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Chapter · January 2016

DOI: 10.1007/978-1-4939-3584-0_6

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Chapter 6

Mesenchymal Stem Cells in Lipogems, a Reverse Story: from Clinical Practice to Basic Science

Carlo Tremolada, Camillo Ricordi, Arnold I. Caplan, and Carlo Ventura

Abstract

The idea that basic science should be the starting point for modern clinical approaches has been consolidated over the years, and emerged as the cornerstone of Molecular Medicine. Nevertheless, there is increasing concern over the low efficiency and inherent costs related to the translation of achievements from the bench to the bedside. These burdens are also perceived with respect to the effectiveness of translating basic discoveries in stem cell biology to the newly developing field of *advanced cell therapy* or Regenerative Medicine. As an alternative paradigm, past and recent history in Medical Science provides remarkable reverse stories in which clinical observations at the patient's bedside have fed major advances in basic research which, in turn, led to consistent progression in clinical practice. Within this context, we discuss our recently developed method and device, which forms the core of a system (Lipogems) for processing of human adipose tissue solely with the aid of mild mechanical forces to yield a microfractured tissue product.

Key words Adipose tissue, Innovative device, Stromal vascular niche, Adipose-derived stem cells, Cryopreservation, Chemical agents, Electromagnetic energy, Lipogems

1 Introduction

Translation of scientific information from the bench to the bedside is a consolidated approach in Science utilized during the last decades, and at the same time it is a concept that has been used so often it risks achieving cliché status. This paradigm has no doubt contributed to the identification of mechanistic bases of a number of diseases, paving the way to modern Molecular Medicine and Therapy. Nevertheless, clinical scientists and public health representatives are increasingly concerned with the low translational efficiency of scientific discoveries of the past generation into tangible clinical improvement. In particular, clinical research is constrained by rising costs, slow results, restrictions in funding, and the need for compliance with cumbersome regulatory issues.

Massimiliano Gnecchi (ed.), *Mesenchymal Stem Cells: Methods and Protocols*, Methods in Molecular Biology, vol. 1416,
DOI 10.1007/978-1-4939-3584-0_6,

The world of stem cells is not immune to these criticisms. So far, human adult stem cells have been isolated from multiple tissues, including bone marrow, dental pulp, placenta, and adipose tissue. Stem cells with attractive features have also been harvested from amniotic fluid and even urinary sediment. A remarkable breakthrough has been seen in our basic knowledge of stem cell biology and the dissection of mechanisms underlying their differentiating potential, paracrine activity, and fate in animal models of disease. Despite this progress, clinical translation of stem cell research to the most challenging diseases currently afflicting society, including heart failure, diabetes, neurodegenerative disorders, stroke, or injuries to the central and peripheral nervous system, is still in its infancy, awaiting affordable strategies and results that may prompt the elevation of cell therapy and regenerative medicine as a consolidated tool to target crucial unmet clinical needs.

Within this context, an urgent question arises: What phenomena do clinicians observe that may attract the attention of basic scientists? Is there any clinical observation from tissue transplantation/cell therapy that is crying for consideration as a novel paradigm in the rescue of damaged tissues, potentially involving laboratory researchers in studies that may lead to further deployment in stem cell biology? Indeed, the relevance of such a reverse, bed-to-bench-side approach, is highlighted by a number of examples, including, just to cite a few: (a) clinical studies showing that cancer-causing retroviruses exert effects similar to AIDS on the human immune system: this helped scientists to identify HIV, a retrovirus in the same family as the cancer-causing retroviruses, as that responsible for the development of AIDS [1]; (b) clinical investigations initially performed on thousands of infants dying annually from a mysterious respiratory ailment: separately, basic studies on surface tension and pulmonary physiology allowed researchers to identify and characterize pulmonary surfactant. As a result, clinical and basic scientists together determined that the deaths were due to a lack of surfactant, which caused the alveoli, or air sacs in the lungs, to collapse [2]. Treatments were then developed in the lab and tested in and applied to patients with great clinical success [3]; (c) clinical research, starting from the anticancer effect of tamoxifen, also demonstrated that the treatment of healthy women can effectively act as a prophylactic strategy to prevent development of breast cancer in high-risk subjects [4]; (d) initial observations linking cardiovascular disease (CVD) to obese patients: over the past several years, these studies fostered an exponential increase in our understanding of adipose biology and its relevance to CVD [5]. Intriguingly, clinical observations in this area still pose remarkable questions for basic scientists: one of the most puzzling is the obesity paradox [5]. How can adipose tissue increase CVD risk factors and yet be protective once CVD develops?

