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Hybrid Stromal Vascular Fraction (Hybrid-SVF): A New Paradigm in Mechanical Regenerative Cell Processing

Tunc Tiryaki, MD*
Steven Roy Cohen, MD†
Serli, Canikyan Turkay, BS‡
Polen Kocak, PhD§
Aris Sterodimas, MD, MSc, PhD¶
Kai-Uwe Schlaudraff, MD‖
Işıl, Akgün Demir, MD**
Annarita Agovino, MD, PhD††
Yaren Kul, BSc‡‡

Background: Enzymatic digestion of extracellular matrix (ECM) from lipoaspirate is the conventional form of harvesting stromal vascular fraction (SVF) called enzymatically digested SVF (E-SVF). Mechanical SVF (M-SVF) isolation has emerged as an alternative method, but it has also some limitations in terms of lower cell viability and diminished cell counts. To enhance the SVF qualitatively and quantitatively, we propose a novel concept called "hybrid-SVF," in which we combine M-SVF with the concentrated parts of adipose tissue after centrifugation, which is called stromal vascular matrix (SVM).

Methods: Hybrid-SVF injection was applied as an adjunctive therapy to fat grafting in 88 patients and 11 samples were evaluated in the laboratory for cell count, viability and cell activity.

Results: Experimental results determined that SVM part showed higher cellular activity. SVM and M-SVF showed higher cellular potency than E-SVF. Clinically, none of the patients required an additional session for fat grafting since there was no significant graft resorption. However, seven patients asked for further volume augmentation due to their individual preferences. No major complication was encountered.

Conclusions: The usage of hybrid-SVF has a very high regenerative potential due to the ECM support and exceptionally high cell yield in addition to preserved cell potency. Although there are ongoing studies focusing on optimizing cell counts and further clinical applications, we believe that our preliminary results might create a paradigm shift in the area of regenerative fat grafting. *(Plast Reconstr Surg Glob Open 2022; 10:e4702; doi: 10.1097/GOX.0000000000004702; Published online 30 December 2022.)*

*From the *Department of Plastic and Reconstructive Surgery, Cadogan Clinic, London, UK; †Department of Plastic and Reconstructive Surgery, University of California, San Diego, Calif.; ‡Department of Genetics and Bioengineering, Faculty of Engineering and Architecture, Yeditepe University, Istanbul, Turkey; §Department of Biomedical Engineering, Faculty of Engineering and Natural Sciences, Istinye University, Istanbul, Turkey; ¶Head Plastic and Reconstructive Surgery Department, Metropolitan General Hospital, Athens, Greece; ‖Department of Plastic and Reconstructive Surgery, Concept- Clinic, Geneva, Switzerland; **Department of Plastic and Reconstructive Surgery, University of Health Sciences, Gaziosmapasa Training and Research Hospital, Istanbul, Turkey; ††Plastic Reconstructive Surgery Department "Casa Austria"Bd, University of Timisoara, Timisoara, Romania; and ‡‡Department of Research and Development, Lipocube Biotech, London, UK.*

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INTRODUCTION

Although autologous fat grafting is a popular technique in plastic reconstructive surgery, it has significant limitations, such as unpredictability and variable rates of graft survival.[1–3] This lingering clinical confusion associated with the viability and predictability of fat grafting is particularly related to the transfers into a hostile recipient bed, where the circulation and wound-healing capacity is impaired by previous fibrosis due to surgery, injections, radiotherapy, or any other acquired pathology.[3,4]

Most adipocytes do not survive after the fat transplantation and undergo apoptosis, especially those located in the center, which leads to eventual loss of graft volume.[5,6] Many steps to overcome these problems have been reported; however, the data show that the resident stem cells in the transferred fat are promoting adipogenesis, thus improving fat graft survival.[7–9]

