Adipose mesenchymal stromal cells Definition, immunomodulatory properties, mechanical isolation and interest for plastic surgery

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Les cellules stromales mésenchymateuses du tissu adipeux : définition, propriétés immunomodulatrices, extraction mécanique et intérêt en chirurgie plastique.

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introduction

Over the last decade, the clinical use of MSCs has begun to skyrocket. Keen and exponentially increasing interest in a wide range of disciplines (hematology, proctology, cardiology, neurology, orthopedics…) has revolved around the notion of "stem cell", that is to say a cell capable of renewing itself ("auto-replication"), of proliferating and of being differentiated into a multitude of cell types. Stem cells are characterized as totipotent when they can generate a living being (embryonic stem cells), as pluripotent when they can be divided into three tissue types (ectoderm, mesoderm and endoderm), and as multipotent when they can only be differentiated in a given tissue type. MSCs belong to the last category and have been named according to their tissue origin: BM-MSC, ASC, UC-MSC, etc... Numerous studies have indicated that MSCs may be used in regenerative medicine in highly diversified clinical situations, leading to a scarcely imaginable medical and media craze initially explainable by their differentiation properties. However, reevaluation of their mechanisms of action was triggered by the massive retention of MSCs in the lungs during intravenous injections, by their low level of persistence in targeted tissues[1-3] and, finally, by demonstration of their limited capabilities of *in vivo* transdifferentiation into mature functional cells. At present, clinical utilization of MSCs is premised on the properties enabling them to produce trophic and immunosuppressant factors. More specifically, due to their immunomodulatory/immunosuppressant properties, the clinical interest of these cells in the treatment of inflammatory and dysimmunitary diseases has become obvious.

The objective of this review is to present today's knowledge base with regard to adipose stromal cells (ASC) and to pinpoint their clinical interest, particularly in plastic surgery. Indeed, ASCs are among the components of the fat utilized in autologous reinjections either to compensate for a lack of substance or for regenerative purposes; indeed, ASC isolation techniques have been developed and may be applied during operations such as plastic surgery.

Mesenchymal stromal cells

Mesenchymal stromal cells (MSC) have been known since the 1970s and were initially isolated from bone marrow (BM-MSC = bone marrow-mesenchymal stem cells)[4]. They are defined by three properties existing in culture: **1-** They are plastic-adherent and proliferate *in vitro*. **2-** They are multipotent, which means that they can be differentiated into diversified cell types derived from the mesoderm[5,6] (bone, cartilage, adipose tissue). **3-** Contrary to hematopoietic stem cells, they do not express a specific surface marker, and as a result, their isolation and characterization have been restricted. The minimum required phenotype consists in the presence of CD105, CD73, CD90 (≥95% positivity) and the absence of expression of the hematopoietic markers CD45, CD34, CD14 or CD11b, CD79a or CD19 (≤2%). Moreover, without an inflammatory stimulus (particularly the interferon gamma [IFN-γ]), MSCs fail to express HLA-DR (a molecule in the class II major histocompatibility complex)[5]. That said, recent studies have shown that MSCs are present in multiple tissues, including adipose tissue. There exist several other names for these cells, of which the most widely known, the designation given by the IFATS (International Federation for Adipose

Therapeutics and Science) are ASC (adipose stromal cells) and ADSC (adipose-derived stromal cells)[7]. Indeed, adipose tissue is a major reservoir for ASCs, easily accessible via the liposuction techniques routinely applied in plastic surgery [8,9].

Nowadays, it has become obvious that the efficacy of MSC injection in different models of tissue lesions and dysimmunitary diseases is largely ascribable to a paracrine effect through which, over a short period of time, MSCs produce anti-inflammatory and immunosuppressive molecules that contribute, in association with trophic factors, to the regeneration of host tissue, as has been shown in the encouraging results of trials on the treatment of complicated anal fistulas in Crohn's disease[10,11].

