EDITORIAL

Like bone marrow, adipose tissue derives from the mesenchyme and is constituted by a highly complex system containing different cell populations, including mature adipocytes, pre-adipocytes, fibroblasts, vascular smooth muscle cells, endothelial cells and adipose-derived stem cells (ASCs). Up to 2001, when ASCs were first isolated, adipose tissue was usually discarded as surgical waste, whereas it now represent a very precious source of mesenchymal stem cells. Indeed ASCs are one of the most promising stem cell population identified so far, since human adipose tissue is ubiquitous and easily obtained in large quantities with little donor site morbidity or patient discomfort. This highly contributes to the increase of their use and to explore different fields of applications for both cell therapy and tissue engineering purposes. Recently, it has been demonstrated that the use of autologous ASCs as research tools and as cellular therapeutics is feasible, safe and efficacious in preclinical and clinical studies of injury and disease.

In this Supplement, the collected papers are concerned with critical issues related to the use of ASCs, both in *in vitro* and in pre-clinical models. Safety, interaction with biomaterials, different potential of ASCs from normal and obese donors and from different harvest site, capacity of these cells to uptake/release anticancer drugs and clinical application in horse tendon pathologies are all concerns addressed in this Supplement.

All the manuscripts include at least one author belonging to the Italian Mesenchymal Stem Cells Group (GISM); it was founded in 2009 to foster a multidisciplinary approach aimed at facilitating the clinical translation of ASC-based products. GISM includes biologists, clinicians, veterinarians, pharmacologists and directors of cell production facilities (http://www.gism.altervista.org).

We are confident that this Supplement will be of interest to the readers of the Journal and also will contribute to the scientific debate regarding this important type of mesenchymal stem cells.
Mesenchymal stem cells (MSC) and adipose-derived stem cells (ASC) were recently proposed for bone maxillofacial reconstruction in association with biomaterials. For this application MSC must be ex-vivo expanded in order to obtain, for a given volume of implanted biomaterial, a relevant number of bone forming cells. Previously conducted pre-clinical studies suggested that a concentration of $6 \times 10^8$ ASC associated with 900 mg of anorganic bovine bone (ABB) could be effective for human maxillary sinus floor elevation.

