

Adipose-derived mesenchymal stem/stromal cells: from the lab bench to the basic concepts for clinical translation

Yesica Romina FRONTINI-LÓPEZ¹; Aldana Daniela GOJANOVICH¹; Diego MASONE^{1,2}; Diego Martín BUSTOS^{1,3}; Marina UHART¹

¹ Laboratorio de Integración de Señales Celulares. IHEM-CONICET, Universidad Nacional de Cuyo Mendoza, Argentina

² Facultad de Ingeniería, Universidad Nacional de Cuyo, Mendoza, Argentina

³ Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Mendoza, Argentina

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Abstract: In the last years, much work has shown that the most effective repair system of the body is represented by stem cells, which are defined as undifferentiated precursors that own unlimited or prolonged self-renewal ability, which also have the potential to transform themselves into various cell types through differentiation. All tissues that form the body contain many different types of somatic cells, along with stem cells that are called 'mesenchymal stem (or stromal) cells' (MSC). In certain circumstances, some of these MSC migrate to injured tissues to replace dead cells or to undergo differentiation to repair it. The discovery of MSC has been an important step in regenerative medicine because of their high versatility. Moreover, the finding of a method to isolate MSC from adipose tissue, so called 'adipose-derived mesenchymal stem cells' (ASC), which share similar differentiation capabilities and isolation yield that is greater than other MSC, and less bioethical concerns compared to embryonic stem cells, have created self-praised publicity to procure almost any treatment with them. Here, we review the current techniques for isolation, culture and differentiation of human ASC (hASC), and describe them in detail. We also compile some advantages of the hASC over other stem cells, and provide some concepts that could help finding strategies to promote their therapeutic efficiency.

Introduction

Stem cells, defined as biological and functional living entities (or 'units') which are responsible for the organism development and the regeneration of organs and tissue systems, are under the spotlight for clinical translation. This is because they are easily expandable *in vitro*, since they self-renew, and because of their ability to differentiate to multiple cell lineages. Adult stem cells represent the most resourceful reservoir of cell progenitors, as they are found in potentially any body tissues. Mesenchymal stem (or stromal) cells (MSC) have been considered one of the most suitable sources for stem cell-based therapies because of their multipotential differentiation, by using these progenitors to replace or repair damaged musculo-skeletal tissues (Murray *et al.*, 2014). However, lately, it has been shown that MSC can differentiate not only into mesoderm-derived lineages but also to neuro-ectoderm lineages (Jaiswal *et al.*, 1997; Mackay *et al.*, 1998; Shiota *et al.*, 2007; Gojanovich *et al.*, 2018), broadening even more their action-span for clinical translation. To date, bone marrow supposes the most studied type of MSC since their identification in 1974 by Friedenstein *et al.* (Friedenstein *et al.*, 1974).

Since the harvesting method for 'bone marrow mesenchymal stem cells' (BMSC) involves the iliac crest puncture, which can be accompanied by physical discomfort, even when the procedure is performed under local anesthesia, and because of possible side effects and morbidity, researchers have looked for alternative sources of MSC. In recent years, new MSC's tissue reservoirs have been recognized in Wharton's jelly (Wang *et al.*, 2009), placenta (In't Anker *et al.*, 2004), amniotic fluid (Tsai *et al.*, 2004), menstrual blood (Borlongan *et al.*, 2010), dental pulp (Pierdomenico *et al.*, 2005), synovial membrane (De Bari *et al.*, 2001) and adipose tissue (Zuk *et al.*, 2001).

Like bone marrow, the adipose tissue derives from the mesenchyme, and consists of an upholding stroma that is in fact more easily accessible. The 'human adipose-derived mesenchymal stem cells' (hASC) are a population of cells with the advantage that they can be isolated from surgical wastes, because the adipose tissue is routinely discarded in abdominal dermolipectomy aesthetic surgery procedures. These hASC are derived from the 'stromal-vascular fraction' (SVF) of adipose tissue and were first described in 2001 by Zuk, who named them 'processed lipoaspirate' (PLA) (Zuk *et al.*, 2001). As human bone marrow stem cells (hBMSC), hASC have the same multilineage differentiation capability, but adipose tissue is a more convenient available source, due to its subcutaneous location, less invasive collection

* Address correspondence to: Marina Uhart,
muhart@mendoza-conicet.gob.ar

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techniques, minimal donor morbidity and higher isolation yield. All of these make hASC the most advantageous reservoir of hMSC and therefore it is being considered as highly promising in up-to-date regenerative medicine.

Along with hASC, the SVF includes other cell types that can be separated from them by adherent culture, since non-adherent cells are eliminated through washing and passaging, and non-proliferative cells are overgrown by hASC (Bourin *et al.*, 2013), which can be easily expanded in number and handled under standard-culture conditions. As stated by the International Society for Cellular Therapy (ISCT) and the International Federation for Adipose Therapeutics and Science (IFATS), the identification of hASC comprises three criteria: adherence to plastic, specific surface antigen expression and *in vitro* differentiation into osteoblasts, adipocytes and chondroblasts (Dominici *et al.*, 2006), which are discussed below.