These observations and clinical limitations have led clinicians to enrich fat grafts with autologous progenitor cells or stromal vascular fraction (SVF).[10,11] SVF is the regenerative cell population obtained via either mechanical or enzymatic digestion of lipoaspirate without culture or expansion. It is a mixture of vascular endothelial progenitors and adipose-derived stem cells, various blood cells, preadipocytes, fibroblasts, and smooth muscle cells,[12] which promote adipose cell replication, incorporate into vessel walls, and decrease the local inflammatory response. Recent studies suggest a positive relationship between SVF-enriched fat grafts and improved operative outcomes.[1,12,13]

The most common method for SVF isolation is the digestion of the adipose tissue extracellular matrix (ECM) with tissue dissociation enzymes. First, the lipoaspirate is digested with a chemical reagent, usually collagenase. After an incubation period, the suspension is centrifuged, and four layers are obtained: the oily liquid, the adipose tissue, the aqueous layer, and the cell pellet.[12,14] The cell pellet is kept and washed out from the active enzyme to obtain approximately 100,000–1,300,000 nucleated cells per gram of lipoaspirate, with more than 80% viability.[14,15]

However, the clinical applications of enzymatically harvested SVF (E-SVF) remain limited because a laboratory setup with dedicated professional staff is needed. Moreover, enzymatic digestion of adipose tissue has been deemed by the FDA and other regulatory bodies, as a "more than minimal manipulation" of tissues, which implies that the final product is considered effectively as a "drug."[16] Therefore, several methods of mechanical isolation of SVF (M-SVF) have surfaced ranging from only shaking, vibrating, or centrifuging, to more comprehensive approaches harnessing mechanical mincing, buffer incubation, and centrifugation together, to give comparable cell counts and yields without the regulatory implications.[16,17]

ECM is known as a complicated system that holds tissues and organs together and regulates cellular communication, migration, and differentiation. Particularly, adipose tissue consists of the ECM and SVF cells such as blood cells, pericytes, macrophages, fibroblasts, vascular endothelial progenitor cells, and adipocytes.[18–20]

Takeaways

Question: There are two methods to obtain stromal vascular fraction (SVF) from lipoaspirate. Enzymatically SVF (E-SVF) has limitations in clinical applications, additionally restricted by regulatory issues. Mechanical SVF (M-SVF) isolation also has limitations such as lower cell viability and decreased cell numbers.

Findings: Hybrid-SVF was obtained via combining stromal vascular matrix (SVM) with M-SVF and injecting directly into the fat grafted area. Results showed that hybrid-SVF has ECM support, high cellular functionality, and cell yield.

Meaning: The hybrid-SVF approach provides high regenerative potential and head-to-head cell yield compared to enzymatic SVF isolation.

In the E-SVF, the ECM can be degraded effectively, and therefore, the cells with regenerative capacity can be included in the SVF.[21] On the other hand, since the mechanical digestion cannot disrupt the integrity of the ECM, the regenerative potential of the M-SVF is significantly lower than that of E-SVF.[22,23]

Traditionally, SVF layers are aligned from the bottom to the top as follows: SVF, disposable liquid material, adipose tissue, and oil layer.[16] Tiryaki et al[16,24] showed that the adipose tissue layer consists of two separate layers, and when these sublayers are examined separately, it was shown that the lower layer's features were similar to those of the SVF layer in terms of cellular activity, viability, and cell count. Thus, isolating and utilizing this layer is shown to have a regenerative potency due to significant cell content and rich matrix support and described as stromal vascular matrix (SVM).

Here, we propose a new concept called "hybrid-SVF" to optimize the mechanical isolation method for SVF both qualitatively and quantitatively. The basic principle behind this new mechanical isolation process is concentration of regenerative cell population in high-volume adipose tissue by precipitating them via centrifugation. This method, while concentrating the mixture of regenerative cells, does not completely damage the ECM structure and offers a chance to evaluate them in future studies. The new point of view called hybrid-SVF (Fig. 1) combines SVM and M-SVF components together and produces approximately the same cell yield but higher regenerative capacity than E-SVF. In this study, the cellular contents and properties of E-SVF, M-SVF, SVM, hybrid-SVF, and the upper layer of adipose tissue after centrifugation, the discarded layer (DL), are compared, and hybrid-SVF was evaluated in terms of safety and ease of use in clinical applications.

