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INTERNATIONAL CONTRIBUTION

Research

Characterization of the Tissue and Stromal Cell Components of Micro-Superficial Enhanced Fluid Fat Injection (Micro-SEFFI) for Facial Aging Treatment

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Abstract

Background: New microfat preparations provide material suitable for use as a regenerative filler for different facial areas. To support the development of new robust techniques for regenerative purposes, the cellular content of the sample should be considered.

Objectives: To evaluate the stromal vascular fraction (SVF) cell components of micro-superficial enhanced fluid fat injection (SEFFI) samples via a technique to harvest re-injectable tissue with minimum manipulation. The results were compared to those obtained from SEFFI samples.

Methods: Microscopy analysis was performed to visualize the tissue structure. Micro-SEFFI samples were also fractionated using Celector,® an innovative non-invasive separation technique, to provide an initial evaluation of sample fluidity and composition. SVFs obtained from SEFFI and micro-SEFFI were studied. Adipose stromal cells (ASCs) were isolated and characterized by proliferation and differentiation capacity assays.

Results: Microscopic and quality analyses of micro-SEFFI samples by Celector® confirmed the high fluidity and sample cellular composition in terms of red blood cell contamination, the presence of cell aggregates, and extracellular matrix fragments. ASCs were isolated from adipose tissue harvested using SEFFI and micro-SEFFI systems. These cells were demonstrated to have a good proliferation rate and differentiation potential towards mesenchymal lineages.

Conclusions: Despite the small sizes and low cellularity observed in micro-SEFFI-derived tissue, we were able to isolate stem cells. This result partially explains the regenerative potential of autologous micro-SEFFI tissue grafts. In addition, using this novel Celector® technology, tissues used for aging treatment were characterized analytically, and the adipose tissue composition was evaluated with no need for extra sample processing.

Level of Evidence: 5

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Cell-based therapy is a promising approach for tissue repair and regeneration in many disorders. Adult stem cells can be isolated from several tissues, such as bone marrow, skin, heart, gut, and skeletal muscle, among others. In the past, adipose tissue has raised substantial interest as an alternative source of stem cells due to its high recovery and accessibility. For the first time, Zuk et al described human adipose tissue as a source of multipotent mesenchymal stromal/stem cells similar to those present in bone marrow.[1] Adipose-derived stromal cells (ASCs) were identified in the stromal vascular fraction (SVF). The SVF is

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composed of different and interrelated cell types: ASC progenitors, pericytes, and endothelial progenitor cells.[2] ASCs are abundant in adipose tissue, comprising approximately 1%. They are defined as plastic-adherent multipotent cells that can differentiate *in vitro* towards mesodermal lineages (osteoblasts, adipocytes, and chondrocytes) and are positive for the mesodermal markers CD73, CD90, and CD105, and negative for the hematopoietic markers CD14, CD34, and CD45.[3] Moreover, ASCs can secrete bioactive molecules capable of stimulating angiogenesis and fat graft revascularization. ASCs also present antifibrotic, anti-apoptotic, and immunomodulatory properties.[4] These characteristics of SVF/ASCs could be involved in some of the effects observed after adipose tissue grafts, such as improved skin tropism, accelerated closure of complex wounds or ulcers, and the enhancement of skin appearance after damage from radiotherapy.[5–7]

The first clinical application of autologous adipose SVF to treat widespread traumatic calvarial defects was reported by Lendeckel et al in 2004.[8] The number of clinical trials evaluating the efficacy and safety of ASCs in tissue reconstruction and regeneration increases every year and, according to the clinical trials database (ClinicalTrials.gov database 2017), there are currently 248 studies registered using ASCs. These studies mainly include treatment for bone and cartilage diseases, autoimmune diseases, and nervous system diseases.

Coleman standardized a procedure for the use of autologous fat tissue as a filler to restore facial volume.[9] The transplantation of viable adipocytes and SVF/ASC-enriched fat grafts yields a combination of volumization and skin regeneration effects. The size of adipose clusters must be below 1 mm to improve vascularization and simplify the superficial injections procedure.[10–13] Accordingly, several lipofilling procedures have been developed during the last 2 decades. These have moved from relatively large cannulas (3-6 mm diameter) with large side portholes (2-4 mm diameter) to those with smaller side portholes (micrograft).[9,10,13–20] The use of micrografts allows for superior engraftment and tissue survival after transplantation and to deliver natural filling via a safer and simpler procedure. Moreover, harvested adipose tissue must be mechanically fluidified before being injected into the patient to avoid lumpiness in the subcutaneous region. The face requires a very complex treatment pattern because the skin greatly differs in thickness, depending on the area and among patients.[14–16] The micro-SEFFI (superficial enhanced fluid fat injection) technique represents a new approach for using microfat grafting in facial rejuvenation procedures. This technique requires minimal manipulation of the tissue and does not require any additional device for the grafting procedure. The SEFFI system uses harvesting cannulas equipped with either a 0.8 mm or 0.5 mm side porthole, while micro-SEFFI system cannulas are equipped with 0.3 mm side portholes. These systems provide three types of tissue with differences in cellularity and fluidity. The SEFFI system is used to treat facial areas with thicker skin, while the micro-SEFFI treats area with thinner skin.[21–23]

ASCs have a role in tissue modeling because of their ability to secrete growth factors and produce new extracellular matrix (ECM) components.[24–26] The harvesting technique may affect the yield, viability, and functions of the isolated cells. For this reason, quality control systems are required to standardize the product obtained via new harvesting techniques and protocols.

In this work, the fluidity, thickness, and composition of mesenchymal stromal cells from adipose tissue harvested with micro-SEFFI cannula were evaluated by conventional microscopy and with a new technology named Celector® (Stem Sel Ltd, Bologna, Italy). Celector® implements the non-equilibrium, earth gravity-assisted dynamic fractionation (NEEGA-DF) method. This method is used to analyze cells based on differences in their dimensional/morphological properties.[27–31] This new analytical technology allows for easier and deeper analysis of the tissue harvested, underlying the presence of red blood cells, adipocytes, cell aggregates, fat droplets, and matrix components liberated from liposuction procedures.