Newly ground-breaking works are aiming to discover hASC natural niche and functions, as well as the possibility to manipulate them for therapeutic purposes. For these studies to be carried on humans, fluid scientists-doctors interactions are essential, as doctors bridge between patients and scientists. The initial contact may start in a scientific or medical congress or workshop, or researchers may contact physicians specifically to ask for tissue donations. Then, the protocol, project and informed consent are revised and approved by a competent bioethical committee. Once a surgery is scheduled, the doctor informs the patient about the possibility to donate the tissue, which otherwise would be discarded, without neither immediate personal reward nor any costs. The scientist has previously provided the clinical or hospital with information on how to collect and store the samples until pick up. After the samples arrive to the laboratory, they are used in research projects, ideally multidisciplinary or experimental-computational hybrid projects, in which the interpretation of the experimental data is used to feedback *in silico* analysis, that in turn generates new questions to be answered experimentally (Fig. 1).

We reviewed the aspects of hASC isolation, characterization, differentiation and some advantages regarding their use over other stem cells. Besides that, we introduce some notions and treatment ideas for hASC's successful clinical applications. This work attempts to bring to physicians concepts that are born from the basic science, trying to merge the knowledge of different disciplines, from molecular-cellular-computational biology to material engineering and modern medicine.

hASC isolation

Many hASC isolation protocols have been published during the last 15 years. Most of them consist of an enzymatic digestion of the adipose tissue to release the cells that form the SVF (Zuk *et al.*, 2001). The later consists of an heterogeneous cell pellet including the hASC of interest plus circulating blood cells, fibroblasts, pericytes, endothelial and immune cells, and pre-adipocytes, among others (Lee *et al.*, 2013; Nguyen *et al.*, 2016). Recently, we published a protocol for hASC isolation, characterization and differentiation, with a detailed step by step description for

these processes (Gojanovich *et al.*, 2018). As all cell culture techniques, hASC isolation must be performed aseptically, in a biological safety cabinet. Briefly, we use about 100 g of freshly harvested subcutaneous adipose tissue obtained from abdomen dermolipectomy surgery of healthy patients, who has signed an informed consent to voluntarily donate the material. Once in the laboratory, the latter is chopped with surgical blades, we made several washes with phosphate-buffered saline (PBS) and centrifuged it to discard mainly the red blood cells, and then we incubate the tissue with an enzyme preparation. After digesting the tissue, we filter it through a 3 mm pore wire mesh to separate the connective tissue. The recovered liquid is centrifuged in order to obtain the SVF. During this process three liquid phases are obtained: (1) an upper yellow lipidic phase, product of the adipocytes rupture during processing; (2) a whitish intermediate phase containing mature adipocytes; and (3) an aqueous lower phase comprising culture medium and a cellular pellet corresponding to the SVF. Carefully, the lower liquid phase and cellular pellet is recovered and centrifuged. Then, the cellular pellet (or SVF) is resuspended in DMEM with 10% FBS, seeded in a 10 cm diameter tissue culture plate and incubated at 37°C and 5% CO₂. The following day, the plated cells are washed with PBS buffer and the medium is changed to wash away the remaining non-adherent cells. Cells are maintained in culture until reaching 90% confluence when they are divided for expansion (Gojanovich *et al.*, 2018).

hASC characterization

Because there is no single marker to characterize the hASC population, it is necessary to perform a multicolor phenotyping analysis with a combination of fluorochrome-labeled antibodies to specific surface antigens by flow cytometry, to evaluate the composition of the isolated population. Since there is no common protocol for isolating and cultivating MSC among laboratories, the ISCT and IFATS issued some criteria for identifying unique populations of these cells. According to this, ASC are characterized by the expression (>80%) of CD13, CD29, CD44, CD73, CD90 and CD105 surface antigens, and must lack expression (<2%) of CD31, CD45 and CD235a, as primary negative markers; while other low or negative markers include CD3, CD11b, CD49f, CD106 and PODXL (Bourin *et al.*, 2013). However, according to Dominici *et al.* (2006), who established the first ISCT consensus, the phenotype characterization of multipotent MSC must include at a minimum the cell surface co-expression of the antigens CD105, CD73, and CD90 (positives) and the absence of hematopoietic and endothelial lineage markers CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR (negatives) (Dominici *et al.*, 2006). Since the hASC isolated in our laboratory are a subtype of hMSC and the characterization kit is intended for use on hMSC, all the surface markers analyzed allow us to perfectly identify our cell population.

Immunophenotyping analysis in our laboratory is achieved by labeling the cells with the BD Stemflow™ hMSC Analysis Kit (Becton, Dickinson -BD-Biosciences, San Jose, CA, USA), which makes possible the characterization of MSC based on the Mesenchymal and Tissue Stem Cell Committee of the ISCT (Dominici *et al.*, 2006). The hASC populations

that we routinely obtain are highly enriched in hASC ($\geq 98\%$) and contain small amounts of other cells (less than 2%) (Gojanovich *et al.*, 2018).

Our characterization is comprehensive enough to accomplish the consensus of IFATS and ISCT, establishing that an ASC phenotyping should include at least two negative and two positive markers in the same analysis (Bourin *et al.*, 2013).