In agreement with these considerations, in the current review we discuss a recently developed method and device, namely Lipogems [6], which yields a microfractured human adipose tissue product that was initially conceived as a device to improve the lipofilling technique for plastic surgery and reconstruction. Since then clinical evidence of unprecedented soft tissue repair after transplantation of the Lipogems product has been observed. Dr. Carlo Tremolada initially invented the method and device to improve our understanding on the inherent features of the tissue product and the putative mechanisms of its rescuing potential through collaboration with basic science research scientists. This reverse story is currently providing novel clues on the mechanisms by which human mesenchymal stem cells (hMSCs)/pericytes residing within the Lipogems product may act to awaken the self-healing patterning by a recipient tissue.

Adhering to the reverse bed-to-bench-side paradigm, the clinical results obtained following the transplantation of the Lipogems product have served as a launching point for subsequent molecular biology studies. Importantly, Lipogems were shown to maintain an intact stromal vascular niche harboring cellular elements with mesenchymal stem cell and pericyte characteristics. Moreover, Lipogems-derived stem cells express transcriptional profiles characterized by self-renewal/stemness patterning, along with a set of genes orchestrating commitment along the neurogenic lineage. Interestingly, the Lipogems product can be reliably cryopreserved, without losing its niche structure and the viability of its embedded stem cells. Further studies have also revealed that the human adipose-derived stem cells (hASCs) residing within the Lipogems product are significantly more responsive to both chemical agents and physical energy than hASCs enzymatically dissociated from the initial lipoaspirate. In particular, exposure to electromagnetic fields induced a significantly higher yield of commitment along the myocardial, endothelial, skeletal muscle, and neural lineages in Lipogems-derived, as compared to enzymatically dissociated hASCs. These findings have helped to unravel several of the mechanistic bases of the healing properties of the Lipogems product and may hopefully serve to further deploy its use in the rescue of damaged tissue.

The fundamental mechanism underlying the clinical efficiency of transplanted freshly obtained Lipogems product (which is basically microfragmeted lipoaspirate thoroughly purified of its oily and hematopoietic components) may involve the secretion of trophic mediators delivering instructive messages that may help create a more compliant “regenerative environment” within the donor tissue. Lipogems may act as a “slow releasing medium” with regenerative factors where they are mostly needed.

2 The Method and the Device

In recent years, surgical techniques including vascular surgery have demonstrated the benefit of transplanted autologous adipose tissue as a filler capable not only of increasing the volume within the tissues receiving vessels compromised by various diseases, but also in restoring the subcutaneous and cutaneous trophisms due to aging or actinic and even radiotherapy damage [7].

A relevant portion of these beneficial effects has been attributed to the rescue-potential of tissue-resident hASCs, sharing consistent phenotypical and transcriptional profiles with hMSCs isolated from different sources, including bone marrow, dental pulp, and term placenta [8, 9]. The use of autologous lipofilling has significantly contributed to the resolution of scarring processes, also favoring engraftment of transplanted skin tissue performed with the aim of optimizing the morphological and functional compliance of the injured soft tissues [8, 9]. In particular, these combined strategies hold promise in promoting the recovery of tissue trophism in a number of challenging conditions, including the diabetic foot, venous leg ulcers, and pressure ulcers.

To date, autologous lipofilling has involved fat harvesting by liposuction usually from the abdomen or inner thigh, followed by centrifugation to partially separate fat from blood, fragments of scarred tissue, and oils released by preadipocytes/adipocytes damaged during the liposuction procedure (Coleman technique) [8]. The processed fat is then placed into syringes with a specially designed blunt cannula for *transplantation*. However, the resulting adipose tissue is a particularly dense product, and it is not suitable for easy passage through narrow needles, which are required to accomplish a delicate subcutaneous graft in extremely fragile or damaged tissues, such as the subcutaneous and cutaneous layers of a diabetic foot or in the delicate and scarred fingers of hands plagued by scleroderma. These clinical presentations require the use of complex and expensive devices (injectors, pumps, specifically designed syringes); nevertheless, they fail to attain a homogenous and finely pliant distribution of transplanted fat tissue [10]. Moreover, results from studies utilizing these current techniques are often variable not only between different patients but even in the same patient and injected area, and rarely is even reabsorption of the injected fat observed (ranging between 20 and 80 %) [10]. This outcome is further worsened by the detrimental effects due to the onset of inflammatory responses triggered by residual oils within the injected fat tissue product.