Current data in the literature show considerable heterogeneity in the description of MSCs. We now know that culture induces modifications in the expression of surface markers in contradistinction to their native state. In addition, the functional properties of MSCs vary according to their tissue origin and method of production[12]. In fact, it is highly likely that the heterogeneity in the clinical results achieved with MSC is to some extent due to differences in production processes. More specifically, processes differ with regard to cellular origin, duration, culture conditions and, consequently, the number of cell doublings (CD) *in vitro* [13,14].

However, current data on the characterization of ASCs in their native state remain insufficient to assess their heterogeneity and plasticity. From a clinical standpoint, relevant information is needed to evaluate the benefit/risk balance not only of plastic surgery by injection of autologous fat, particularly in the breast, but also of applications involving cell therapy.

Adipose stromal cells

In culture, ASCs as well as BM-MSCs are capable of multiplying [their multiplication can be quantified by determining the number of population doublings (PD)] and of being differentiated into adipocytes, osteoblasts and chondrocytes. Also in culture, while ASCs, BM-MSCs and MSCs of the umbilical cord (UC-MSC) present similar morphology and comparable membrane phenotypes, ASCs possess a greater capacity of clonogenicity, as is shown by a more elevated rate of *colony-forming unit fibroblast* (CFU-F), whereas UC-MSCs have a higher number of PDs than ASC, and BM-MSCs are the cells that multiply the least[15]. Notwithstanding their resemblances, MSCs differ in phenotype according to their environmental niche or their function within a tissue. For example, and unlike BM-MSCs, ASCs do not express the adhesion CD106 molecule[15,16]. Moreover, ASCs possess greater pro-angiogenic capacities than BM-MSCs, which is why they have been tested in cases of lower limb ischemia[17]. In addition, ASCs possess appreciable functional properties as regards the inhibition of inflammatory and immune responses[18].

While these different characteristics have been defined on the basis of ASCs obtained in cultures, as of now native ASCs remain little known. They possess altogether specific characteristics such as expression *in vivo* of the CD34 marker[19-21]. Given the fact that hematopoietic and endothelial markers are not expressed, CD34 plays a major role in the isolation and characterization of ASCs (Figure 1). The CD34 molecule is a transmembrane glycoprotein belonging to the sialomucine family, and its function has yet to be thoroughly elucidated[22,23]. Non-specific to a cell type, it is predominantly expressed by hematopoietic stem cells, endothelial cells[24] and endothelial progenitors[25]. As a marker, it is lost during culture [22,26], which is why, in the past, certain studies described ASCs as CD34-negative[27].

Over the last decade, given the development of fundamental knowledge pertaining to these cells, criteria have been put into place to define mesenchymal stromal cells and to determine their differences with so-called stem cells[6]. The previously cited criteria included phenotype conditions[5] such as the presence of markers CD73, 90, 105 and the absence of markers CD45, 34, 14 or 11b. While it remains applicable to the BM-MSCs on which it is based, this definition has been called into question and it has become obvious that it is inapplicable to ASCs. Indeed, native ASCs express CD34[20], which is the key marker of their isolation[19,28], rendering the criteria of Dominici et al effectively inapplicable to adipose tissue[5]. Additional indications were consequently given in view of amending the definition of ASCs, which are phenotypically defined by the absence of markers CD45 (pan-hematopoietic marker), CD235a (which is conducive to the elimination of the erythrocytes remaining after addition of lysis buffer), CD31 (endothelial cells) and the presence of CD34. An expert group went on to explain that ASCs must also be positive with regard to the stromal markers CD73 and 90, and that their characterization can be enhanced by the presence of CD10, CD26, CD49d, CD49e as well as CD146 (MUC18)[29].