A keystone issue to guarantee the quality and safety of Advanced Therapy Medicinal Products containing expanded MSC and ASC is their chromosome stability in culture: this topic has been widely investigated and conflicting results have been published. Abnormal karyotype of human ex-vivo expanded MSC and ASC was found by some authors, while, at the same time, several other studies showed the MSC and ASC karyotype to be normal. It is therefore important that all the results obtained on MSC and ASC karyotype analysis be published. Given this context, the aim of this manuscript, aim of this manuscript is to verify the karyotype stability of ASC in view of their applications in clinical trials. ASC obtained from the adipose tissue of 4 donors were expanded over extended culture time. Based on previous ASC expansions we hypothesized to be able to obtain $6 \times 10^8$ cells by passage 7. Karyotype analysis of 30 metaphases was planned to be investigated at passage 2, 7, and 15 in all the cultures. No abnormalities were found in the karyotype of two donors at all the passages tested, while a translocation was found in 2 metaphases of a donor at passage 7, but not at passage 15, and in the fourth donor in 5 metaphases a trisomy was found at passage 15. Chromosomal abnormalities were detected only after extended ASC expansion. Whether these anomalies can be related to risk for the patient’s safety will have to be demonstrated by in-vivo studies.
Today adipose tissue is not just considered as the primary energy storage organ, but it is also recognized as an important endocrine tissue and an abundant source of mesenchymal stem cells (adipose-derived stem cells, ASCs). During the last decade, several studies have provided preclinical data on the safety and efficacy of ASCs, supporting their use in cell-based therapy for regenerative medicine purposes. Little is known about the effect of obesity on ASCs properties. Since ASCs differentiation and proliferation are determined by their niche, the differences in body fat distribution and the obesity-related co-morbidities may have several consequences. In this study we compared ASCs of subcutaneous adipose tissue from obese (obS-ASCs) and non-obese (nS-ASCs) donors in order to compare their immunophenotype and osteogenic and adipogenic potential. Moreover, in order to evaluate the possible difference between subcutaneous and visceral fat, obS-ASCs were also compared to ASCs derived from visceral adipose tissue of the same obese donors (obV-ASCs). Our results show that subcutaneous and visceral ASCs derived from obese donors have an impaired cell proliferation, clonogenic ability and immunophenotype. Nevertheless, obS-ASCs are able to differentiate toward osteogenic and adipogenic lineages, although to a small extent with respect to non-obese donors, whereas obV-ASCs lose most of their stem cell characteristics, including multi-differentiation potential. Taken together our findings confirm that not all ASCs present the same behavior, most likely due to their biological microenvironment in vivo. The specific stimuli which can play a key role in ASCs impairment, including the effects of the obesity-related inflammation, should be further investigated to have a complete picture of the phenomenon.
Co-culture of mesenchymal stem cells (MSCs) and articular chondrocytes (ACs) has been proposed for autologous cartilage cell-based therapies, to overcome the issues associated to limited availability of articular chondrocytes (ACs). To evaluate the potentiality of a co-culture approach in aged osteoarthritic patients, MSCs from infrapatellar fat pad (IFP-MSCs) and knee subcutaneous adipose tissue (ASCs) were co-cultured with donor-matched osteoarthritic, expanded and cryopreserved, ACs in a 75%/25% ratio. Co-cultures were prepared also from nasal chondrocytes (NCs) to evaluate their possible use as an alternative to ACs. Pellets were differentiated for 14 days, using mono-cultures of each cell type as reference. Chondrogenic genes SOX9, COL2A1, ACAN were less expressed in co-cultures compared to ACs and NCs. Total GAGs content in co-cultures did not differ significantly from values predicted as the sum of each cell type contribution corrected for the co-culture ratio, as confirmed by histology. No significant differences were observed for GAGs/DNA in mono-cultures, demonstrating a reduced chondrogenic potential of ACs and NCs. In conclusion, a small percentage of expanded and cryopreserved ACs and NCs did not lead to IFP-MSCs and ASCs chondro-induction. Our results suggest that chondrogenic potential and origin of chondrocytes may play a relevant role in the outcome of co-cultures, indicating a need for further investigations to demonstrate their clinical relevance in the treatment of aged osteoarthritic patients.
Many strategies, including those based on genetically modified Mesenchymal Stromal Cells (MSCs), have been developed in recent years in order to obtain high concentrations of anticancer drugs effective on tumor mass. In previous studies, we showed that human and murine bone marrow-derived MSCs (BM-MSCs) and human skin-derived stromal fibroblasts (hSDFs) acquired strong anti-tumor capacity, both in vitro and in vivo, once primed with Paclitaxel (PTX). In this report we investigate whether adipose tissue-derived MSCs (AT-MSCs) behave similarly to BM-MSCs in their uptake and release of PTX in sufficient amounts to inhibit tumor proliferation in vitro. According to a standardized procedure, PTX primed AT-MSCs (AT-MSCsPTX) were washed and then subcultured to harvest their conditioned medium, which was then tested to evaluate its in vitro anti-tumor potential. We observed that AT-MSCsPTX were able to uptake PTX and release it in a time-dependent manner and that the released drug was active in vitro against proliferation of leukemia, anaplastic osteosarcoma, prostatic carcinoma and neuroblastoma cell lines. These data confirm that AT-MSCs, as well as BM-MSCs, can be loaded in vitro with anti-cancer drugs. While the harvesting of BM-MSCs requires invasive procedures, AT-MSCs can be prepared from fat samples taken with little patient discomfort. For this reason, this source of stromal cells represents an important alternative to BM-MSCs in developing new tools for carrying and delivering anti-cancer drugs into tumor microenvironments.
Skin substitutes are epidermal, dermal or complete bilayered constructs, composed by natural or synthetic scaffolds and by adherent cells such as fibroblasts, keratinocytes or mesenchymal stem cells. Silk fibroin is a promising polymer to realize scaffolds, since it is biocompatible, biodegradable, and exhibits excellent mechanical properties in terms of tensile strength. Moreover, fibroin can be added of others components in order to modify the biomaterial properties for the purpose. The aim of this work is to prepare silk fibroin films for adipose-derived stem cell (ADSCs) culture as a novel feeder layer for skin tissue engineering. Pectin has been added to promote the protein conformational transition and construct strength, while glycerol as plasticizer, providing biomaterial flexibility. Eighteen formulations were prepared by casting method using fibroin, pectin (range 1-10% w/w), and glycerol (range 0-20% w/w); films were characterized by Fourier transform infrared spectroscopy and differential scanning calorimetry assay, to select the optimal composition. A stable fibroin conformation was obtained using 6% w/w pectin, and the best mechanical properties were obtained using 12% w/w glycerol. Films were sterilized, and human ADSCs were seeded and cultured for 15 days. Cells adhere to the support assuming a fibroblastic-like shape and reaching confluence. The ultrastructural analysis evidences typical active-cell features and adhesion structures that promote cell anchorage to the film, thus developing a multilayered cell structure. This construct could be advantageously employed in cutaneous wound healing or where the use of ADSCs scaffold is indicated either in human or veterinary field.
TWO BONE SUBSTITUTES ANALYZED IN VITRO BY PORCINE AND HUMAN ADIPOSE-DERIVED STROMAL CELLS

