Minimally Invasive Surgical Protocol for Adipose Derived Stem Cells Collection and Isolation - Ovine Model

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Many methods of stem cells collection and isolation from various tissue types harvested either from small or large experimental animals or from human tissues have been published so far, all evaluating them as a potential source of adult mesenchymal stem cells with applicability in various pathologies or tissue bioengineering. The present study purposed to describe a minimally invasive surgical protocol for adipose tissue collection from sheep’s inter-scapular area. The procedure was carried out on adult sheeps, in aseptic conditions. A light sedation protocol with Detomidine was performed, the recovery from anesthesia being carried out with Atipamezole. Throughout the sedation, the surgical procedure and the recovery from anesthesia, the vital functions of the animal were monitored. The adipose tissue samples collected in sterile tubes with culture medium (Dulbecco’s modified Eagle’s medium - DMEM/10% FBS10 - fetal bovine serum, 2% antibiotic/antifungal), have been successfully used by our research team for adipose tissue derived stem cells (ADSCs) isolation for further use in cardiac valves tissue engineering.

Keywords: adipose tissue, sheep, stem cells, Detomidine, Atipamezole

Many methods of stem cells isolation from various tissue types harvested from small or large experimental animals or from human tissues have been published so far, all evaluating them as a potential source of adult mesenchymal stem cells with applicability in various pathologies or tissue bioengineering. Mesenchymal stem cells are characterized by the ability to renew and differentiate both into mesodermal tissues (osteoblasts, adipocytes, chondrocytes and myocytes) and other cell types, such as astrocytes and neurons [1-3]. In this regard, the isolation of mesenchymal stem cells from bone marrow [4,5], adipose tissue [6-8], umbilical cord blood [9], peripheral blood [10,11], as well as from the amniotic fluid [12] and the dermal tissue [13] have been published.

Due to the easy protocol of collection, isolation and the great potential of proliferation and tissue differentiation in multiple cell lines, adipose tissue proved to be an attractive source of multipotent stem cells with potential use in regenerative medicine and tissue engineering [14]. From this point of view, the ovine model has been found to be of real importance in pre-clinical studies in the fields like orthopedic [15], urology surgery [16] and heart valves tissue engineering. Starting with the first collection and isolation of fat cells performed on mice by Rodbell [17], the method was applied thereafter to other animal models, being later modified for use in human models. Benea et al (2018) found on a rabbit model that collagen scaffolds seeded with adipose stem cells derived from liposuction fluid could be a valuable option in cartilage regeneration [19]. Hurmuz et al (2016) suggested that adipose stem cells derived from the human infrapatellar fat pad and isolated by a non-enzymatic method, have similar morphological and phenotypical characteristics with mesenchymal stem cells [20].

In 2017, Berea and colleagues obtained a 3D scaffold-free construction with potential use in bone tissue engineering using a mixture of human ADSCs with Endothelial Cells and Fibroblasts [21]. Realizing the great regenerative potential of ADSCs, in 2016, Tremolada et al developed a new technology in which the fat tissue resulted from liposuction is washed and micro-fragmented without enzymes or other additives. They reported that the resulting product has been shown to have a great regenerative potential based on adipose derived mesenchymal stem cells, containing pericytes within an intact stromal vascular niche. Lipogems technology is actually used with promising results in numerous clinical applications, plastic and reconstructive surgery and degenerative joint diseases [22].

For decades, sheep have been chosen as one of the most used large animal model in the medical research, due to the similarities in size and physiology to humans, but also for the easy handling during surgical procedures, unlike rodents [23].

Ovine subcutaneous fat tissue, regardless of the anatomical harvest area, is an attractive source of multipotent stem cells with a great regenerative potential and wide applicability in tissue engineering. According with Mrugala et al (2008), ovine ADSCs have similar properties and morphology to stem cells isolated from bone marrow [24].

Grzesiak et al (2011) reported that adipose mesenchymal stem cells isolated from the sternum region have less differentiation potential comparing with those isolated from back, perineum or tail base, suggesting that the isolation from the ovine’ sternum should be avoided [25].