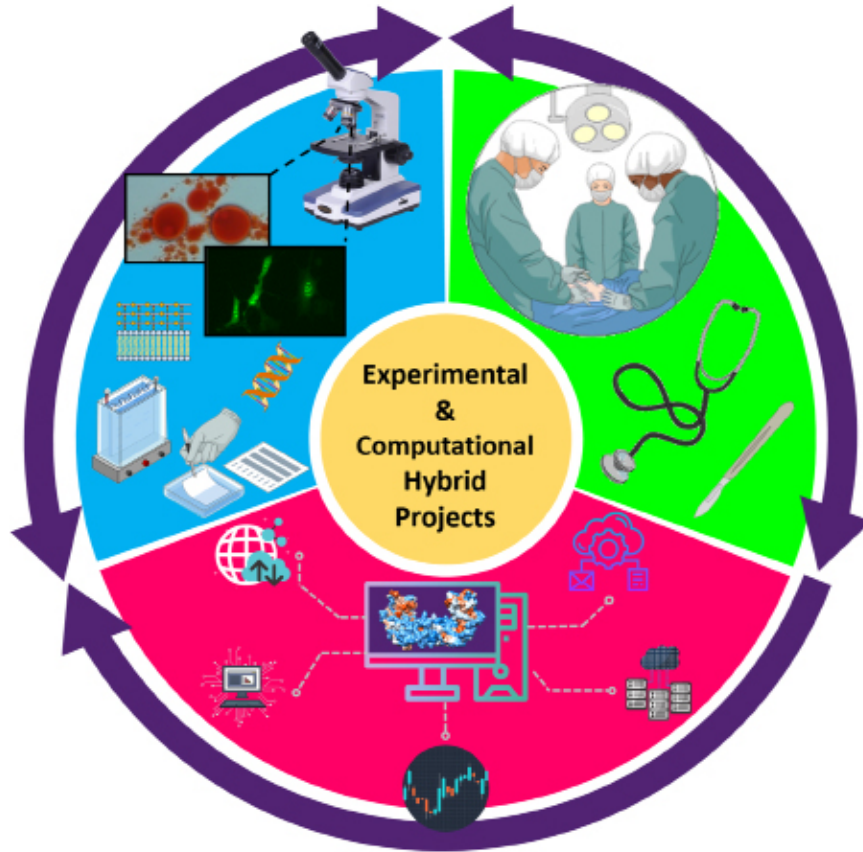


FIGURE 1. Diagram representing scientific research-clinics positive feedback. Clinical problems guide the construction of experimental and computational hybrid projects. Computational biology is used to predict experimental outcomes, optimize experiments planning and use of resources. Experimental biology (both *in vitro* and *in vivo*) will then generate new questions that feedback in silico studies, and will also generate innovations for clinical applications.

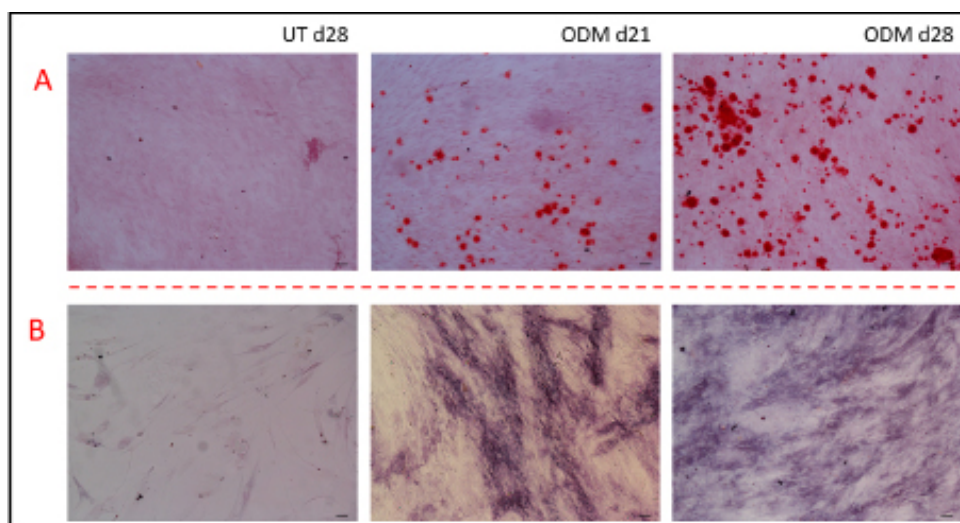


FIGURE 2. Osteogenic differentiation of adipose-derived mesenchymal stem/stromal cells. Untreated (UT) cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), and osteogenic differentiation medium (ODM), containing DMEM+10% FBS, plus 10 mM β -glycerophosphate (β -GP), 100 nM dexamethasone (DEX) and 50 μ g/mL 2-phospho-L-ascorbic acid (AA2P) at days 21 (d21) and 28 (d28) after induction. (A) Alizarin Red Staining of the hydroxyapatite mineralization nodules and (B) Alkaline phosphatase staining as an osteogenic biomarker protein (Scale bars: 50 μ m).

hASC differentiation

Several groups have demonstrated that hASC can differentiate into mature adipocyte cells through induction with a drug cocktail, which varies between protocols, but usually contains dexamethasone, insulin, 3-isobutyl-1-methylxanthine (IBMX) and indomethacin or thiazolidinedione (TZD) (Bunnell *et al.*, 2008). Dexamethasone is a glucocorticoid receptor agonist, which sensitizes the insulin signaling (Tomlinson *et al.*, 2010); insulin is a well-known hormone that stimulates glucose uptake; IBMX is a phosphodiesterase inhibitor used to increase the cAMP levels (Bunnell *et al.*, 2008); both indomethacin and TZD are PPAR γ agonists-ligands (Tontonoz & Spiegelman, 2008).