MATERIALS AND METHODS

Between January 2019 and September 2021, hybrid-SVF injection was applied as an adjunctive therapy to fat grafting in 88 nonobese healthy patients either to correct soft tissue defects or for facial rejuvenation, by the same surgery group at a single surgery center. Informed

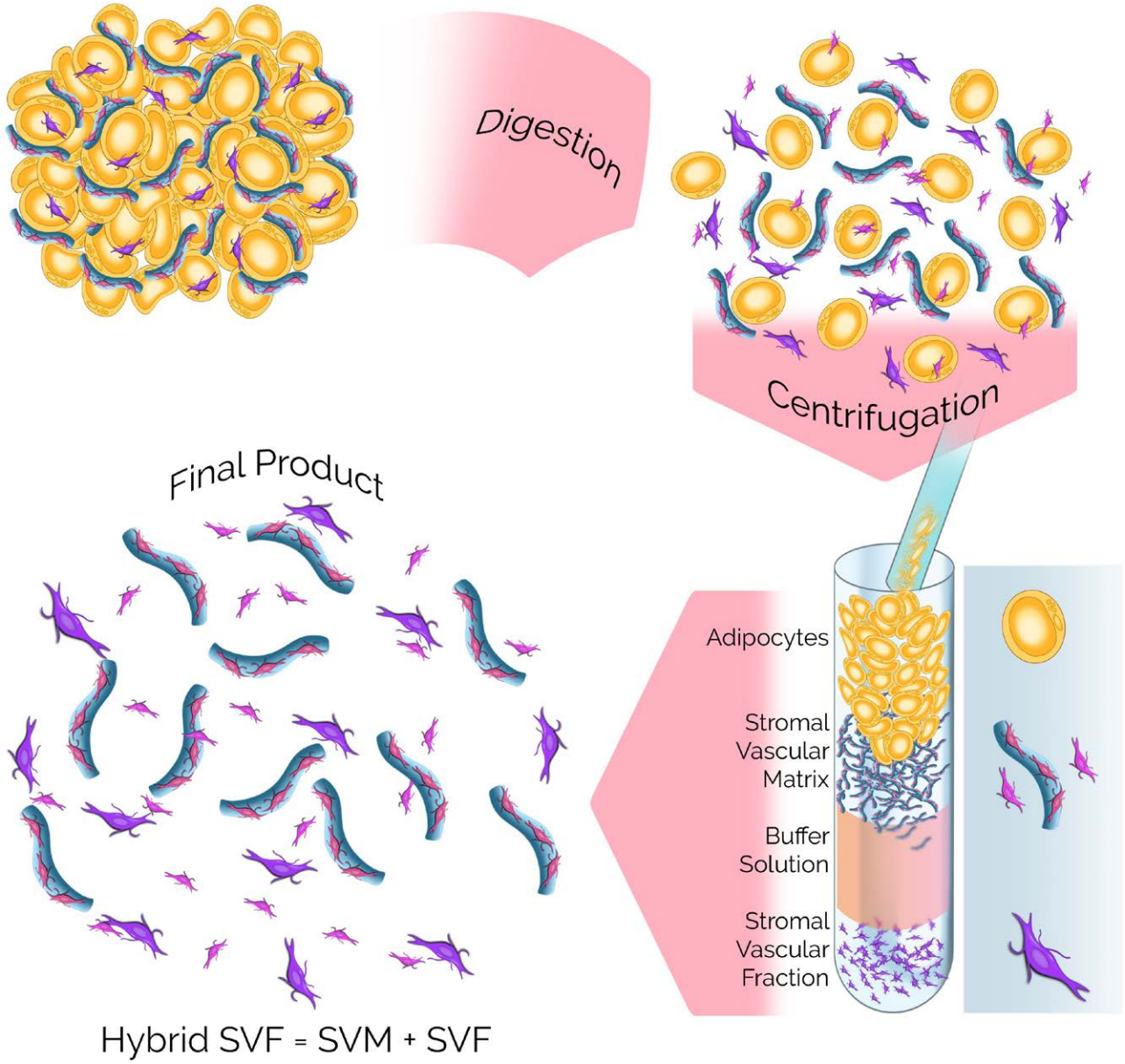

Fig. 1. Schematic illustrations of hybrid-SVF. After mechanical digestion and centrifugation stages, adipose tissue was obtained in four different layers: adipocytes, SVM, Buffer solution, and M-SVF. Hybrid-SVF is a highly concentrated regenerative cell cocktail made up of a layer of stromal vascular matrix in the middle and stromal vascular fraction in the bottom.

consent was obtained from all the patients. Of these 88 patients, 11 patients were chosen randomly and divided into two groups [hybrid-SVF (M-SVF, SVM, and DL) group and E-SVF group] to calculate cell counts and evaluate the cell viability and activity rates. Our protocol conformed to the guidelines of the 1975 Declaration of Helsinki, and was approved by individual institutional review board.

Surgical Technique

The operations were performed under local anesthesia with or without sedation, and the grafts were harvested primarily from the lateral thighs, and rarely from the lower back and abdomen. After the donor site was infiltrated with a solution of saline and epinephrine, a 2.4-mm cannula and traditional Coleman injection cannula were used, for the aspiration and fat transfer, respectively. Once harvested, half of the lipoaspirate was digested by using the Mechanical Isolation SVF kit (Lipocube SVF; Lipocube Biotech, London), whereas the other half was utilized for a tissue-shaping procedure using traditional microribbon lipo-structuring techniques. To standardize the volume of graft reinjected, a 1 mL per 7 cm of cannula excursion standard is used. Once Mechanical Isolation SVF kit was applied according to the manufacturer's protocol, adipose tissue is separated into four different phases after the centrifugation step, as shown in Figure 2B. During the clinical cases, the light-colored DL part of the