Lastly, other recent studies have suggested the existence of several stromal sub-populations in adipose tissue[19,30,31], one of them being known as a pericyte population due to its perivascular mural location, which puts it in contact with endothelial cells[30,32]. This population is largely defined by the presence of the marker CD146[32]. Some authors have suggested that MSCs with differing tissue origins might be derived from a common, pericyte-like progenitor cell[19]. It is worthwhile to note that contrary to the aforementioned IFATS definition, these authors differentiate ASCs ($CD34^{pos}$ cells) from pericytes ($CD146^{pos}$ cells)[29]. However, only in a $CD34^{pos}$ fraction did Maumus et al[20] observe a population presenting clonogenic properties, a finding in contradiction with those of teams focusing on pericytes[30-32]. Figure 1 presents an analysis of MSC sub-populations in adipose tissue following an adaptive selection strategy.

The immunomodulatory properties of MSCs

There exist many studies reporting the contrasting immunomodulatory properties of MSCs. In animals and humans alike, results *in vitro* differ. That said, it has been proven that MSCs are not constitutively immunosuppressant; only after stimulation by inflammatory signals[33] from the micro-environment such as the inflammatory cytokines IFN-γ or TNF-α do they acquire such properties[34-36]. During inflammatory response, immune cells such as T lymphocytes, monocytes or macrophages produce the IFNγ necessary to MSC activation[37,38]. Moreover, MSCs are only weakly immunogenic; *in vitro,* they fail to activate allogeneic T lymphocytes. However, they are not immune-privileged, and can be recognized by the immune system of an allogeneic host and are consequently liable to be destroyed by effectors such as activated NK (natural killer) lymphocytes[39]. Their effects are not HLA-restricted, which means that the MSCs exercise their suppressive properties whatever the HLA genotype of the recipient's immune cells[40]. MSCs do not constitutively express either MHC Class II, which they can nonetheless express in an inflammatory context, or costimulatory molecules such as CD40, CD80 and CD86 [41,42]. They act upon all effectors of innate or adaptive immunity (Figure 2) and alter cell proliferation (blocking in phase G0/G1 of the cell cycle[43,44]) as well as other immune cell functionalities.

<u>*Lymphocytes cells*</u>. Lymphocytes are the leucocytes implicated in adaptive immune response, and two main lines may be distinguished: T and B. With regard to T lymphocytes,

MSCs inhibit proliferation [45,46], cytotoxicity and IFN-γ production [45,46]. MSCs induce differentiation into regulatory T lymphocyte (Treg)$CD4^{pos}CD25^{high}FOXP3^{pos}$ [47,48,49,50,51]. MSCs can also impact secretory profile and, consequently, lymphocyte function. Indeed, numerous studies have reported repolarization of the Th1 lymphocyte (IFNγ synthesis) towards a Th2 profile (IL-4 synthesis), a process enabling tissue regeneration. Placenta MSCs have been described as allowing a switch from a Th1 profile towards a Th2 profile[45] by means of the IL-10-priducing monocyte induced by HGF. *In vitro*, adipose tissue MSCs have been described as inhibiting proliferation of naive T-CD4+ lymphocytes and as blocking polarization in Th1 and Th17[48]. Other studies have reported similar inhibition of Th1 polarization[44,52]. That said, works comparing the MSCs of different tissue sources have yielded discordant results on T lymphocytes. Puissant et al reported similar effects on proliferation of T lymphocytes in the spinal cord and in adipose tissue [18], while Najar et al reported similar results with ASCs as compared to umbilical cord MSCs and BM-MSCs[53]. Ribeiro et al demonstrated the superiority of ASCs over BM-MSCs and UC-MSCs in the inhibition of $T\text{-}CD4^{pos}$ and $T\text{-}CD8^{pos}$ lymphocytes and NK[54]. As for Xishan et al, they highlighted the superiority of BM-MSCs over ASCs as inhibitors of the proliferation and activation of T lymphocytes[44]. As concerns B lymphocytes, the role of MSCs remains open to debate[55]. It now appears more and more evident that it depends on their activation status, which itself depends on the interactions of the MSCs with other immune cells[56]. In a non-inflammatory context, MSCs do not inhibit B lymphocyte proliferation, and they even permit the survival and generation of regulatory B cells ($CD38^{high}CD24^{high}$ $IL\text{-}10^{high}$), whereas in an inflammatory context stimulated by IFNγ, MSCs inhibit B lymphocyte proliferation and immunoglobulin synthesis by blocking plasmocytic maturation[57,58].