Since there is no consensus about the optimal adipocyte differentiation protocol, we first tested several cocktail formulations on the 3T3-L1 cell line, a well-established murine pre-adipocyte cell line widely used for studying adipogenesis (Gojanovich *et al.*, 2016). The best drug combination included dexamethasone, insulin, IBMX and rosiglitazone (a high-affinity PPAR γ ligand, member of the TZD family). Next, we adapted the NIH 3T3-L1 adipogenesis protocol described in Gojanovich *et al.*, (2016) for hASC adipogenic differentiation by introducing cycles of induction and maintenance medium, as detailed in Gojanovich *et al.* (2018).

To evidence the lipid droplets accumulation, we routinely use Oil Red O (Biopack, Buenos Aires, Argentina), which specifically stains triglycerides and cholesteryl oleate (Ramírez-Zacarias *et al.*, 1992; Masone *et al.*, 2017; Gojanovich *et al.*, 2018).

The osteogenic differentiation of hASC requires a specific treatment of confluent monolayer with dexamethasone, β -glycerophosphate and ascorbic acid during three to four weeks to change their characteristic fibroblastoid morphology to a multilayer culture of elongated cells, typical of this differentiation (Bunnell *et al.*, 2008; Langenbach & Handschel, 2013). Based on literature information, combined with our results, the optimal concentration for osteogenic differentiation drugs are 100 nM dexamethasone, 50 μ g/mL 2-phospho-L-ascorbic acid and 10 mM β -glycerophosphate (Langenbach & Handschel, 2013), replacing the osteogenic medium every two or three days. Briefly, dexamethasone induces hASC osteoblast differentiation by activating WNT/ β -catenin signaling-dependent RUNX2 expression; ascorbic acid facilitates differentiation by promoting Type I collagen (COL1A) secretion; and β -glycerophosphate favors extracellular matrix (ECM) mineralization by serving as a local source of inorganic phosphate ions.

Detection of calcium deposition after induction of differentiation can be detected by Alizarin Red S staining (Biopack, Buenos Aires, Argentina), since the dye forms a complex with the $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ of the ECM in a chelation process, visualized as red mineralizing nodules (Bunnell *et al.*, 2008) (Fig. 2A). To determine the alkaline phosphatase (ALP) activity as an osteogenic protein biomarker, we perform ALP staining using BCIP $^{\circ}$ (5-bromo-4-chloro-3-indolyl phosphate, Calbiochem, San Diego, CA, USA) which is hydrolyzed by the enzyme forming an intermediate product, that is then oxidized by the NBT (nitro blue tetrazolium, BDH Chemicals Ltd., Poole England, UK), generating an insoluble violet precipitate (McGadey, 1970) (Fig. 2B).

To assess chondrogenic differentiation, the micromass culture technique (Denker *et al.*, 1995) is usually employed. Normally, a concentrated cell suspension is seeded in a 24 well plate and allowed to attach at 37°C for 2 hours. Cell-to-cell interaction in high cell density micropellets is critical to achieve a propitious chondrogenic process (Vinatier *et al.*, 2009). After that, the chondrogenic medium containing TGF- β , insulin, transferrin, sodium selenite, dexamethasone, proline, and ascorbic acid is gently added so that the cell nodules are not detached (Zuk *et al.*, 2001). It is well documented that TGF- β increases the proteoglycans and Type II collagen (COL2A) synthesis rates and also cell proliferation (Awad *et al.*, 2003; Vinatier *et al.*, 2009); insulin, transferrin and sodium selenite, commonly known as ITS supplement medium, serves to enhance nutrition transportation into the inner part of the cartilage, promotes chondrocyte dedifferentiation process, and also production of polysulfonate glycosaminoglycans (GAGs) and COL2A (Liu *et al.*, 2014); dexamethasone increments the proline uptake and upregulates aggrecan, proteoglycans and COL2A biosynthesis via SOX-9 transcription factor expression (Awad *et al.*, 2003); proline and ascorbic acid are necessary as substrate and co-factor, respectively, of the prolyl-lysyl hydroxylase, a key enzyme in collagen biosynthesis (Temu *et al.*, 2010). Cultures must be maintained with the chondrogenic medium during one month, with medium changes every two or three days.

The presence of ECM proteins characteristic of hyaline articular cartilage, like proteoglycan, can be confirmed by Alcian Blue, Toluidine Blue O or Safranin O staining (Jubb & Eggert, 1981). Those are basically cationic dyes that form complexes with the anionic glycoconjugates of the cartilage ECM, such as GAGs and proteoglycans. The intensity of the staining is proportional to the amount of glycoconjugates in the hyaline cartilage (Terry *et al.*, 2000).

Clinical applications

Since the beginning of the 21st century, when the ASC were discovered, they appeared as particularly useful in the field of regenerative medicine because of their many advantages compared to other types of stem cells (Zuk *et al.*, 2001). First of all, ASC can be easily harvested with minimal donor morbidity through liposuction or dermolipectomy surgery. The harvest method implies a higher yield on ASC isolation, and their capability of differentiation is similar to other MSC (Feisst *et al.*, 2015). Moreover, ASC are not as questioned as embryonic stem cells because they are derived from adult adipose tissue (Dai *et al.*, 2016).