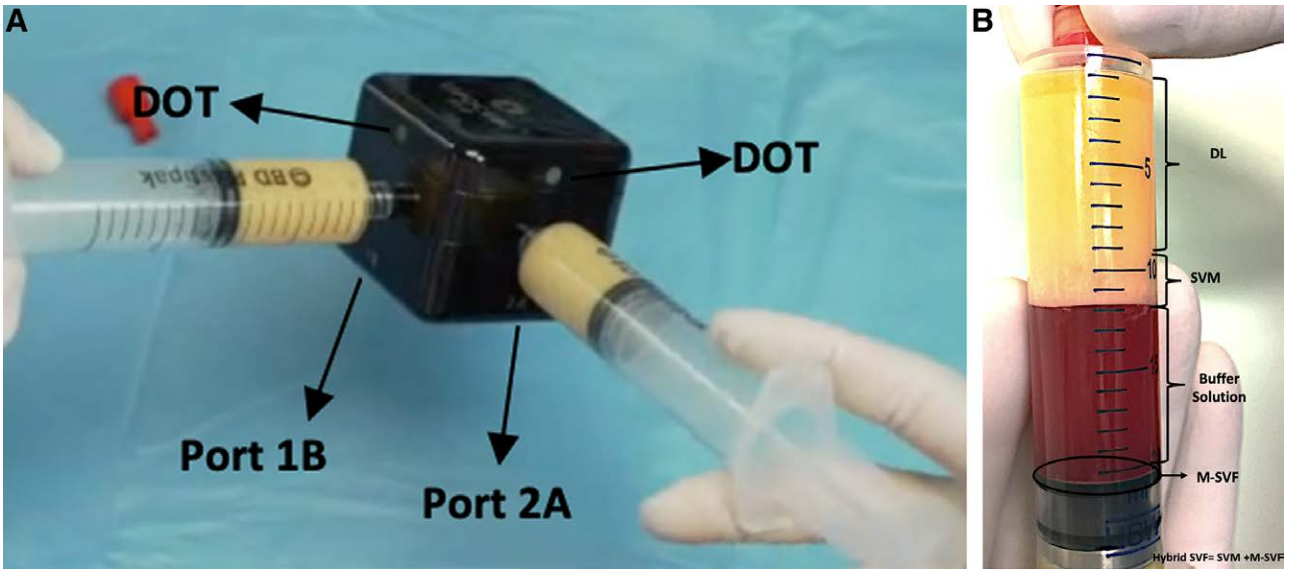

Fig. 2. Mechanical SVF isolation with SVF kit from Lipoaspirated fat tissue and configuration of different layers of processed input. A, Mechanical isolation SVF kit process. B, After centrifugation, the top oil and fat layers (the light color part of the adipose tissue) are called DLs. The very bottom ECM-rich portion of the adipose tissue is called SVM, and the SVF over the top of the cell-adhesive gasket is called M-SVF. Hybrid-SVF is the fusion of concentrated tissue-based stromal vascular fraction, which is called SVM, with cellular-based mechanically isolated SVF materials.

adipose tissue is discarded, and the darkest-colored lowest part (called SVM) is taken to the injector via three-way cocks. The buffer part of the separated fraction is also discarded. The M-SVF part collected in the concave gaskets was resuspended with the previously separated SVM part. The prepared hybrid-SVF was injected directly into the fat-grafted area to enrich the grafted material, with a ratio of 3:1, meaning 1-mL hybrid-SVF was injected for every 3 mL of fat grafted per location. Effort was made to distribute the SVF evenly throughout the grafted area.

Stromal Vascular Fraction Isolation

In this study, 60 mL of lipoaspirate was harvested from 11 patients, chosen randomly from our 88 patient clinical series and divided into three 20-mL aliquots, which were submitted to enzymatic and mechanical digestion for SVF and SVM isolation. In the E-SVF group, lipoaspirate was enzymatically digested using GMP-graded collagenase NB6 (Serva Electrophoresis, Heidelberg, Germany) at a concentration of 0.1 U/mL and a ratio of 1:1 (v/v) in a 37 °C heated orbital shaker at 250 rpm for 30 minutes, washed and centrifuged twice at 300 g for 5 minutes, and the pellet was resuspended in a saline solution.

In mechanical isolation groups, isolation was performed using a Mechanical Isolation SVF kit (Fig. 2A). The lipoaspirate was put into syringes and connected to a closed device with three different sets of blade grids on three luer-lock ports on a rotating canal. The lipoaspirate was inserted into the first port and passed ten times through the first blade grid with 1000-µm holes. The rotating canal's direction was switched to the second port, and the lipoaspirate was passed through the second blade grid with 750-µm holes and the blade grid with 500-µm holes for complete dissociation. The pistons of the syringes are detached, and the detached syringes containing the dissociated lipoaspirate are then centrifuged at continuous differential centrifugation speeds for 10 minutes for 2000*g* with the Luer-lock tips directed inward so that the SVF can be collected in concave gaskets.[16]

In the M-SVF group, the supernatant was totally discarded, and the pellet was resuspended in phosphate buffered saline solution, which is the darker-colored lowest part of the adipose fraction with concentrated ECM content after centrifugation step was discarded sequentially (Fig. 2B). The upper DL part of the adipose fraction was taken to another injector for cell count, viability, and characterization. The SVM and DL were incubated at 37 °C with GMP-graded collagenase NB6 (Serva Electrophoresis, Heidelberg, Germany) at a concentration of 0.1 U/mL and a ratio of 1:1 (v/v) for 30 minutes in an orbital shaker at 250 rpm, washed and centrifuged twice at 300 g for 5 minutes, and the pellet was resuspended in phosphate buffered saline solution.