METHODS

Sample Collection and Experimental Setup

The study was conducted for 7 months in 2016 (from June until December 2016). Adipose tissue was harvested consecutively from 8 healthy donors of an average age of 46 years (range, 25 to 55 years). Seven donors were female, and one was male (Table 2). Adipose tissue was harvested from different locations using SEFFI (0.5 mm and 0.8 mm side portholes diameter) and micro-SEFFI cannula (0.3 mm side portholes diameter) after informed consent was obtained in accordance with the Declaration of Helsinki guidelines (Table 1). From case 5, we obtained a lipoaspirate sample with only the cannula with 0.3 mm side portholes because the patient did not have enough adipose tissue to allow harvesting with all 3 types of cannula. Fat aspiration was performed while the patient was maintained under local anesthesia, and intravenous sedation was monitored. The following standardized protocol was used. Cold Ringer's lactate solution (500 mL) was mixed with lidocaine (500 mg), sodium bicarbonate (5 mg), and epinephrine (1 mg) and then injected into the selected donor site at a ratio of 1:1 to the average amount of tissue to be harvested. Manual aspiration of the adipose tissue was performed with a 10 mL syringe alternatively mounted with each of the 3 different multi-perforated side port cannulas. The fat depots selected as the preferred

Table 1. Number of Samples Obtained from Liposuction Using SEFFI and Micro-SEFFI Cannulas

Sample Type	Number of Samples	Average Volume (Ml/Sample)	Anatomical Location			Gender	
			Abdomen	Hip	Trochanter	Female	Male
0.8	8	10.13	3	3	1	7	1
0.5	5	7.10	2	1	1	5	0
0.3	9	7.94	3	4	1	7	2

The average volume obtained, harvesting site, and sex of the donors are shown.

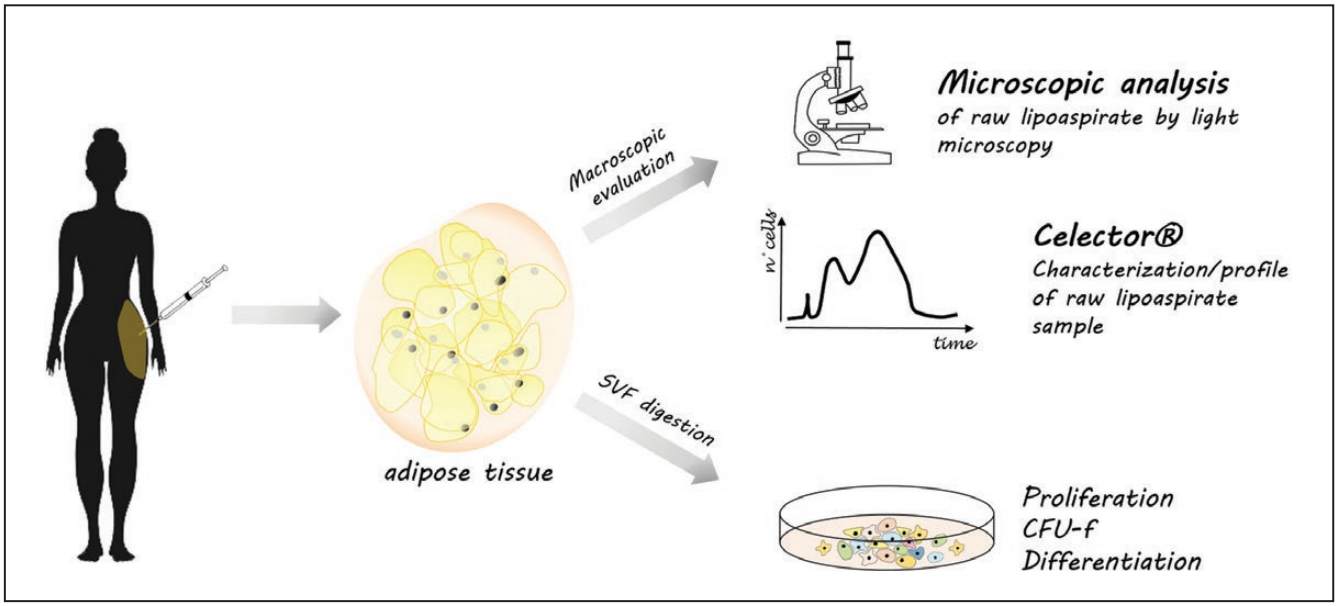

Figure 1. The experimental design flow is demonstrated. Adipose tissue was harvested using 3 types of cannulas with different porthole diameters (0.3 mm, 0.5 mm, and 0.8 mm portholes). Drops of fresh tissue were visualized under a light microscope to elucidate the structure and composition of the adipose tissue. In parallel, a small portion of fresh tissue (~500 µL) was analyzed by Celector® to obtain its profile and tissue composition, while the remaning fresh tissue was digested with Collagenase II to obtain an SVF fraction and release mesenchymal stromal cells. These cells were then characterized by their CFU-F formation ability, proliferation ability, and differentiation potential.

harvesting sites were in the abdomen, hip, and peritrochanteric region. After aspiration, the fat was mixed with cold Ringer's solution to rinse the anesthetic and to facilitate tissue precipitation. To reduce the possibility of light oxidation of the adipocytes, the syringe was then capped and maintained in a dark environment under a sterile cloth. The tissues harvested with 0.3 mm, 0.5 mm, and 0.8 mm side port cannulas were kept in separate labeled syringes. The tissue was centrifuged for 1 minute at 3500 rpm, providing an estimated sedimentation force of 448 g. The liquid portion collected at the bottom of the syringe as well as the free oil on top of the tissue were then eliminated.

Samples were then rapidly transferred to the lab and analyzed as follows: samples were observed under an inverted microscope and with the Celector® analytical instrument to examine tissue structure and composition. In parallel, a portion of each sample was digested, and cells were isolated (Figure 1).

Tissue Characterization

Microscopic Analysis of Lipoaspirate Tissue

One drop of fresh lipoaspirate tissue from each type of cannula (0.3 mm, 0.5 mm, and 0.8 mm side portholes) was mounted onto a histological slide and covered with a transparent coverslip. The tissue was then visualized under a light microscope (Leica, Buccinasco (MI), Italy), and pictures were taken.