To overcome these critical problems, Carlo Tremolada and colleagues recently developed and designed a novel method and device (Lipogems™) [6] which: (a) completely resolves the issues associated with the techniques previously utilized for fat

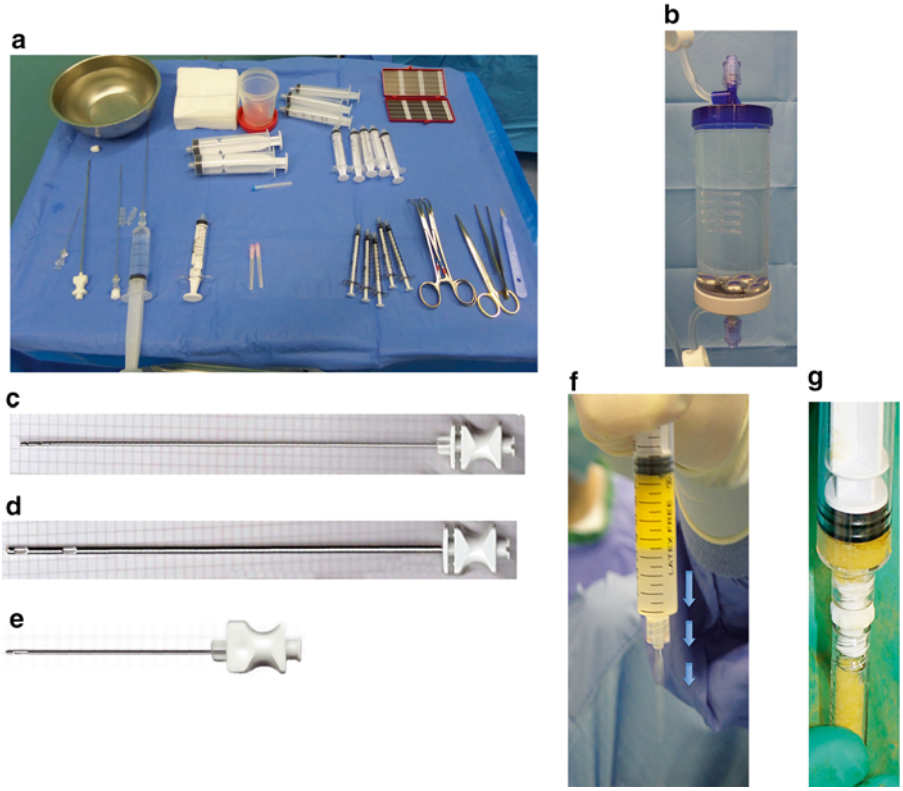

Fig. 1 The Lipogems system. (**a**) Example of the surgical kit provided. (**b**) View of the standard 225-ml device. (**c**) Infiltration cannula (19 G blunt 1 mm multiholes). (**d**) Harvesting cannula (13 G blunt 2 × 3 mm multiholes). (**e**) Injection cannula (19 G blunt 1 × 2 mm hole). (**f**) After collecting lipogems, the syringe is positioned vertically to decant and excess saline is discarded. (**g**) Decanted lipogems tissue is passed from the 10-ml syringe to a 1-ml syringe with a special plastic disposable connector. The 1-ml syringe is ideal for injection into soft tissue

harvesting; (b) facilitates injection through very narrow needles and subsequent homogenous distribution of the fat product (Lipogems product) within the recipient tissue; (c) avoids uneven tissue filling despite a variable rate of volume reabsorption depending on single patients and area of grafting; and (d) avoids post-transplant inflammatory processes.