The overall nucleated cell number and the viability of E-SVF, M-SVF, SVM, and DL were analyzed by a flow cytometer (Muse CellTM Analyzer) using the Muse Count & Viability Kit after erythrocyte lysis. Moreover, cells were labeled with acridine orange (AO) and propodium idodie (PI) and examined under fluroscence microscopy. Viable cells get stained with AO/PI fluorescent green under dark-field fluorescence microscopy, whereas nonviable cells are stained with fluorescent orange.

The characterization of adipose-derived stromal/stem cells (ASCs) was performed to determine ASC cell phenotype in the suspensions (CD34+/CD90+, CD73+/CD105+). The regenerative cell population in four groups were stained with 5 mL of monoclonal antibodies (BD Biosciences, Le Pont de Claix, France). Surface markers

CD34+, CD31−, CD73+, and CD105+ were measured by flow cytometry, according to the manufacturer instructions.

One lipoaspirate sample was randomly selected from each of 11 patients for adipogenic differentiation and gene expression analysis for E-SVF, M-SVF, and SVM groups. To induce adipogenic differentiation, 1×10^4 cells/cm^2 were seeded in 12 well plates. Adipogenesis differentiation was performed by StemPro Adipogenesis Differentiation kit. The medium was replaced every 3 days for 3 weeks according to the manufacturer protocol and was evaluated by oil red staining and investigated by phase-contrast microscopy. Gene expression profiles were examined by adipocyte-specific adiponectin, Ppar, C/EBPa, and C/EBPb genes. Total RNA isolation from E-SVF, M-SVF, and SVM was performed using the Total RNA Purification Plus Kit (Norgen, Calif.) according to the manufacturer procedure. For the conversion of extracted RNA into cDNA, the QuantiTect Reverse Transcription Kit (Qiagen, France) was utilized. The mRNA expression levels of Adiponectin, Ppar, C/EBPa, and C/EBPb genes were determined using the QuantiTect SYBR Green PCR kit (Qiagen, France). The reaction mixture composed of SYBR green PCR mix, universal primer, RNase-DNase free water, and 500 ng for each sample, and reactions were carried out using the iCycler RT-PCR equipment according to the manufacturer protocol (Bio-Rad, Hercules, Calif.). The 18S rRNA reference gene was used to do relative quantification during the study. Absolute quantification was examined using the standard curve.

Statistical Analysis

Statistical analyses were performed using IBM SPSS (Statistical Package for the Social) Statistics 28.0. Two-tailed paired *t* tests were used to compare enzymatically digested SVF, stromal vascular matrix, mechanically digested SVF, discarded material, and hybrid-SVF samples, and values of *P* less than 0.05 were considered significant. After ANOVA test, post-hoc paired comparisons Bonferroni correction was used to compare characteristics of regenerative cell population via enzymatically digested SVF, SVM mechanically digested SVF, discarded material, and hybrid-SVF samples.

RESULTS

Cell Count and Viability

SVF yield was calculated by dividing the number of viable nucleated cells in SVF per gram of processed fat. The quantity of adipose tissue to be processed was the same among E-SVF, M-SVF, SVM, and DL groups. According to the isolation methods, total nucleated cell number, cell viability, and cell images after isolation are shown in Supplemental Digital Content 1 (**See figure**, **Supplemental Digital Content 1**, which shows the nucleated cell count and their viability, and cell images after isolation (**http://links.lww.com/PRSGO/C300**). There were no significant differences regarding the cell viability parameters assessed in E-SVF, SVM, DL, and the hybrid-SVF (which is composed of SVM and M-SVF) groups ($P < 0.05$). Morever, the viable nucleated cell numbers of the E-SVF, M-SVF, SVM, and DL groups were determined as 1.85(±0.1) x10^6, 0.8(±0.31) x10^6, 1.1(±0.6) x10^6 and 0.04(±0.008) x10^6, respectively. According to results, hybrid-SVF cell yield was calculated as 1.9(±0.5) x10^6 which is approximately the same as the enzymatic digestion group.