Celector® Characterization

Celector® was used as a quality control device to study the tissue composition and fluidity of the lipoaspirate sample. Celector® is a novel technology for cell characterization,

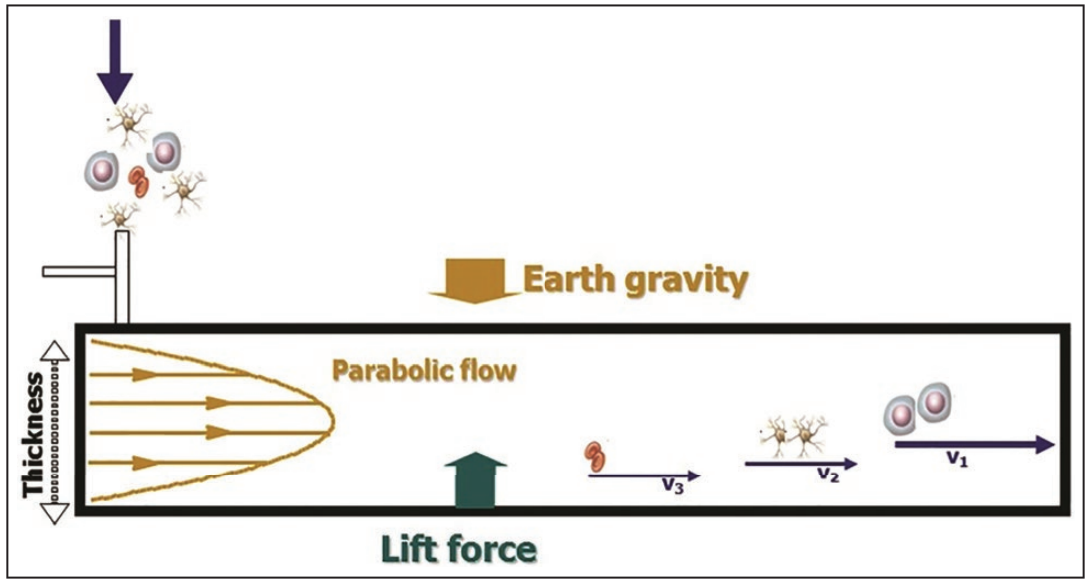

Figure 2. NEEGA-DF fractionation mechanism. Separation occurs in a capillary channel with a parabolic profile of mobile phase flow. During the elution, the opposing force on the cells is gravity, which acts perpendicularly to the flow, and the opposing lift forces depend on morphological features. A heterogeneous cell suspension is injected into the capillary channel; cells with different morphology reach a different position and are thus eluted at well-defined velocities.

quality control, and sorting that has the key advantage in that it can sort cells from both raw tissues and *ex vivo*-cultured cells without any additional sample manipulation (Stem Sel ltd., Bologna, Italy). Celector® consists of a fluidic system and a biocompatible capillary separation device that implements a patented technology for cell analysis[30] of dispersed tissue or single cells. A micro-camera detector placed at the exit of the separation channel (USB 2.0 board-level camera - mvBlueFOX-MLC, Matrix Vision, Oppenweiler, Germany) monitors the elution process, generating a recorded plot of the eluted cell number as a function of time (the fractogram). A fraction collector is connected to the instrument to eventually collect viable cells. The instrument was placed inside a laminar flow hood to provide sterile working conditions, and sterile solutions were used to preserve sterility during fractionation.

NEEGA-DF Method

Celector® implements the NEEGA-DF proprietary separation method to ensure high purity, viability and recovery of the analyzed cells.[29] The scheme of the NEEGA-DF fractionation method is shown in Figure 2. Cells are eluted in the capillary device by the flow of the mobile phase with a parabolic profile for flow velocities. The mobile phase flows at a higher velocity at the center of the channel than close to the channel walls. Cells with different morphological features (dimension, shape, or surface properties) reach a specific position across the channel thickness during transportation due to the combined action of gravity, acting perpendicularly to the flow, and opposing lift forces that depend on the morphological features of the sample act in the opposite direction. Cells at a specific position in the channel acquire well-defined velocities and are therefore eluted at specific times. To generalize, larger cells reach a higher position inside the capillary and, as a consequence, acquire higher velocities within the capillary device (Figure 2: v1 > v2 > v3). This soft fractionation mechanism guarantees the preservation of the native physical features of the cells, as there is no contact between the eluted cells and the separation device; thus, viable, intact cells can be collected at the channel outlet.

Fractionation Procedure

At the beginning of the experimental day, decontamination of the fractionation system was performed by flushing the system with cleaning solution. Next, the system was washed copiously with sterile, demineralized water. Cells, mesenchymal cells in particular, have the capacity to adhere to plastic. For this reason, to block non-specific interaction sites on the plastic walls, the fractionation system was flushed with a sterile coating solution made of 1% bovine serum albumin (BSA) and 1% penicillin/streptomycin (PS) in phosphate buffered saline (PBS). Finally, the fractionation system was filled with the sterile mobile phase. All solutions were provided by Stem Sel Ltd.

A 100 µL volume of the lipoaspirate sample obtained from the micro-SEFFI cannula was injected into the system. Tissue was eluted at a flow rate of 1 mL/min to separate the cell and matrix components from the tissue.

Optical Analysis

Eluted cells were monitored using a micro-camera detector placed at the separation channel exit. The cell count software (Stem Sel Ltd) counts and processes all eluted cells as a function of time. Dimension inclusion/exclusion criteria are set by the operator to refine the counting procedure. For lipoaspirate samples, the dimension setting was defined from 7 µm to unlimited µm. In this way, the software was able to recognize sizes ranging from small cells (ie, red blood cells) to large cell aggregates entrapped in the ECM.

Adipose Tissue-Derived Cell Characterization

Cell Isolation

Adipose tissue was processed as previously described.[32] Briefly, the tissue was digested in a 0.075% collagenase II solution (Sigma-Aldrich Co., St. Louis, MO, USA) prepared in Dulbecco's Modified Eagle's Medium (DMEM, Lonza, Walkersville, MD, USA) for 30 minutes at 37°C under agitation. Digested samples were then washed with isotonic PBS and then resuspended in growth medium to block digestion (DMEM-H, 10% FBS, 1% penicillin and streptomycin, all from Lonza). Between each washing step, the sample was centrifuged at 300 g for 10 minutes. The pellet was then passed through a 100 µm strainer to remove debris and undigested tissue. Next, the SVF was resuspended in growth medium. The nucleated cells were counted using methyl violet solution. The cells were cultured at 5000 cells/cm^2 in growth medium.