Probably related to these advantages, it is noteworthy the number of clinical trials being accomplished at the moment with ASC. If we look for “adipose-derived stem cells” on the US National Institutes of Health (NIH) website (<http://www.clinicaltrials.gov>), we can find that there are 112 studies, among which 42 are currently open. The great potential of stem cells to treat several human diseases like cancer, Parkinson’s disease, diabetes mellitus, Crohn’s disease, heart failure, autism, burns, amyotrophic lateral sclerosis, spinal cord injury, erectile dysfunction, premature ovarian failure, osteoarthritis, autoimmune diseases, etc. is well-known. A huge commercial “stem cell based therapies” industry is

being built based on this potential, and it is alarming the increased number of companies that conveniently sell stem cells treatments that have not been proved in clinical trials because of the lack of regulation (Martell *et al.*, 2010) in many countries. Indeed, a “stem cell tourism” has emerged to travel to less regulated countries to get treatments that are not approved in the more regulated ones.

In Argentina, there is still no legislation regarding the clinical use of stem cells. Nevertheless, the Instituto Nacional Central Único Coordinador de Ablación e Implante (INCUCAI) has declared that “the only stem cell treatment that has demonstrated safety and scientific efficacy is transplantation of hematopoietic progenitor (stem) cells in cases of onco-hematological diseases and out of this treatment there is no clinical evidence or treatments established at national and international levels, who have demonstrated the effectiveness of using stem cells to cure other diseases”.

Along with their isolation-related qualities, hASC are highly attractive in the medical field, since they do not present surface molecules of class II major histocompatibility complex (MHCII), which allows them to be transplanted across allogenic barriers without eliciting an immune response (Le Blanc *et al.*, 2003; Tse *et al.*, 2003; De Miguel *et al.*, 2012; Huang *et al.*, 2016). If the hASC are to be used in clinics, it is mandatory to replace all traditional culture reagents by defined xeno-free reagents or, alternatively, more recently developed techniques which avoid the use of FBS or other animal sera are to be used (Cimino *et al.*, 2017).

Hereafter, we describe a series of hASC’s potential clinical applications in different medical areas, including their pros and cons (Fig. 3).

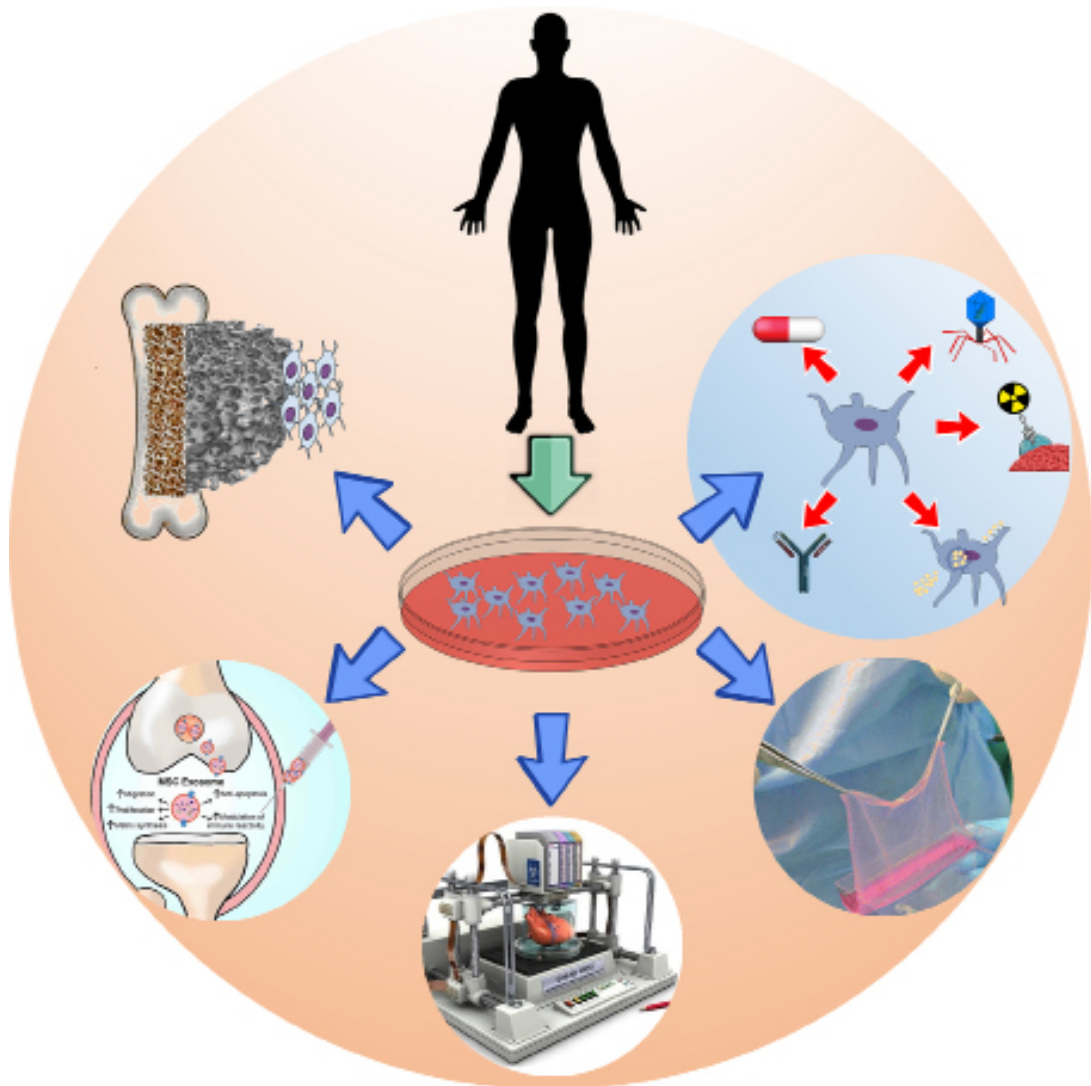


FIGURE 3. Human adipose-derived mesenchymal stem/stromal cells (hASC) potential clinical applications in different medical areas. As detailed in the article, hASC can be used to treat a wide variety of diseases. The fields with current greater advances are plastic and reconstructive surgery, bone and joint repair, 3D tissue printing and oncological therapies.