Monocytes, macrophages, dendritic cells. Monocytes represent a population of blood leucocytes capable of being differentiated in tissues into macrophages and dendritic cells, which are antigen-presenting. These cells are implicated in innate cellular response. Much of what we know about macrophages is derived from studies on mice[59]. In an oversimplified manner, classical or pro-inflammatory M1 macrophages, with their response that can be compared to type Th1 in the nomenclature of T lymphocytes, are described as markedly differing from M2 macrophages, which are alternatively considered as activated or anti-inflammatory and can be compared to type Th2 responses[60,61]. It would nonetheless seem that in between these two extremes, macrophages are more substantially heterogeneous[62]. While M1 macrophages are induced *in vitro* through stimulation by IFNγ, lipopolysaccharide (LPS) or TNFα, M2 macrophages are stimulated by IL-4 or IL-13 [61]. There exists no single signature permitting characterization of a macrophage as M1 or M2; different clusters of arguments on each side warrant examination. In humans, at their surface M1s strongly express class II HLA-DR MHC molecules, and weakly express CD206 (mannose receptor, implicated in phagocytosis[63]); they have a pro-inflammatory secretory profile (IFNγ, TNFα, IL-1β) and moderate capacities for phagocytosis. Conversely, on their surface M2s weakly express HLA-DR and strongly express CD206[64]; they have an anti-inflammatory (IL-10) secretory profile and an elevated capacity for phagocytosis[65]. MSCs help to orient the polarization of macrophages with a pro-inflammatory phenotype towards those with an anti-inflammatory phenotype, thereby favoring tissue regeneration[65,66,67,68]. Indeed, when BM-MSCs are cultivated with macrophages, the latter increase CD206 expression, augment their capacities for phagocytosis and reduce their syntheses of pro-inflammatory cytokines while increasing their syntheses of anti-inflammatory cytokines[69]. MSCs facilitate the survival of monocytes and differentiation into macrophage M2 $CD206^{pos}CD163^{pos}$, cells synthesizing IL-10 and CCL-18, which plays a part in the attraction of T-regulatory lymphocytes[70]. Chiossone et al

found *in vitro* that monocytes cultivated with BM-MSC permit maturation into M2 macrophages, which strongly express CD11b, CD206 and CD163[67]. Lastly, MSCs can alter the differentiation and maturation of dendritic cells[71].