Cell Analysis

To characterize the stemness of the isolated cells, 5,000 cells from the SVFs were plated in a 6-well plate to visualize colony forming unity fibroblasts (CFU-Fs). After 14 days, cells were fixed for 20 minutes in 10% formalin solution and then stained with methylene blue for 10 minutes. Colonies colored blue were visualized under a light microscope.

Cell Proliferation Assay

The proliferative potential of the adipose cells was evaluated by the alamarBlue assay according to the manufacturer's instructions (AlamarBlue® Cell Viability Assay - Thermo Fisher Scientific, Waltham, MA, USA). Briefly, 10,000 cells were plated in triplicate in a 96-well plate in growth medium for 24 hours. Next, 10% alamarBlue solution in DMEM/high glucose was added to the cells for 4 hours. Fluorescence was monitored at a 530 nm to 560 nm excitation wavelength and a 590 nm emission wavelength directly from the plate (Victor system, Victor Multilabel Plate Reader - Perkin Elmer, Boston, MA, USA). The medium was replaced, and the procedure was repeated every day at the same time until the cells reached confluence. The fluorescence values were normalized to have the same fluorescence as a starting point.

Differentiation Potential Towards Mesenchymal Lineages

The differentiation potential towards mesenchymal lineages (adipogenic, chondrogenic, and osteogenic) was tested on ASCs derived from the different cannulas.

Adipogenic Differentiation

ASCs were cultured in growth medium until they reached 70% confluence, at which point the medium was replaced with adipogenic induction medium (hMSC, Mesenchymal Stem Cell Adipogenic Differentiation Medium, Lonza). The cells were cultured for 2 weeks, and the medium was replaced every 3 to 4 days, shifting from induction to maintenance medium until the end of the treatment. To visualize fat droplets, the cells were fixed in formalin and stained with Oil Red O solution.

Osteogenic Differentiation

ASCs were cultured in growth medium until they reached 90% confluence, at which point the medium was replaced with osteogenic induction medium (StemPro Osteogenesis Differentiation kit, Gibco - Thermo Fisher). Growth was continued for 28 days, with the medium being replaced every 3 to 4 days until the end of the differentiation. The matrix deposition was stained using Alizarin Red solution (40 mM, pH 4.1, Sigma-Aldrich Co.).

Chondrogenic Differentiation

One-hundred-thousand cells were resuspended in 5 µL of PBS, plated, and then incubated for 1 hour at 37°C in a humidified incubator. The pellet was cultured for 1 day in growth medium, and the medium was then replaced with chondrogenic medium (StemPro Chondrogenesis Differentiation kit, Gibco - Thermo Fisher). The differentiation assay lasted 21 days, and the medium was changed every 3 to 4 days. Micromasses and differentiated adherent cells were visualized under a light microscope using Alcian blue staining.

RESULTS

Lipoaspirate Harvesting Procedure

Adipose tissues from SEFFI and micro-SEFFI were freshly processed at an interval of 2 to 3 hours after harvesting. Lipoaspirates harvested using SEFFI and micro-SEFFI systems were macroscopically similar (Figure 3). The samples stratified in different layers: an upper layer of oil due to the lysis of mature adipocytes, a middle layer of adipose tissue, and an infranatant containing saline and red blood cells (RBCs). The oily layer was removed, and adipose tissue

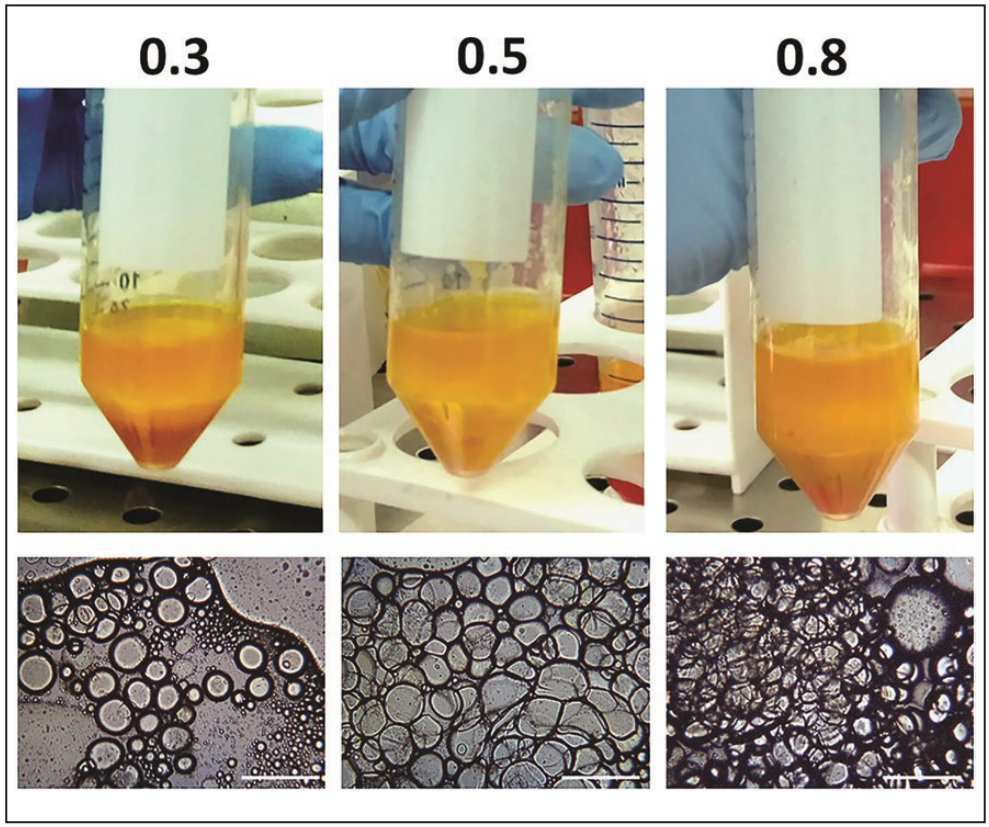

Figure 3. Lipoaspirate sample appearance: representative images of fresh tissue harvested with the micro-SEFFI (0.3) and SEFFI (0.5 and 0.8) systems. The samples showed classical phase separation with an oily top layer, adipose tissue in the middle, and an infranatant containing RBCs on the bottom layer (top). Representative images of fresh adipose tissue visualized under the light microscope (scale bar = 100 µm) (bottom). Round adipocytes are well defined in the 0.3 mm sample, while the complexity of the tissue increased from the 0.5 mm to the 0.8 mm sample.