Oncology

Several uses of hASC for oncolytic therapy have been proposed on the basis of their ability to home/migrate. But, what is it the homing/migration of the hASC and why does it happen? In 2009, Karp and Teo defined homing as “the arrest of MSC within the vasculature of a tissue followed by transmigration across the endothelium” (Karp & Leng Teo, 2009). MSC express cytokines, chemokines and growth factors receptors on their surface, that allow them to migrate to the site of disease or injury by chemotactic gradients, which guide them to the damaged tissue (Cho *et al.*, 2009). Although the homing/migration of the ASC has been tested in most cases, it seems that this capability is dependent on the injection method, and even when they manage to reach the damaged tissue, the amount grafted there is too low compared to the amount injected (De Becker and Riet, 2016). Controversially, Anjos-Afonso *et al.* (2004) transduced murine MSC with a lentiviral eGFP containing vector to track them within mice and found that mMSC migrate mostly to the lung, but also to other organs like liver, muscle and kidney, even in the absence of injury, and that they acquire the residing tissue morphology and antigen expression (Anjos-Afonso *et al.*, 2004).

For cancer treatment, MSC have been proved as vehicles to deliver several anti-cancer therapies. Some examples include the expression of anti-cancer proteins (such as interleukins, pro-apoptotic proteins, etc.), the production of pro-drugs activating enzymes, the delivery of oncolytic viruses and nanoparticles containing chemo- or radiotherapies (Bhere and Shah, 2015). Even though these treatments seem promising, we must consider that they can be toxic even to the MSC themselves and also that the MSC can enhance the tumor severity because of their angiogenic capacity, or they may even generate a new tumor elsewhere (Fiala, 1968; Blanton *et al.*, 2009). Recently, a novel strategy is being tested, which consists in MSC encapsulation (Murua *et al.*, 2008). This approach requires not only the expertise of physicians and molecular biologists, but also bioengineers, thus a multidisciplinary team to deal with a bigger challenge.

Drug screening and disease modeling

Despite their source, the extent of stem cell's applications covers a wide scope. The advantages of their use in the field of drug screening rely on the promise of developing a more trustworthy, accelerated and profitable drug discovery process. Until 2015, less than 10% of the drugs that enter Phase I clinical trials, end up being approved successfully for disease treatment (Seruga *et al.*, 2015). This information highlights the need to expand and improve the spectrum of new drug testing.

Most primary drug screening tests are carried out with cells grown on two-dimensional tissue culture plates or flasks. The aim of this method is to identify the largest number of hits, but it fails to mimic the niche complexity. That is when appears the need of using methods, which simulate better the *in vivo* situation.

Since the use of animal models in this research area tends to lead to drug failure and side-effects when translated to humans, due to interspecies differences (Miranda *et al.*, 2018), it is necessary to address new approaches. In this

matter, 3D assays could shorten the gap between primary screening and human trials, providing tools such as cellular aggregates or spheroids, the new developed constructs termed “organoids” and the most recently created organ-on-a-chip platforms (Ranga *et al.*, 2014; Miranda *et al.*, 2018).

Cellular aggregates or spheroids for screening purposes are a powerful tool since there is no need to provide any adhesion matrices for cell adhesion. As an obstacle, they present heterogeneity and cellular disorganization, which leads to poor control of the size and differentiation of the cells within the spheroids (Ranga *et al.*, 2014; Miranda *et al.*, 2018).

The optimal assessment of drug effects and disease modeling should be carried out on models which best mimic the whole-organ composition, physiology and microenvironment, which implies a heterogeneous but uniformly distributed and organized cellular composition. For that matter, organoids, defined by Lancaster and Knoblich (2014) as “a collection of organ-specific cell types that develop from stem cells or organ progenitor cells to form an organ-like tissue exhibiting multiple cell types that self-organize to form a structure not unlike the organ *in vivo*” (Lancaster *et al.*, 2014), are the new gold standard in drug screening and disease modeling. Their potential over spheroids lies in that they better simulate the migration, angiogenesis, and bioavailability of drugs when crossing biological barriers (Ranga *et al.*, 2014). On the other hand, the current limitation of organoid culture is the scale-up production, since their formation is time-demanding (Miranda *et al.*, 2018).

The need to fill the gap between *in vitro* testing and humans has led to the development of multi-organ platforms. Organs-on-a-chip provides a device which comes to improve the assessment of the interaction of the drugs and their metabolites with different parts of the body, recapitulating some other aspects of the human physiology like absorption, metabolic processing and toxicity. Nevertheless, since their accessibility is limited, its application is still restrained (Miranda *et al.*, 2018).