2.1 The Lipogems Kit

The Lipogems system is a dedicated kit including four sets of instruments that have been optimized to yield the best technical performance and maximize ease of use at each step (Fig. 1a, b).

a. *Anesthesia Kit*: includes a dedicated, disposable blunt 19 G cannula (Fig. 1c) to be attached to any Luer Lock syringe (best 10–60 ml) to infiltrate the adipose tissue before harvesting with saline and diluted epinephrine (1:500,000) with minimal pain and local trauma. This step greatly facilitates subsequent

tissue harvesting through local vasoconstriction and subsequent hemostasis (induced mechanically and by the diluted epinephrine). Local (optional) anesthesia with very diluted lidocaine (0.02 %) may be added to the solution. The harvest area should be infused to obtain a certain degree of tissue firmness (as a general rule, 50 ml per 10×10 cm area of skin), and about 12 min should elapse before collecting the adipose tissue.

b. *Harvesting Kit*: contains a disposable blunt 13 G multi-hole smooth cannula with Luer Lock fitting, specially designed to ensure an optimal compromise between invasiveness and speed of fat tissue harvesting (Fig. 1d). Special autoblocking 10- or 60-ml Luer Lock syringes are provided (optional) to ease the manual harvesting of lipoaspirate which is transferred to larger 60-ml syringes by a special disposable transfer device. The 60-ml syringes are positioned vertically to decant the sample into a tray (a plastic disposable one is also optional) and excess fluid (blood and tumescent solution) is discarded to speed up the next stage.

c. *Processing Kit* (Figs. 2 and 3): this is the true heart of the Lipogems Device and Method. It includes a disposable device with a complete set of fittings and connections for the saline

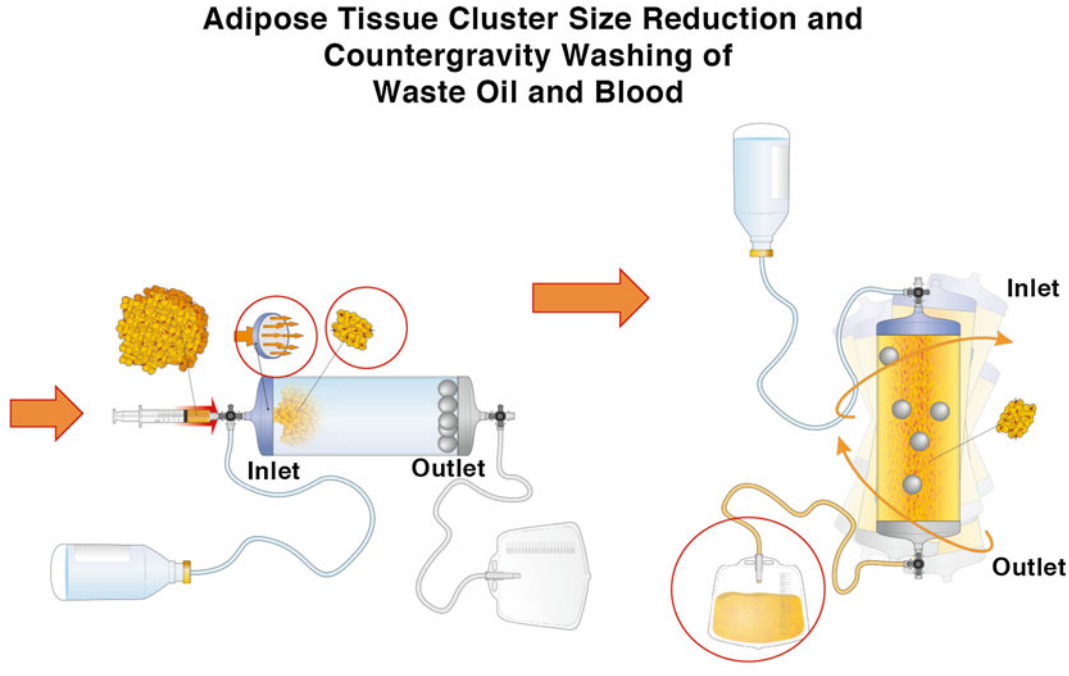

Fig. 2 The Lipogems device: first volumetric cluster reduction and washing of the lipoaspirate. The Lipogems device is powered by gravity and is completely closed and filled with saline. The lipoaspirate is pushed into the inlet (first reduction in volume) and the device is shaked for some minutes to completely wash the Lipogems tissue and remove any waste oil and blood