was aspirated with a serological pipette to measure the exact amount of tissue. We observed that the lipoaspirate samples from micro-SEFFI (0.3 mm) were smoother than those from the 0.8 mm cannula, while samples from the 0.5 mm cannula had a similar consistency. Images from light microscopy analysis of the fresh tissue confirmed these observations. Tissue from the 0.8 mm cannula consisted of complex structures composed of larger adipocytes trapped in the ECM, while the micro-SEFFI (0.3 mm) sample was smoother as shown by the presence of individual and smaller adipocytes (Figure 3). Tissue obtained from the 0.5 mm SEFFI had a composition in between the 0.8 cannula and the micro-SEFFI.

A microscopic analysis of tissues was also performed through Celector®. Celector® technology can analyze unprocessed lipoaspirate tissue harvested with the micro-SEFFI cannula. As explained above, this sample is smooth and easy to inject with no need for further fluidification. The samples from the 0.5 and 0.8 cannulas were also analyzed.

The adipose tissue was injected into the system, and the tissue composition was visualized via live imaging. Figure 4A illustrates the typical fractionation cell count profile for the cell suspension distribution. The x-axis shows the elution time in minutes, and the y-axis represents the biological material visualized by the camera, which is recognized and counted by the cell count software (Stem Sel Ltd, Italy). This software recognizes elements with sharp edges and defined dimension via parameters chosen by the operator. To analyze the heterogeneous lipoaspirate sample, we set an interval between 7 µm (the diameter of RBCs, the smallest element present) to unlimited size because lipoaspirate samples are composed of large aggregates and fat droplets with unpredictable/unknown dimensions.

From the live images, we identified debris and unretained RBCs at the very beginning, followed by large cell aggregates, ECM fragments (Figure 4B i, ii), single cells, and small fat droplets (Figure 4B iii, iv). The micro-SEFFI sample was rich in RBCs that eluted throughout the fractionation process. All the fractionated samples eluted between 2 and 25 minutes.

Characterization of Target Cell Component

ASCs were derived from all donors using 3 types of cannula. Neither the type of surgical procedure nor the