Bone regeneration

The main challenge in the field of bone repair is to improve the osseointegration of implants in large bone injuries. Unlike small bone defects, where the human body has the innate ability to repair, bone healing in large bone injuries is delayed or absent. Before detailing some clinical strategies to reduce post-operative complications associated with graft-versus-host disease in bone transplants, it is necessary to explain what we mean when we speak of osseointegration. This concept was first introduced by Professor Per-Ingvar Branemark (1969), who explained this process as “a direct structural and functional connection between ordered living bone and load-carrying endosseous implant at the light microscopic level” (Jayesh and Dhinakarsamy, 2015). In order to enhance osseointegration, it is of outstanding relevance to define three features that should have bone grafts: (1) **osteogenesis**: is the process by which new bone is synthesized, it requires cells with bone differentiation capacity; (2) **osteinduction**: MSC are recruited to differentiate, this recruitment needs the regulation of growth factors and cytokines; (3) **osteoconduction**: is the three-

dimensional growth of capillaries, perivascular tissue and stem cells from the receiving area of the host to the graft, generating a scaffold with the niche characteristics that favors the host-graft interface for regeneration (Zárate-Kalfópulos and Reyes-Sánchez, 2006).

Some cases in which MSC *per se* have been enough to repair bone defects (Lee *et al.*, 2015; Fu *et al.*, 2016; Zou *et al.*, 2016) have been shown, but in general, the gold standard therapeutic strategy is to combine the components to induce bone growth and regeneration, taking into account the three above mentioned characteristics that should have bone grafts (Romagnoli and Brandi, 2014). Most suitable scaffolds must have pores that allow cell infiltration and extracellular matrix deposition; permit incorporation and control drugs, growth factors and bioactive components release; and should be comprised of resorbable materials so that they would be replaced by newly-formed bone over time (Romagnoli and Brandi, 2014).

There are basically two types of polymer scaffolds. Natural polymers are derived from biological sources, such as collagen, hyaluronic acid and alginate. These have the advantages of being biocompatible and bioactive, promoting cell attachment and growth. However, their liabilities are that they present a high risk of pathogen transmission, are difficult to engineer, and they exhibit batch-to-batch variability. On the other hand, synthetic polymers are far more malleable and have defined chemical formulation, so they can be easily sterilized. Nevertheless, despite their stability, synthetic materials can elicit undesirable inflammatory responses *in vivo* since they are not bioactive (Agarwal and García, 2015).

Joint repair

To understand the requirements of joint repairment it is necessary to recognize what are the symptoms of articular cartilage damage. Pathologies like osteoarthritis and rheumatoid arthritis begin to manifest by pain, swelling, limited motion and go on with cartilage degeneration (Nam *et al.*, 2018). Progress of the illness severity is accompanied by changes that occur within the articular cartilage, especially regarding its composition.

There are mainly three types of cartilage: elastic cartilage, hyaline cartilage and fibrocartilage. Cartilage from joints is composed by hyaline cartilage, whose smoothness and flexibility are intermediate between elastic- and fibrocartilage (Nam *et al.*, 2018). To add more complexity, the articular hyaline cartilage has different compositions of ECM according to its depth. Chondrocytes located at the surface of the articular cartilage secrete a protein named lubricin, which lubricates the joint. In the middle zone, chondrocytes mainly produce aggrecan and, in deeper regions, ECM is mostly composed by proteoglycans (Sakata *et al.*, 2015; Nam *et al.*, 2018).

Since chondrocytes are the only cell type and constitute 1-5% of the articular cartilage, and the joint, unlike most tissues, is avascular, it is almost impossible to repair an articular lesion regenerating hyaline cartilage. So when there is an imbalance between the catabolic and anabolic factors that lead to cartilage degradation, chondrocytes are replaced by fibroblast-like cells at the beginning and afterwards hyaline cartilage is replaced by fibrocartilage. These events prompt osteophyte formation, inflammation, rigidity and additional

pain (Sakata *et al.*, 2015; Nam *et al.*, 2018).

Animal models are always the first choice in the attempt to imitate the endogenous condition, but because of size differences, joint thickness and healing potential, the results are difficult to extrapolate to humans (Sakata *et al.*, 2015). Despite this drawback, MSC are more suitable since their therapeutic potential can be explained by two properties: (1) their capability of homing/migration, incorporation and further differentiation within the host, and (2) a battery of morphogens, chemokines and factors secreted by the MSC (Pak *et al.*, 2018). This second characteristic is being currently harnessed to treat essentially knee and hip joints in osteoarthritis by intra-articular injection (Desando *et al.*, 2013; Zhang *et al.*, 2016; Cosenza *et al.*, 2017; Seong *et al.*, 2017).

Some other therapies applied for joint regeneration are microfractures, which consists in a procedure using special instruments that are inserted through small incisions in the underlying bone to induce the healing response, and 'autologous chondrocyte implantation' (ACI) in which chondrocytes are isolated from the low-weight-bearing part of a healthy joint and culture expanded *in vitro*. The liability of these methods relies in that microfractures can lead to fibrocartilage formation, and primary monolayer chondrocytes cultures tend to dedifferentiate them, which contributes to loss of its chondral characteristics, acquiring fibroblastoid morphology (Nam *et al.*, 2018).