MSC action mechanisms are multiple and for the most part inducible by soluble factors[72]. For example, MSCs synthesize the immunosuppressive enzyme indoleamine-2,3 dioxygenase (IDO), which catabolizes tryptophane into kynurenine[73]. This molecule, which is crucial to inhibition of the proliferation of T lymphocyte effectors by human MSCs, acts by depletion of the medium into an essential amino acid, tryptophane, and by producing kynurenine, which is toxic for T lymphocytes. We now know that the percentage of inhibition of T lymphocyte proliferation is correlated with augmentation of the kynurenine/tryptophane ratio, which reflects IDO activity[12]. Not constitutively present in MSCs, IDO is triggered in response to IFNγ and potentialized by TNFα[35]. In the same way, the effects of MSC on B lymphocytes are mediated by IDO. In fact, tryptophane supplementation enables restoration of B lymphocyte proliferation and immunoglobulin synthesis[58]. In addition, MSCs produce the cyclo-oxygenase 2 (COX-2) enzyme, which plays a part in the synthesis of prostaglandine E2 (PGE2) from arachidonic acid. PGE2 in association with IDO plays a part in inhibiting the proliferation of T[53] and NK[39] cells. Indeed, PGE2 has been reported as being one of the mechanisms enabling differentiation of macrophages into M2 macrophages, whereas this differentiation is lost in the presence of COX2 inhibitors[67]. Németh et al went so far as to underline the key role of the PGE2 produced by BM-MSCs in reprogramming the M2 macrophages secreting IL-10. In these studies, interleukin 10 is considered as responsible for sepsis resolution and consequently for improved survival in mice[74]. Following exposure to TNF-α, MSCs secrete the protein TSG-6 (TNF-α stimulated gene/protein 6) which acts by negative retro-control on the macrophages by decreasing their synthesis of pro-inflammatory factors, thereby curtailing recruitment of polynuclear neutrophil[75,76]. And as regards TSG-6, due to its powerful anti-inflammatory effect in mice it elicits a diminution of infarcted territory in myocardial infarction[3] by reducing the deleterious effects of the excessive inflammation associated with the massive infiltration of polynuclear neutrophils. MSCs also produce a specific form of HLA, HLA-G5, which is considered as responsible for the induction of regulatory T lymphocytes[49]. Importantly, and even though in mice MSCs exhibit the same immunosuppressant and anti-inflammatory properties as human MSCs, the implicated mechanisms markedly differ according to species, thereby underscoring the key importance of studies conducted from human tissues[77]. While in humans, IDO is the main mechanism inhibiting T lymphocyte proliferation, in mice it is iNOS[78].

In order to objectify MSC heterogeneity, and more specifically to compare the immunological properties of the different MSCs, it is crucial to apply a standardized methodology allowing researchers to ascribe observed effects to the MSCs themselves (for example, to the type of source tissue being used) without being subject to bias due to the experimental conditions of an immunological test (for example, a variation in the type of immune cells being used). That, in any event, is how it has been shown that the immunosuppressant potential of ASCs is greater than that of BM-MSCs[12].

Stromal vascular fraction (SVF) and how it can be isolated. From fat grafting to SVF grafting…

In vivo, ASCs are embedded between adipocytes in the extracellular matrix. In order to study them and to have them amplified *in vitro*, the extracellular matrix has got to be digested by proteolytic enzymes. Adipose tissue (AT) is digested at 37°C over a period that varies from one author to the next in a buffer containing collagenase enabling digestion of the collagen fibers of the extracellular matrix. Once the different cellular components have been separated, it is time to centrifuge the product of enzymatic digestion so as to isolate the stromal vascular fraction (SVF). SVF contains all cells except the adipocytes. In fact, extraction is divided into three phases; from top to bottom, they involve the adipocytes, the digestive environment and, finally, SVF (containing hematopoietic cells, endothelial cells and stromal cells)[9]. It matters to remember that SVF contains approximately 2 to 10% of ASC[79].

ASC amplification in clinical projects involving cell therapy treatment subsequently initiates a stage of culturing under standard conditions of good manufacturing practices; the cells are considered as advanced therapy medicinal products[80].

Direct utilization of SVF (without a cell culture phase) in the operating room is of particular interest to plastic surgeons. Indeed, based on the concept of enzymatic digestion by collagenase, different machines are now at surgeons' disposal (see the paragraph on « plastic surgery context »)[81]. Contrary to cell therapy, which implicates a "pure" cellular population, SVF contains a heterogeneous collection of cells, of which the probable interactions may occasion non-elucidated disparities in clinical efficacy. Moreover, given the fact that methods for obtaining SVF have yet to be standardized, there exists a high likelihood of ending up with cellular cocktails of which the composition is diverse, variable and heterogeneous. In France, autologous use of SVF in the operating room is authorized without legal constraint, the reason being that during an operation, the surgeon can manipulate autologous tissues during maneuvers such as fat transplant or reinjection. Contrary to cultivated MSCs, which enter into the framework of "advanced therapy medicinal products", with pronouncedly more rigorous constraints, autologous tissues retain their basic cellular functions.