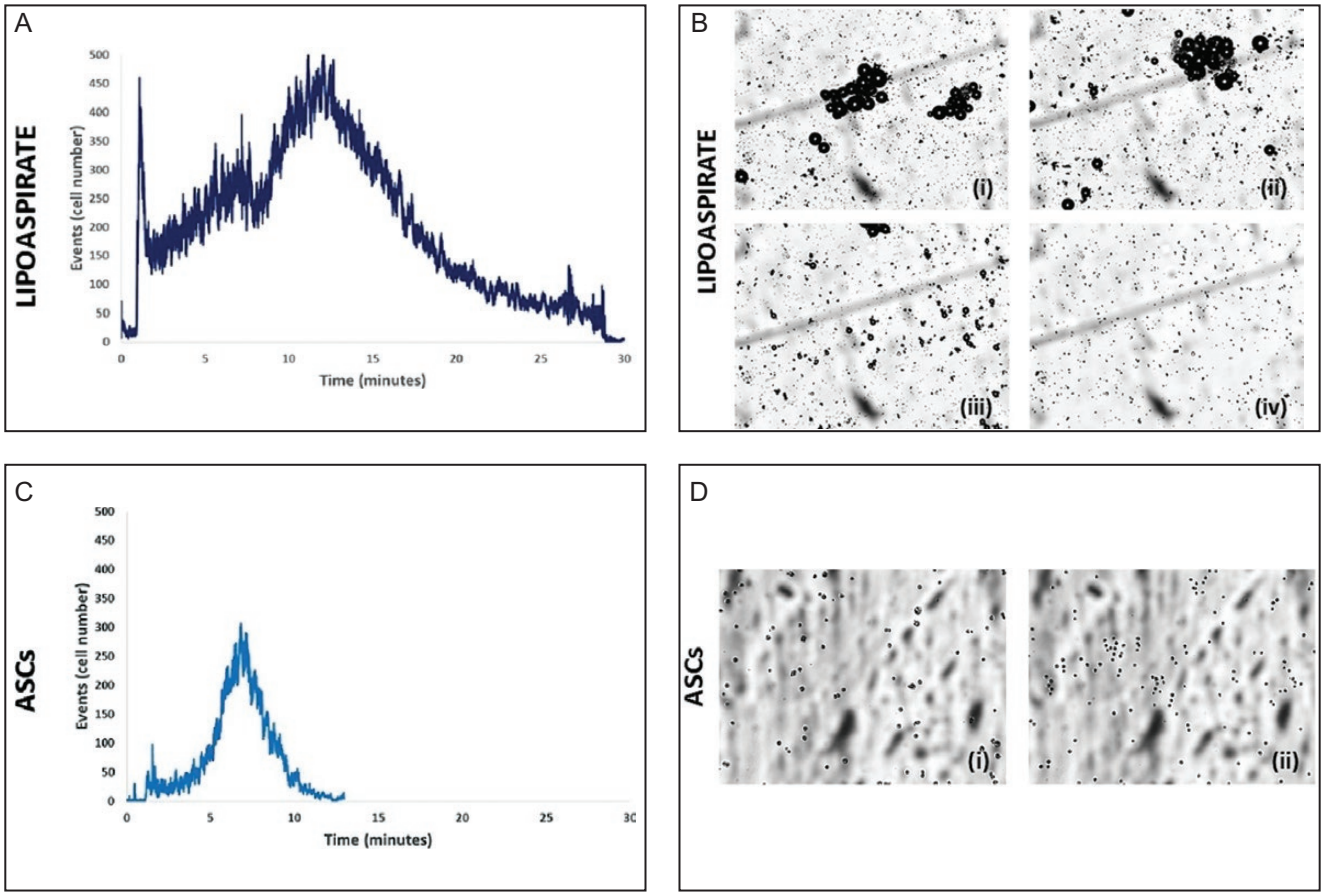

Figure 4. Separation profile of fresh lipoaspirate from the micro-SEFFI cannula and expanded ASCs by Celector®. (A) Separation profile of the raw lipoaspirate by Celector®. The graph shows the cellular profile of the eluted lipoaspirate sample with cell events vs time. In the first minute, unretained cells and debris are eluted, 2 distinct peaks are then visible. (B) Representative images of a raw lipoaspirate sample. Analysis by Celector® showed large agglomerates of ECM, fat droplets, adipocytes, and single cells at 3 minutes of elution time (i), agglomerate and small aggregates of cells and RBCs at 5:30 (ii) and 8:30 (iii) minutes, and single cells and RBCs at 15 minutes of elution (iv). (C) Graph showing expanded ASCs. ASCs eluted between 3 and 10 minutes have similar Gaussian-like separation profiles. (D) Recorded images of ASCs. The images show larger cells eluting at 3:30 minutes (i) and smaller and more compact cells eluting at 4:30 (ii).

harvesting site of the subcutaneous adipose tissue significantly affected the total number of viable cells in the SVF. The SVF consists of pre-adipocytes, a population of adult mesenchymal stem cells, and endothelial cells. The number of cells obtained from the SVF at the isolation time was counted using methyl violet solution, and the cellularity was compared among the different cannulas (Table 2). The adipose tissue harvested with a 0.3 mm side port cannula had a lower cellularity than that harvested with the 0.8 mm cannula (86,403 cells/mL vs 187,019 cells/mL) but was comparable to that harvested by the 0.5 mm cannula (86,403 cells/mL vs 51,900 cells/mL) (Figure 5D). Once plated in a tissue flask, only adherent cells were evaluated and further studied. The cells appeared elongated, with a semi-fibroblastic shape. No morphological differences were observed among cells derived from the different cannulas (Figure 5A–C).

To understand the stemness capacity of the SVF, cells were plated immediately after isolation in a 6-well plate and monitored via a light microscope every 3 days. ASCs started to form colonies from single attached cells. No substantial difference was observed in the number of CFU-Fs among samples (Figure 5F).

The ASCs showed a normal proliferative profile. According to the plotted curve, ASCs derived from each different cannula began to proliferate immediately and plateaued after 96 hours (Figure 5E). At the beginning of the culture period, the cells had a fibroblast-like morphology, while they had a large cytoplasm and were most likely in senescence after a few passages (data not shown).

The cells differentiated into 3 mesodermal lineages, proving their stemness capacity. ASCs cultured in adipogenic medium illustrated the formation of lipid droplets in their cytoplasm as observed by staining red via Oil Red

Table 2. Cellularity of Mononuclear Cells Derived from Liposuction

Patient	Age	Gender	Cannula	Volume (mL)	Cellularity (cells/mL)
1	53	Female	0.8	6	17,917
			0.5	6	11,250
			0.3	6	7,500
2	55	Female	0.8	7	528,571
			0.5	5	64,500
			0.3	5	13,500
3	48	Female	0.8	10	167,500
			0.5	7.5	30,000
			0.3	7.5	7,000
4	48	Female	0.8	13	390,385
			0.5	12	134,375
			0.3	12	123,958
5	25	Male	0.3	7	41,429
6	43	Female	0.8	20	59,000
			0.3	22	10,341
7	50	Female	0.8	5	135,000
			0.3	2	162,500
8	51	Female	0.8	5	195,000
			0.5	4	19,375
			0.3	1	325,000

O, while those in the osteogenic medium had mineralized foci, detected as red area staining with Alizarin red. Chondrogenic differentiation was detected by the presence of the glycosaminoglycan within the ECM when stained with Alcian blue solution (Figure 6).

The expanded ASCs were also analyzed by Celector® to obtain their elution profile. These ASCs were eluted between 4 and 10 minutes, and no substantial difference was observed among ASCs derived from the different cannulas (Figure 4C). Live images of the ASCs showed larger cells eluting in the ascending part of the curve (first minutes) and smaller and more compact cells in the descending part of the curve (Figure 4D).

DISCUSSION

Adipose tissue is a promising source for stem cells. The ASCs contained in the SVF have demonstrated regenerative potential in clinical studies.[33–35] Different protocols have been developed to isolate stromal cells from lipoaspirate samples using enzymatic or non-enzymatic procedures. Even though these protocols are quite similar to each other, the differences in enzyme concentration, number of washing steps, centrifugation parameters, hemolysis, and culture conditions can affect the efficiency and reproducibility of the isolation. Moreover, surgeons have distinct techniques for harvesting adipose tissue, increasing the variability among samples. In the past, many automated, closed devices to isolate stromal cells from adipose tissue, based on collagenase digestion or mechanical forces, were invented in this fast-growing market.[36] Thus, new quality control systems are essential to evaluate the isolated adipose tissue, ASCs, and their regenerative potential.