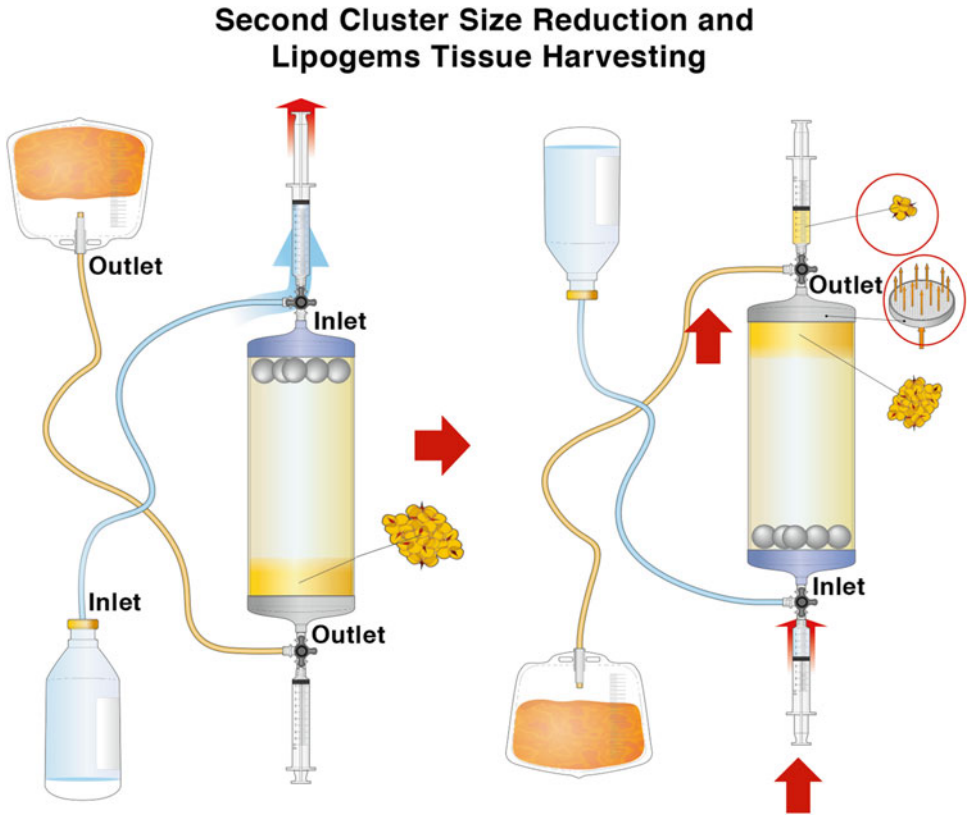

Fig. 3 The Lipogems device: second volumetric reduction of adipose clusters. Once the fat is completely washed and the solution is clear (5–15 min) the device is reversed (*grey cap up*) and a second cluster reduction is performed to obtain the final Lipogems product

bags (1 l saline bags are recommended for the 60-ml device and 3–5 l for the 240-ml device) and a large waste bag. The Lipogems device, aided by the forces of gravity and *minimal tissue manipulation* (as it is defined from a regulatory stand point), allows volumetric reduction (micro-fracturing) of the adipose cluster in the initial lipoaspirate (Figs. 2 and 3).

Between 40 ml and 130 ml of lipoaspirate (ideally 100 ml) are processed during each procedure with the standard 225 ml device (10–25 ml in the 60 ml device). To avoid cell damage, the device is carefully prefilled with saline to avoid the presence of air throughout all the steps, also producing a completely closed system. Tissue processing starts with a first cluster reduction obtained by pushing the aspirated fat from the syringe into the device through a first size reduction filter while allowing a corresponding quantity of saline to exit toward the waste bag (http://youtu.be/wCGM3smxTG8). During a subsequent shaking step, stainless steel marbles inside the device emulsify oil residues which are subsequently removed

together with contaminating blood components and cellular debris by the gravity counter-flow of the saline solution, while the washed reduced fat clusters migrate to the top of the device (http://youtu.be/wCGM3smxTG8). When the solution inside the device appears clear and the lipoaspirate is yellow, the device is turned upside-down by 180°, with the fat tissue product now facing a narrower size reduction filter. The second adipose cluster reduction is obtained by passing the floating adipose clusters through this second-size reduction filter by pushing additional fluid from the lower opening of the device using a 10-ml syringe (http://youtu.be/wCGM3smxTG8). At the end of the procedure, which is performed in a surgical room and only lasts 10–15 min., the device releases a *micro-fractured fluid fat tissue product* (clusters of 300–600 μm in diameter) that can easily flow through a small caliber needle. Lipogems can also easily pass through a standard 25 G sharp needle and homogenously disperse within the recipient tissue after transplantation. The same product can also be subjected to controlled freezing for tissue banking purposes.

