, disturbed healing wounds, ulcers and burn wounds [41,42]. We have previously shown that low-energy ESWT enhances proliferation and differentiation of ASC lines *in vitro* [43,44]. These *in vitro* studies corroborate the clinical success of ESWT in wound healing, nerve regeneration and vascularization [45,46].

In our experimental set-up, low-energy ESWT was applied to freshly isolated SVF cells from human adipose tissue aiming to equalize and enhance cell properties and functionality. To limit the degree of manipulation of the cells during the SVF isolation process we applied in a second approach ESWT directly on the freshly obtained human adipose tissue and compared it with ESWT on isolated SVF cells.

Based on this, we studied cellular adenosine triphosphate (ATP) content, immunophenotype, cell yield, viability, colony-forming unit fibroblast (CFU-F) assay and protein secretome of the SVF. Furthermore, we cultured ASC from these SVF and investigated proliferation and differentiation potential toward the adipo-, osteo- and chondrogenic lineage.

Methods

SVF/ASC isolation

The use of human adipose tissue was approved by the local ethical board with patient's consent. Subcutaneous adipose tissue was obtained during routine outpatient liposuction procedures under local tumescence anesthesia. SVF isolation was performed as modified from Wolbank *et al.* [47] as follows. Briefly, 100 mL liposuction material was transferred to a blood bag (400 mL Macopharma) and washed with an equal volume of phosphate-buffered saline (PBS) to remove blood and tumescence solution. Afterward, for tissue digestion PBS was replaced with 0.2 U/mL collagenase NB4 (Serva) dissolved in 100 mL PBS containing $\text{Ca}^{2+}/\text{Mg}^{2+}$ and 25 mmol/L N-2-hydroxyethylpiperazine-N0-2-ethanesulfonic acid (HEPES; Sigma) and the blood bag was incubated at 37°C under moderate shaking (180 rpm) for 1 h. The digested tissue was transferred into four 50-mL tubes (Greiner). After centrifugation at 1200g for 7 min, the cell pellets were incubated with 100 mL erythrocyte lysis buffer (154 mmol/L ammonium chloride [Sigma], 10 mmol/L potassium bicarbonate [Sigma], 0.1 mmol/L ethylenediamine-tetraacetic acid [EDTA; Biochrom] in aqua dest) for 3 min at 37°C to eliminate red blood cells. The supernatant was aspirated after centrifugation for 5 min at 500g. The pellets were pooled, washed with PBS and filtered through a 100- μ m cell strainer (Greiner). After another centrifugation step at 500g for 5 min, the supernatant was removed and the isolated SVF cells were cultured in endothelial growth medium (EGM-2; Lonza) at 37°C, 5% CO_2 and 95% air humidity or resuspended in EGM-2 for further analyses. To obtain the adherent cell fraction including ASC, SVF were seeded on a plastic surface in expansion media (EGM-2), and cultured to a subconfluent state. Media was changed every 3 to 4 days. Cells were detached with Accutase (PAA) for 5 min at 37°C and collected in a tube. After centrifugation the pellet was resuspended in EGM-2 and cells were quantified with trypan blue exclusion in a cell counter (TC-20; Biorad).

In vitro ESWT

For *in vitro* shock wave treatment, an unfocused electrohydraulic device was used (DermaGold 100; MTS

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