The clinical context in plastic surgery

On a parallel track, it has become increasingly evident that given the presence of MSCs, adipose tissue transfer presents considerable interest in regenerative surgery. Also known as fat grafting, lipofilling and lipomodelage, this treatment contributes to the regeneration of transplanted tissue. As was demonstrated for the first time in 2007 by Rigotti et al, it is of particular interest as a means of regenerating the sequels of radiotherapy. Indeed, this team showed that the injection of adipose tissue in severe radiation lesions (LENT-SOMA clinical score 3 and 4) in 20 patients permitted neoangiogenesis and improved tissue hydration. Using this technique, regeneration of the radiation dermatitis zone was observed, and it enabled simple reconstructions by split skin grafting instead of the usual, more debilitating reconstructions[27]. By producing trophic support the MSCs facilitated neoangiogenesis; reduced tissue inflammation was in all likelihood the key explanatory factor of clinical success[82]. Magalon et al availed themselves of MSCs' properties and successfully applied the treatment to patients suffering from scleroderma, an autoimmune pathology

leading to tissue fibrosis and microangiopathy clinically translated as Raynaud syndrome. Proof of its efficacy has been given by reinjection of human adipose tissue in nude mice in whom cutaneous scleroderma lesions were induced by bleomycine injection; following fat transfer, reduced tissue fibrosis and neoangiogenesis were observed[83]. As regards humans, a feasibility and safety study was conducted in twelve women and no adverse event was reported, while hand function was improved and pain, edema and Raynaud syndrome were reduced[84-86]. Two randomized controlled studies are ongoing and will yield a high level of evidence on the role of this treatment in this type of pathology (https://clinicaltrials.gov/shows/NCT02396238; NCT02558543). In addition, stromal vascular fraction containing the ASCs is of particular interest to plastic surgeons on account of their capacity to improve fat graft survival. Indeed, during autologous fat transfers, the fat survival rate is variable, ranging from 20 to 80%[87]. The enrichment of fat with SVF, a concept known as "cell-assisted lipotransfert" that was developed by Matsumoto et al in 2006, has led to improved adipose graft survival associated with better vascularization[88]. The same operation was carried out by Yoshimura et al for breast augmentation, and the same results were achieved[89]. The popularity of this technique has proved conducive to the development of new systems through which stromal vascular fraction (SVF) is extracted from adipose tissue for immediate use in the operating room as a means of enriching the tissue to be transferred, the goal being to improve fat retention. The Cytori® machine currently seems to yield the best results in terms of viable cells and the highest rate of clonogenic cells (CFU-F). In addition, it has helped to produce the lowest residual enzyme activity, which represents a significant criterion for clinical use[81]. And yet, notwithstanding the initially promising results obtained with this machine, discordant results ensued; in some studies no benefit was shown[90]. That much said, a high level of proof in evidence-based medicine of the clinical interest of MSCs in fat survival was achieved in 2013 by Kolle et al[91]. In their randomized controlled study, they convincingly demonstrated that the enriched fat in MSCs cultivated *in vitro* led to pronouncedly improved graft survival, with a fat retention rate of 80.9% in the "enriched" group versus 16.3% in the placebo group. Unfortunately, their rigorous demonstration of the efficacy of these cells is not transposable in clinical routine. In plastic surgery, it would be hard to envisage firstly the extraction of adipose tissue, secondly a cell culture phase, and thirdly a second intervention with MSC-enriched tissue in accordance with indications for reconstruction; multiple constraints, for the most part economic, render this type of operation impractical. Moreover, in the aforementioned study a major methodological bias prevents extrapolation of the results inasmuch as the tissue was reinjected in large quantities (30mL) in monobloc surgery, which meant that it remained distant from any vascularization. This is contrary to the basic principles persuasively codified by Coleman[92]. On the same token, the quantity of cells reinjected after a culture phase with SVF extraction and direct utilization in a clinical setting is disproportionate. However, a well-conducted review of the literature applying rigorous methodology and including 25 studies and 696 patients came to the conclusion that fat graft enrichment with SVF cells actually improved graft survival (64 vs. 44%, $p>0.0001$) for small-scale reinjected volumes[93].