The entire procedure is performed manually and sped up by gravity. We are currently developing a fully automated Lipogems device in order to further speed up and simplify the overall process and reduce operator dependent variability.

d. *Infiltration Kit*: If the Lipogems tissue is to be used immediately for clinical purposes, the system includes a specially designed 19 G blunt cannula (Fig. 1e), which permits minimally invasive delivery of the Lipogems product into subcutaneous and cutaneous tissues, as well as intramuscularly, as it has been recently performed at the level of the anal and urethral sphincters, for the correction of fecal incontinency [11]. The cannula is inserted after first puncturing the skin or mucosa with a standard 18 G or larger needle. A 1-ml Luer Lock cannula is recommended for controlling injections especially on the face. Its peculiar small caliber and blunt tip allow the delivery of Lipogems with minimal discomfort. Before infusion, the 10-ml syringe is vertically positioned and excess fluid is discarded (Fig. 1e) and then the Lipogems tissue product is transferred with a 1-ml syringe fitted with a special disposable Luer Lock connector provided in the kit (Fig. 1g).

2.2 Features of the Lipogems Product

Immunohistochemical analysis revealed that the Lipogems product encompasses a remarkably preserved vascular stroma with slit-like capillaries wedged between adipocytes and stromal stalks containing vascular channels with evident lumina. Seventy-two hours following initial fat harvesting, at 4 °C, the Lipogems product still exhibited an intact niche, while unprocessed lipoaspirate from the same donor exhibited an unorganized environment with compressed

and distorted microchannels [6]. The exact mechanism(s) accounting for the niche preservation within the Lipogems product remains to be established. We cannot exclude that maintenance of the adipose stem cell microenvironment is the result of (a) a lipoaspiration performed with an ad-hoc designed cannula more gently impacting with the site of harvest; (b) the mild mechanical forces and low pressure (gravity) applied within the processing device throughout each step; and (c) the prompt removal of oil, cellular debris, and lysates that may chemically act to degrade the niche architecture over time. In fact, comparative immunohistochemistry revealed that at 72 h after harvesting, the expression of CD146 was significantly increased in the Lipogems product, as compared with the unprocessed lipoaspirate; CD34 was similarly expressed in both samples. Since CD34 is a marker of endothelial differentiation and CD146 is co-expressed by endothelial cells and pericytes, these results indicate that pericytes, a mesenchymal cell that is thought to have stem-like properties, explains the significantly increased expression of CD146 found in the Lipogems product. Accordingly, the expression of α-smooth muscle actin (ASMA), a well-established marker of mural cells, was also higher in the Lipogems product than in the unprocessed lipoaspirate [6]. The number of cells expressing S-100 protein, a marker for adipocytes and preadipocytes, was similar in both conditions.

Further phenotypic analyses were performed on freshly harvested Lipogems product, the product previously stored at 4 °C for 24 h, or the product thawed after 7 days of cryopreservation at −180 °C under liquid nitrogen. Following a collagenase digestion to release the stromal vascular fraction (SVF) and to remove adipocytes, cellular viability close to 100 % was observed in all samples, as shown by the trypan blue dye exclusion test, with no differences between groups. Comparative flow cytometry analyses of selected stem cell markers in non-expanded cellular components from the lipoaspirate and the Lipogems product revealed significantly higher expression of the CD146+/90+/34− pattern [6] (identifying cells with pericyte characteristics [12]) in the Lipogems SVF when compared to the original lipoaspirate. The SVF fraction from the Lipogems product also exhibited a significantly higher percentage of CD146+/34+ elements than the unprocessed lipoaspirate. This expression pattern points to a pericyte subset that may be transitional between pericytes and supra-adventitial adipose stromal cells, and/or a set of endothelial (progenitor) cells [13, 14]. The percentage of $CD90^{+}/CD29^{+}/CD34^{-}$ elements, unambiguously identifying the hMSC population, was remarkably higher in the Lipogems product, when compared with the lipoaspirate. In further support of the differences between the two cellular products, the percentage of hematopoietic-like elements positive for CD14, CD34, and CD45 was significantly lower in the Lipogems than in