In this work, we focused on the analysis of micro-SEFFI samples due to the novelty of the harvesting system as well as its advantages in aesthetic surgery, such as tissue fluidity. Lipoaspirate samples were analyzed by light microscopy, and large aggregates of adipocytes and ECM were captured. Analysis of fresh tissue composition is limited because it loses components, such as blood, fat droplets, single cells, and ECM fragments, when it is embedded and histologically stained. Therefore, new quality control

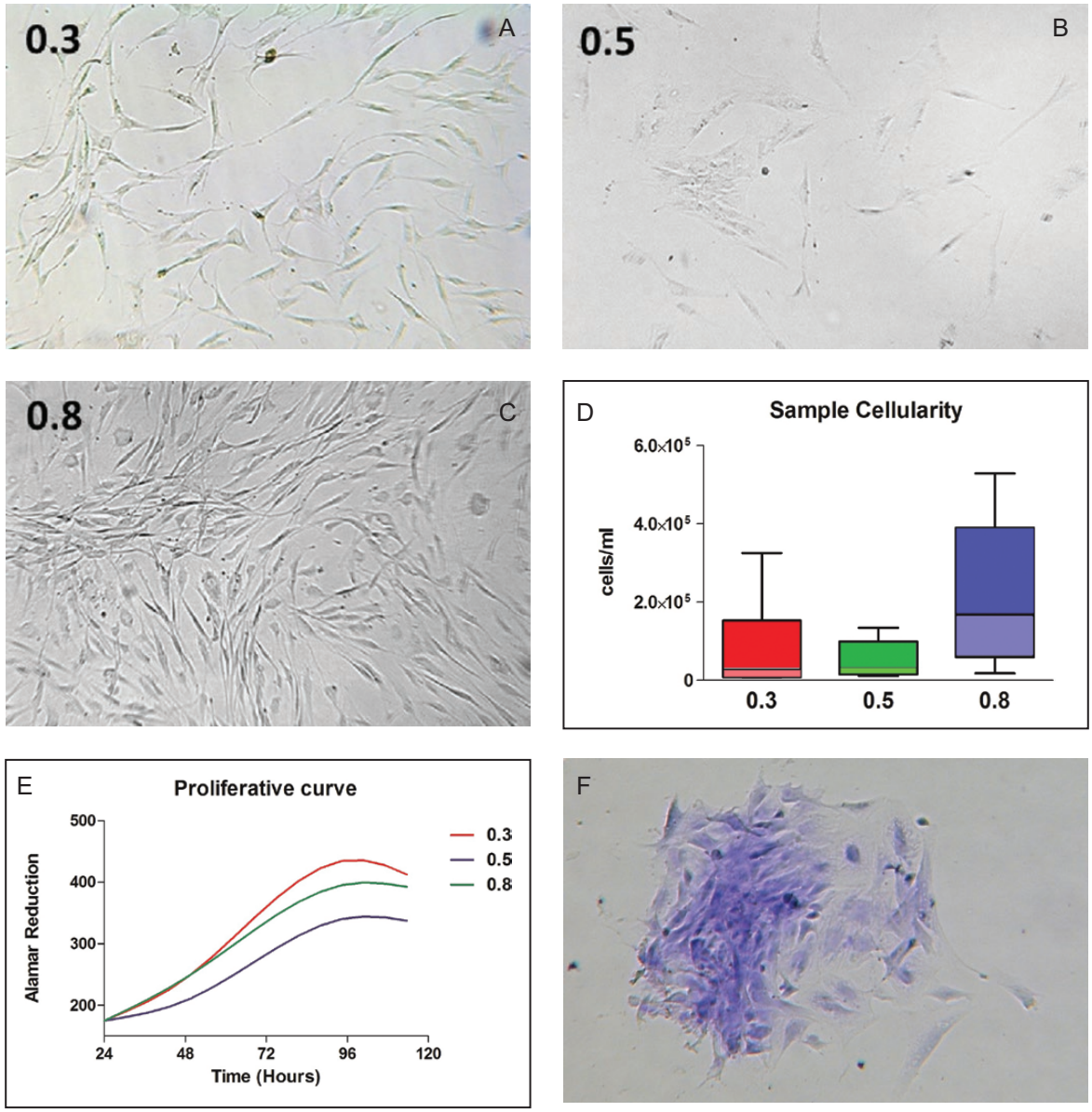

Figure 5. Characterization of lipoaspirate-derived ASCs. (A, B, C) Representative images of ASCs. These samples were derived by cannulas 0.3 mm, 0.5 mm, and 0.8 mm in diameter; ASCs showed classical mesenchymal morphology with a spindle shape (magnification 4X). (D) Cellularity of the lipoaspirate samples. The number of cells derived from the SVF fractions per mL of harvested adipose tissue decreased with the size of the side portholes. (E) Proliferative curve of ASCs. ASCs collected from the 0.3 mm, 0.5 mm, and 0.8 mm cannulas showed no difference among sources. Cell proliferation was measured using the Alamar blue assay, and reduction capacity was monitored over 5 days. (F) Freshly isolated ASCs (p0) grew in colonies (CFU-Fs) as shown by cells colored blue by methylene blue staining in the representative image (magnification 4X).

systems are needed to improve the characterization of adipose tissue before injection into the patient as a lipofilling or for regenerative purposes (eg, immunomodulatory effect for cartilage repair).[37–40]

The Celector® system provides additional information about the composition of liposuction aspirate derived from the micro-SEFFI cannula. Thanks to the fractionation profile and live images of the eluted tissue, the instrument gives fingerprint-like information about the tissue. The fractogram is composed of 2 main peaks and an initial void of unretained elements, such as debris and RBCs. The heaviest component, such as large cell aggregates composed of adipocytes and ECM, elute between 3 and 10 minutes, while the majority of cells elute in the second peak between 10 and 20 minutes, as determined by the elevated values on the y-axis (Figure 4A). These elution events are composed of small fat droplets and likely RBCs, as shown by the small dimensions.