In our investigation, after several harvesting attempts, performed by the research team from different anatomical regions (inter-scapular, paravertebral - lumbar and coccygeal, groin, perimammar, great omentum and perirenal), the most appropriate anatomical location for...
collection and ADSCs isolation was the inter-scapular region. The harvesting region was chosen for several reasons: the easier access to the dorsal side of the animal on the operating table, a low infectious risk pre- and post-operatively (the ventral/inguinal region was avoided because of the high infectious potential), a better represented fat tissue at this level than other regions, such as the paravertebral area, and last but not least, a high consistency of viable cells [26].

This study aimed to present a minimally invasive surgical protocol used by our research team for adipose tissue collection and isolation of ADSCs in cardiac valves tissue regeneration.

Experimental part
Material and methods

The minimally invasive procedure was carried out on six adult sheeps, weight 20±3 kg age between 6 and 12 months. The surgical interventions were performed intra-vitally on light sedation, in aseptic conditions, at the Experimental Station of the University of Medicine and Pharmacy of Tg-Mures. The Ethics board of UMF Tg-Mures approved the study with the reference no.131/2016.

Anesthesia: For sedation, an α-2 adrenoceptor agonist was used, intravenously Detomidine (10-20µg/kg) and inhaled Sevoflurane subsequently by mask (fig. 2-a). The cardiopulmonary function was monitored during the surgery.

Protocol: The experimental animal was placed in lateral decubitus and the inter-scapular region was shaved and aseptized with Betadine solution (fig. 2-b). After isolation with sterile fields, a 2 cm skin incision was performed with the subcutaneous dissection (fig. 2-c). Given that the stem cells in the adipose tissue are of pericyte type, the collection of a well-vascularised fat tissue was targeted. After the adipose tissue collection, ADSCs were isolated using the collagenase method: samples of 0.5-1 cm³ were collected in sterile tubes with culture medium DMEM/10% FBS10 -fetal bovine serum, 2% antibiotic/antifungal). (fig. 2-d) the ADSCs being isolated and cultured in vitro as described by Zuk (2001) [29] and Gimble (2003) [3] for subsequent use in heart valves tissue regeneration. The ability of isolated cells to differentiate into multiple cell lines has been demonstrated by using differentiation kits (PromoCell, Inc) containing mixtures of growth factors causing adult stem cells to differentiate separately in adipocytes, osteoblasts and chondrocytes, this part of the research being described in another study published by our research team [30].

The procedure was finalized with the closure of the incision in anatomical plans, simple Blair-Donatti sutures being used (Prolene 4-0), (fig. 2-e), followed by wound disinfection with silver nitrate solution (fig. 2-f).

Recovery from anesthesia: For recovery from anesthesia, i.m. Atipamezole was used, in a dose of 1:1 relative to the Detomidine dose. An i.m. analgesic has also

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**Fig. 1.** The chemical structures of Detomidine and Atipamezole [27,28]

**Fig. 2.** Minimally invasive surgical protocol: a - sedation; b - preparing the inter-scapular region for incision; c - surgical incision for adipose tissue site accessing; d - adipose tissue samples collection in sterile tubes with culture medium; e - incision closure with simple sutures Blair-Donatti; f - wound disinfection with silver nitrate solution.
been administered, all this time, the animal’s vital functions were monitored.

Results and discussions

During the sedation, no signs of bradycardia or hypotension were registered, common adverse events when using this type of anesthetic drugs in animals. Moreover, vomiting, muscle twitching or hypothermia were not registered as well after recovery from sedation. Atipamezole, an α2 adrenergic receptor antagonist, with a high specificity for theα2-adrenergic receptors, assured the reversal of sedative and analgesic effects of Detomidine, waking up the animals within 10 min. It was not registered any case of postoperative wound infection or dehiscence, all experimental animals recovered quickly and favorably without any type of complication. The harvested adipose tissue was successfully used for ADSCs isolation, resulting in 6 cell cultures. After 3 weeks harvested adipose tissue was successfully used for ADSCs and favorably without any type of complication. The results and discussions

The proposed surgical protocol is an easily to perform procedure for adipose tissue collection and ADSCs isolation, without postoperative complications. The anesthesia protocol provided the required sedation and analgesia during the surgery, ensuring as well a fast recovery of the experimental animals and without complications.

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