the unprocessed lipoaspirate [6]. Accordingly, a significantly higher percentage of hMSC-associated expression elements, compared with hematopoietic stem cell-related markers, was recently observed in another independent study on the characterization of the human micro-fragmented fat tissue product obtained with the Lipogems method and device [15].

3 The Expansion of Lipogems-Derived hASCs

We have provided evidence that Lipogems-embedded stem cells can be easily transferred and expanded in culture, without any manipulation. After placing the Lipogems product in regular D-MEM medium, containing 10 % fetal calf serum, hASCs were released from the tissue clusters, attaching to the tissue culture plastic, and reached 70–80 % confluence within 7–12 days [6]. Therefore, even in a GMP setting, the Lipogems product can be immediately transferred to a tissue culture environment for expansion, while in the same setting the conventional enzymatic processing of the lipoaspirate, and related washing of blood and oil contaminants, would require considerably longer periods and additional manipulation (usually 40–50 min per sample), prior to placing the released cells into culture.

Culturing Lipogems-derived hASCs also provided evidence that these cells exhibit the typical developmental potential of hMSCs, including commitment along osteogenic, chondrogenic, and adipogenic lineages [6, 15]. Adipogenic differentiation showed multiple adipocytic multivacuolar cells; the size increased with the time of induction. Osteogenic differentiation was confirmed by morphological changes, as early as the first week of induction and at the end of the induction period, by the formation of mineralized matrix, as demonstrated by Alizarin Red staining. Chondrogenic differentiation was observed after a 3-week induction period, as shown by the appearance of abundant extracellular matrix, and the presence of human type II collagen. The Lipogems tissue itself, not only its derived hMSCs, differentiated toward cartilage in vitro, acting as a natural scaffold and exhibiting interesting mechanical properties [16].

Of particular interest, Lipogems-derived hASCs expanded in culture retained their ability to express a set of genes, including vascular endothelial growth factor (VEGF), KDR, encoding a major VEGF receptor, and hepatocyte growth factor (HGF), involved in the orchestration of vasculogenesis and proper capillary formation [6]. Moreover, Lipogems-derived stem cells were found to express genes that constitute the core circuitry of self-renewal such as Oct4, Sox2, Nanog, and neurogenic lineage genes such as NeuroD1, Pax6, and Sox3 [15].

4 The Lipogems Product Is Efficiently Cryopreserved and Can Be Obtained from Cadaveric Donors

As reported above, the Lipogems product can be cryopreserved even in liquid nitrogen, without altering either the stromal vascular niche structure or the viability of the embedded stem cell elements (hMSCs and pericytes) [6]. Conversely, the release of viable hASCs from cryopreserved lipoaspirates is a rare, low-yield, and non-reproducible phenomenon.

This observation implies that excess Lipogems product resulting after a transplantation procedure, or the product itself, may be subjected to banking for future use, without losing its tissue status.

Intriguingly, a micro-fractured fat tissue product still harboring viable hASCs can also be harvested from cadaveric donors with the Lipogems device [6]. In cadaveric fat tissue (≤30 h post-mortem) there were approximately 75 % fewer total viable cells within the SVF after either enzymatic digestion or Lipogems processing, when compared with adipose tissue harvested from living donors. The yield of cells released from the cell clusters of the Lipogems product following its treatment with collagenase is similar to that observed after a direct enzymatic digestion of the unprocessed lipoaspirate, indicating that Lipogems processing does not affect hASC recovery. Flow cytometry analysis of cultured hASCs derived from cadaveric Lipogems provided evidence that the vast majority (~80 %) of cells expressed characteristic hASC markers, exhibiting phenotypic patterns similar to those detected in hASCs obtained from the Lipogems product of living donors.

Similar to the product derived from living donors, the cadaveric Lipogems product can be cryopreserved; after thawing, viable cells are released and can be grown and expanded in culture.