Last but not least, the expensiveness of industrial systems of enzymatic isolation of SVF and the cost price of academic studies render these treatments difficult to provide in clinical routine. It will consequently be necessary to find new means of producing SVF cells usable in the operating room, either with the goal of increasing the volumetric retention of the graft or so as to undertake regenerative surgery, as has been done with regard to scleroderma. Techniques for mechanical extraction of SVF cells meet the needs of our discipline and are likely to be representative of the future[94].

New hope was born in 2013[94] with the first description of mechanical digestion of adipose tissue, a process the authors imperfectly termed "nanofat grafting". In this technique, the tissue is emulsified, transferred to syringes (n=30) and filtered at 500 µm; in the end, SVF cells are produced. (If the term "nanofat grafting" is imprecise, the reason is that no adipocytes are involved, and that the cells involved, which are not nanometer-sized, corresponded according to the authors to ASCs.)

A second description of mechanical digestion reported by Raposio et al consisted in tissue vortexing followed by long centrifugation; once again, the procedure presumably produced ASCs[95]. As concerns these two techniques, it was explained that they enabled ASC production to take place, even though neither one of them had provided a scientific demonstration. Moreover, the composition of the SVF cells produced by the two techniques differed, as did the percentage of ASC. In 2016, on the other hand, we demonstrated the possibility of ASC production by mechanical digestion of adipose tissue in accordance with existing benchmarks[96]. In our study, we compared the first two mechanical extraction techniques described in plastic surgery to enzymatic digestion, which is the reference method. We showed that SVF cell viability was inferior in the mechanical digestion groups associated with the traumatism related to the methodology ; more precisely, the number of ASCs was 10 to 12 times inferior to the reference method. We came to the conclusion that the nanofat method described by Tonnard was of greater interest than the method detailed by Raposio insofar as it led to the production of SVF-enriched ASC, as was attested by a higher degree of CFU-F. Once the SVF is cultured subsequent to these three extraction techniques, we have observed that the ASCs possessed the same differentiation capacities and the same capacity to inhibit LT proliferation.

Numerous technical variants may modify SVF composition and shall necessitate characterization studies of the products used in a clinical setting. On a parallel track and as previously underlined by the authors, it will behoove researchers to agree on a definition of emulsification techniques and on the main lines of research to be undertaken in view of analyzing the reinjected product [97].

In conclusion

ASC and SVF have enabled new clinical indications to see the light of day in our discipline. Mechanical SVF production directly accessible in our operating rooms is probably representative of the future. A number of emerging techniques necessitate reiterated attempts at characterization of the products reinjected in our patients; for example, functional studies should allow us to determine whether or not the cells obtained indeed possess the same immunomodulatory and trophic support properties as cultivated ASCs. Following which, a phase of clinical experimentation will be necessary in order to clearly define the roles of these promising techniques in the therapeutic arsenal of the plastic surgeon.

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Figure 1. Analysis of stromal cells in adipose tissue by flow cytometry

After enzymatic tissue digestion and labelling of the SVF cells, MSC analysis is carried out by an appropriate gating strategy using Kaluza software.

Each analysis involves a preliminary selection of cells with (A) exclusion of cell debris, (B) selection of living cells, (C) singlets, (D) exclusion of hematopoietic and endothelial cells (lin: CD45 / CD11b / CD235a) and (E) analysis of the isolated populations from a diagram CD146 / CD34.