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Nowadays, the repair of large bone defects is an important goal in orthopaedic and dental fields. Tissue engineering, applied to increase the bone regeneration process, combines suitable scaffolds with either terminally differentiated cells or Mesenchymal Stromal Cells. In vitro studies with Adipose-derived Stromal Cells (ASCs) may identify new bioactive supports, to be tested in preclinical model. In this study, we evaluated the biocompatibility and the osteoinductive properties of two bone substitutes, RegenOSS39 (RO-1) and a new generation scaffold (RO-2), on both porcine and human ASCs. Porcine ASCs need a prolonged initial phase to adapt to both substitutes; indeed, their growth was initially reduced respect to cells cultured in their absence. In contrast, human ASCs were not negatively affected. However, no toxicity of RO-1 and -2 was observed on both ASC populations which are able to stick to both biomaterials. RO-1 and -2 supported osteogenic differentiation of porcine and human ASCs in a different manner: the presence of RO-1 up-regulated both alkaline phosphatase (ALP) activity and collagen production of human ASCs, whereas in porcine ASCs, RO-2 seemed to up-regulate ALP activity, while the production of collagen is mainly stimulated by the presence of RO-1. We suggest to use not just human ASCs, but also animal ones to select suitable scaffolds to generate bio-constructs in vitro, which then need to be tested in animal model before reaching the market.
ALLOGENEIC ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS IN COMBINATION WITH PLATELET RICH PLASMA ARE SAFE AND EFFECTIVE IN THE THERAPY OF SUPERFICIAL DIGITAL FLEXOR TENDONITIS IN THE HORSE.

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Overstrain tendonitis are common pathologies in the sport horses. Therapeutic approaches to tendon healing do not always result in a satisfactory anatomical and functional repair, and healed tendon is often characterized by functional impairment and high risk of reinjury. Recently, mesenchymal stem cells (MSCs) and platelet rich plasma (PRP) have been proposed as novel therapeutic treatments to improve the tendon repair process. MSCs are multipotent, easy to culture and being originated from adult donors do not pose ethical issues. To date, autologous MSCs have been investigated mainly in the treatment of large bone defects, cardiovascular diseases, osteogenesis imperfecta and orthopaedic injuries both in human and veterinary medicine. The clinical applications in which autologous MSCs can be used are limited because patient-specific tissue collection and cell expansion require time. For clinical applications in which MSCs should be used right away, it would be more practical to use cells collected from a donor, expanded in vitro and banked to be readily available when needed. However, there are concerns over the safety and the efficacy of allogeneic MSCs. The safety and efficacy of a therapy based on the use of allogeneic adipose tissue-derived mesenchymal stem cells (ASCs) associated with platelet rich plasma (PRP) were evaluated in 19 horses affected by acute or subacute overstrain superficial digital flexor tendonitis (SDFT). The application of allogeneic ASCs neither raised clinical sign of acute or chronic adverse tissue reactions, nor the formation of abnormal tissue in the long term. After a follow-up of 24 months, 89.5% horses returned to their previous level of competition, while the reinjury rate was 10.5%, comparable to those recently reported for SDFT treated with autologous bone marrow derived MSCs. This study suggests that the association between allogeneic ASCs and PRP can be considered a safe and effective strategy for the treatment of SDF tendonitis in the horse.