5 Lipogems-Derived hASCs Vigorously Respond to Both Chemical and Physical Stimuli

An interesting finding in stem cell biology is that stem cell multi-/pluri-potency and fate can be modulated not only by naturally occurring or synthetic chemical agents [17–23], but also by physical energy, as observed following exposure to electromagnetic fields [24–27].

We have previously shown that the expression of vasculogenic genes can be remarkably enhanced following exposure of enzymatically dissociated hASCs to a mixture of natural molecules including hyaluronic, butyric, and retinoic acids [28]. Both Lipogems-derived hASCs and hASCs resulting from enzymatic digestion of lipoaspirates harvested from the same donor were found to spontaneously express VEGF, KDR, and HGF mRNAs

to a similar extent. However, exposure of Lipogems hASCs to the above mixture resulted in significantly higher transcription of these genes, as compared to the effect yielded from enzymatically dissociated stem cells. Studies are currently in progress to assess whether such a greater vasculogenic potential may result in enhanced tissue healing in animal models of vascular disease.

Lipogems-derived hASCs were also highly responsive to the action of electromagnetic fields. In particular, exposure of Lipogems-derived hASCs to a Radio Electric Asymmetric Conveyer (REAC), an innovative device designed to asymmetrically convey radioelectric fields of 2.4 GHz to either the human body [29, 30] or cultured cells [26, 27], remarkably enhanced the transcription program of multilineage, tissue-restricted genes [27], including: (a) cardiogenic genes prodynorphin, GATA-4, and Nkx-2.5; (b) vasculogenic transcripts VEGF, HGF, and von Willebrand factor (vWF); (c) neurogenin-1, and (d) myoD, involved in neurogenic and skeletal myogenic commitment, respectively.

Stem cell exposure to REAC also finely tuned the expression of stemness-related genes, inducing an early increase in Nanog, Sox2, and Oct4 transcription during the first 4–12 h, followed by a significant down-regulation of transcript levels below the control value after 24 h of treatment [27]. It is now evident that the down-regulation of stemness genes after their initial induction is a critical step in cell progression toward a differentiated state [31–35]. Relevant to these observations, comparative transcriptional analyses in REAC-exposed cells revealed that both the early overexpression and the subsequent inhibition of stemness genes were significantly more pronounced in Lipogems-derived hASCs than in the enzymatically dissociated counterpart [27]. This distinctive feature was reflected in the differentiating ability of Lipogems-derived hASCs. In a stem cell population exposed to the electromagnetic field, flow cytometry analysis of β-3-tubulin, myoD, and α-sarcomeric actinin highlighted a neural, skeletal myogenic, and cardiogenic commitment, respectively; and provided evidence that the percentage of each lineage commitment from Lipogems-derived hASCs significantly exceeded the percentage detected from enzymatically dissociated hASCs [27].

On the whole, these data indicate that the Lipogems product may be an ideal source of stem cells capable of optimizing their multipotency expression and differentiation potential in the presence of either chemical or physical stimuli. The mechanisms underlying these results still remain to be elucidated. We are currently investigating the possibility that lipoaspirate processing with the Lipogems device (avoiding the use of collagenase and other enzymes) may have preserved the cell surface environment and glycocalyx composition better than other methods based on enzymatic dissociation. This would account for the enhanced hASC responsiveness to chemical and physical interventions.

6 Future Directions

It is increasingly evident that a multidisciplinary approach to basic and clinical research should accelerate translation to the bedside. While basic research findings drive the development of clinical research studies, and data from these studies improve our understanding of human health and disease; it is also true that data collected in the clinical setting may guide the direction of basic research questions and hypotheses. Ultimately, both would be expected to lead to improvements in medical treatment, diagnostics, and preventive care.

Studies are in progress to verify whether the Lipogems product by itself, or the chemical/physical preconditioning of the product (or its expanded hASCs) prior to transplantation, may result in improved tissue rescue in defined animal models of disease, including heart failure, neurodegenerative diseases, skeletal muscle dystrophy, diabetes, bone, and cartilage defects.

In the affirmative, the Lipogems method and device may become an attractive *system* for multifaceted tissue/cell therapy interventions.

Weblink to Supplemental Animation. Lipoaspirate processing with the Lipogems device. http://youtu.be/wCGM3smxTG8

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