A new state-of-the-art technology to help cure joint injuries is the 3D bioprinting of cell droplets embedded on a scaffold. The deposition of the bioink, in turns, has to be controlled, cytocompatible, bioresorbable and satisfy the ECM features. Most suitable scaffolds for cartilage regeneration are composed of hydrogel, since gel consistency allows the mixing of the cells with growth factors and provide the lubrication characteristic, contributing to the cells commitment towards the chondrogenic phenotype (Roseti *et al.*, 2018).

Plastic and reconstructive surgery

One of the most commonly performed procedures in this medical field is the transplantation of autologous fat graft, where lipoaspirated adipose tissue is reinjected without any previous process to separate different populations of cells. It is usually used in reconstructive and aesthetic surgery to fill defects and promote graft volume for tissue augmentation (Kaufman, 2007). This method has been improved by Matsumoto, who enriched the lipoinjection with SVF cells in order to promote a better engraftment of the implant (Matsumoto *et al.*, 2006). The engraftment is thought to occur by the ASC's promoting revascularization properties during the acute phase of transplantation (Blanton *et al.*, 2009). This procedure is advantageous because the adipose tissue from one reservoir of the body is removed and reimplanted in another site during a single surgery (Lin *et al.*, 2008). On the other hand, this kind of reimplantation implies a serious risk, since it is likely to occur necrosis or resorption of the grafted fat (Coleman, 1995; Eto *et al.*, 2012).

MSC are also applied in the treatment of chronic non-healing cutaneous lesions such as *fistulae* in Crohn's disease, skin necrosis, tissue ischemia caused by previous irradiation, and burns. It has been suggested that hASC can improve skin healing because they have the ability to differentiate

into endothelial progenitor cells, since they secrete vascular endothelial growth factor (VEGF), and have, indeed, a periendothelial location within the adipose tissue (Traktuev *et al.*, 2008; Hsiao *et al.*, 2012). This skin recovery characteristic is also attributed to the fact that hASC have immunomodulatory effects, which is given by their capacity to secrete cytokines, which confers them pro-coagulant and neo-angiogenic properties (Moll *et al.*, 2012; Kapur and Katz, 2013). Another interesting feature about the ASC in wound healing is that they have shown to diminish excessive scar formation, improve color and scar pliability, associated with their anti-inflammatory response (González *et al.*, 2009; Yun *et al.*, 2012).

Finally, when the injured areas are too large or relatively deep, the use of scaffolds immersed in a cell suspension (Kim and Jeong, 2014) is required. In Argentina, the first clinical trial for the treatment of large burn injury, approved by the INCUCAI is now on progress. This trial consists in the local and topical application of cadaveric BMSC through a fibrin-based polymer spray covering an acellular dermal biological matrix (Mansilla *et al.*, 2012). Importantly, this trial is being performed with allogenic MSC instead of autologous ones.

An unsolved question regarding their use in skin regeneration is whether the application of living MSC is needed, since according to Wang *et al.*, (2017) their usefulness seems to be mainly based on their secretory vesicles.

Conclusions

From 1912, when Alexis Carrel published the first drawing of a stem cell colony obtained *in vitro* from a chicken embryo (Carrel, 1912), to the finding in 2006 of the four factors to induce pluripotency in somatic cells by Shinya Yamanaka (Takahashi and Yamanaka, 2006), we have gone through a relatively slow period of learning on stem cells. But, in the last 12 years, along with advances in cell biology instrumentation and informatics, we have witnessed a great leap in our knowledge of stem cells potential.

Currently, there are almost five thousand clinical trials with stem cells from different sources. Of these, at least two thousand are being carried out with adult MSC, which highlights their utility. As we have already explained, ASC are a subtype of MSC, showing lots of advantages over other stem cells. Starting from the bioethical concerns regarding the use of embryonic stem cells, and continuing with ASC's greater yields, the identical capability of differentiation to other MSC and lack of MHCII in their surface, that allows allogenic transplantation without rejection, they suppose one of the most taking-into-account models to be applied in regenerative medicine.

ASC treatments have been proposed for a variety of diseases including musculoskeletal (osteoarthritis, rheumatoid arthritis, osteogenesis imperfecta), cardiac (heart failure, arrhythmia, myocardial infarction), neurological (Parkinson's disease, Amyotrophic Lateral Sclerosis, multiple sclerosis, Alzheimer's disease, schizophrenia), metabolic (Type I and II Diabetes), and hepatic diseases (liver cirrhosis). Several of the ongoing approaches in regenerative medicine, consider strikingly diverse parameters that influence the biological outcome within the niche of the target tissue. This adds complexity to the equation by introducing

various professions (biologists, physicians and materials engineers), to combine their expertises in a single area (tissue engineering), for the creation of more effective treatments (Beldjilali-Labro *et al.*, 2018).

One of greatest concerns regarding the ASC applications in medicine is that they may promote tumor growth, which has been shown in some studies where tumor cells proliferate more under ASC-conditioned medium or in co-culture of ASC with prostate cancer cells (Yu *et al.*, 2008; Prantl *et al.*, 2010). This fact is not surprising, since it has been proved that they secrete VEGF, which could stimulate metastases by generating new blood vessels within the tumor.

Although the use of ASC is quite promising, we must not disregard the need of adequate scientific groundings, for both their *in vitro* and *in vivo* handling, and stick to stringent laboratory good practices, like the use of non-xenogenic animal-derived products, to ensure optimal clinical results.

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