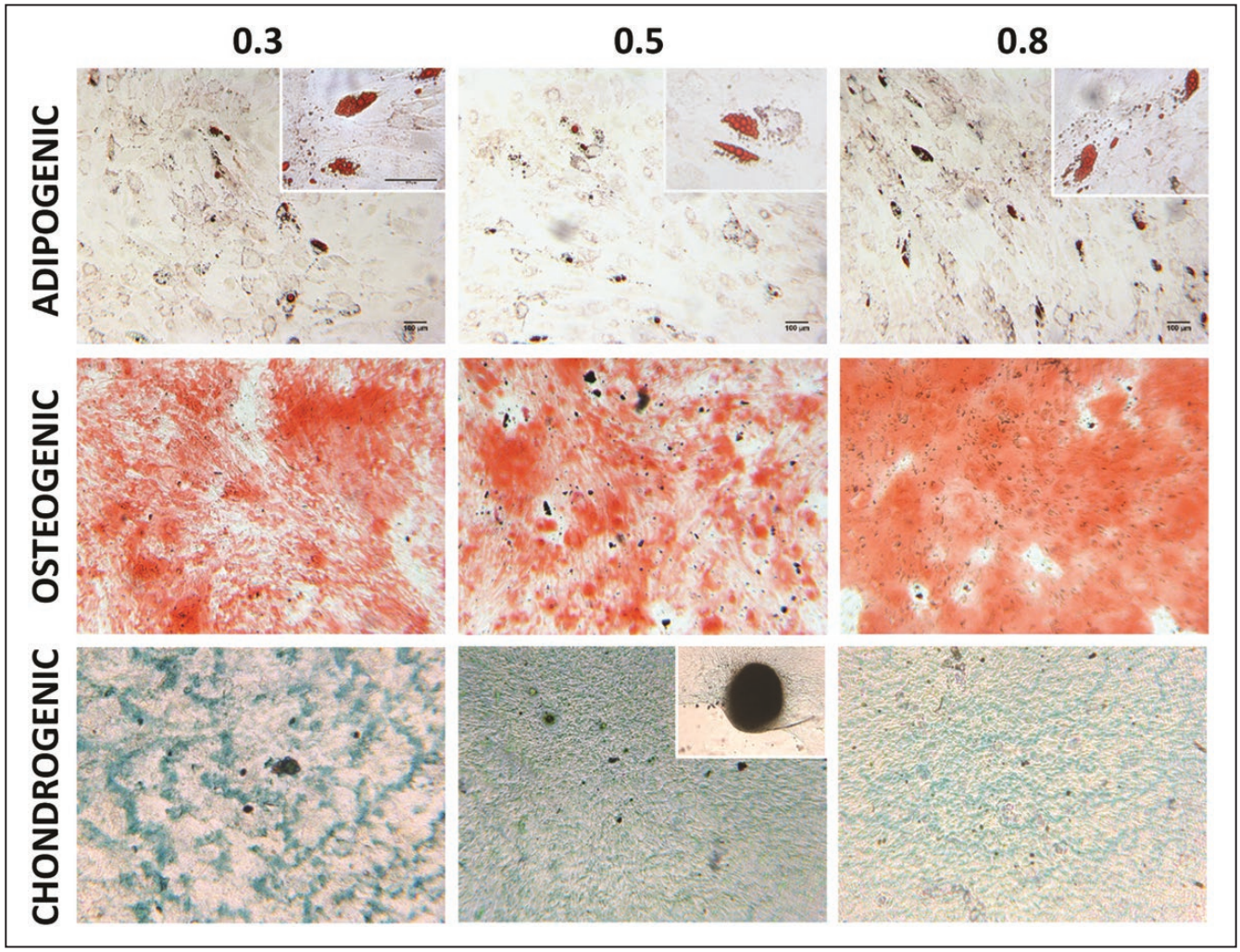

Figure 6. Mesenchymal differentiation of ASCs. ASCs derived from different cannulas (0.3 mm, 0.5 mm, and 0.8 mm side porthole sizes) demonstrated the ability to differentiate towards adipogenic, osteogenic, and chondrogenic lineages, respectively. Fat droplets colored with Oil Red O staining, deposition of mineralized matrix visualized by Alizarin Red staining, and glycosaminoglycan in the ECM colored in blue by Alcian blue. The sample from the 0.5 mm cannula also showed the formation of typical chondrogenic micromasses.

After the first results obtained via microscopic observation and Celector® characterization, the biological analysis focused on stromal cells. We investigated the possibility of isolating mesenchymal stromal cells from adipose tissue harvested with SEFFI and micro-SEFFI cannulas. In the past, different types of cannulas have been presented to the market as new tools to harvest adipose tissue, with a focus on mesenchymal stromal cells and pericytes for their regenerative role.[23] Adipose tissue harvested with the SEFFI system is designed to deliver viable adipocytes and SVF/ASCs to restore and regenerate the tissue. Stromal cells and pericytes secrete a wide variety of factors with anti-fibrotic, anti-apoptotic, immunomodulatory, and neo-vascularization proprieties. For the first time, we demonstrated the ability to isolate ASCs from lipoaspirate samples harvested by micro-SEFFI cannulas. These samples were compared to those obtained via the SEFFI procedure. We confirmed that the sample cellularity decreases with the size of cannula portholes.[23] ASCs isolated from 0.3 mm, 0.5 mm, and 0.8 mm porthole cannulas demonstrated the stemness capacity of ASCs to form CFU-Fs when plated after isolation. To confirm the stemness potential of the isolated ASCs, differentiation towards mesenchymal lineages was performed. ASCs from SEFFI and micro-SEFFI (0.5 mm, 0.8 mm, and 0.3 mm cannulas) were proven to be capable of differentiation towards adipogenic, osteogenic, and chondrogenic lineages. To further investigate whether differences in stemness potential exist among cells derived with the different cannulas, further studies are needed, especially those screening stem cell genes involved in self-renewal, such as Oct4, Sox2, and Nanog.

CONCLUSIONS

This study aimed to prove the presence of mesenchymal stromal cells with stemness characteristics in lipoaspirate samples harvested with cannulas with small side portholes. The current work describes a complete characterization of tissue and cell components for the quality control of lipoaspirate samples obtained from the micro-SEFFI cannula used for aging treatment. The tissue was first observed via conventional microscopy techniques, and a novel method was used to analytically characterize and evaluate adipose tissue compositions with no need for extra processing. The fractionation profile and live images provided a quality evaluation of the tissue, underlining the presence of red blood cells, dimensions of cell aggregates, and numbers of ECM fragments, single cells, and fat droplets liberated during the aspiration procedure. Moreover, we demonstrated the presence of ASCs in lipoaspirate SVF obtained from the micro-SEFFI cannula. Even though the sample size was limited, we proved that stromal cells that can be isolated and further expanded on the fact that once re-injected into the patient, stromal cells can assist with tissue regeneration. We are planning to further investigate the analytical use of Celector® for clinical lipoaspirate samples as a novel method for controlling the quality of the composition of tissue samples and isolated ASCs.

Disclosures

Ilaria Vigliotta and Silvia Zia are employees of Stem Sel. Andrea Zattoni, Barbara Roda, and Pierluigi Reschiglian are associates of Stem Sel. The other authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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