Surface Marker Characterization by Flow Cytometer

Adipose-derived stem cells were defined as CD31− (endothelial marker), CD34+ (stem cell marker), CD90+ (stem cell marker), and CD73+ (stem cell marker). The SVM group showed significantly higher ASC concentration (17.0%, 6.8%) than any other groups.

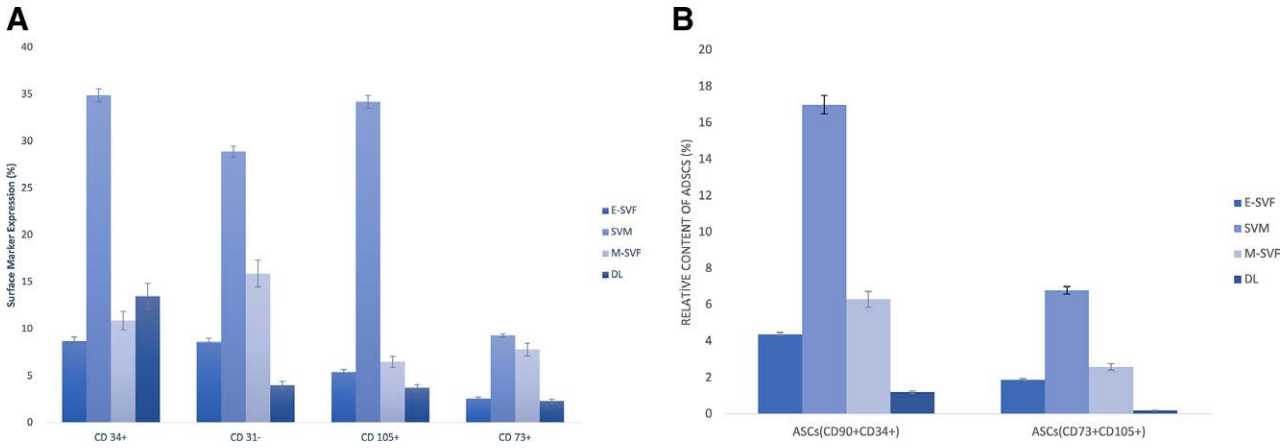

Fig. 3. The flow cytometer results of E-SVF, M-SVF, SVM, and DL groups. A, The ratio of CD34+, CD31−, CD105+, and CD73+ cells in the SVF population was determined. The results showed that the SVM group has high levels of CD34+ (34.9%), CD31+ (28.9%), CD105+ (34.2%), and CD73+ (9.3%) cell surface markers. B, CD90 (+) (stem cell marker), CD34+ (endothelial marker), CD105+ (stem cell marker), and CD73+ (stem cell marker) were used to identify adipose-derived stem cells (stem cell marker). when compared with the other groups, the SVM group had substantially higher ASC concentration (17.0%, 6.8%). M-SVF (6.3%, 2.6) cells had higher levels of ASCs' surface marker expression than E-SVF (4.4%, 1.9) cells, whereas DL (1.2%, 0.2%) cells had no or few ASCs. ($P < 0.05$; n = 11).

M-SVF (6.3%, 2.6) cells showed higher ASCs surface marker expression than E-SVF (4.4%, 1.9), and the DL (1.2%, 0.2%) showed none or low numbers of ASCs (Fig. 3A).

Relative percentage of CD34+, CD31−, CD105+, and CD73+ on the SVF population was measured. Results demonstrated that the SVM group has the following cell surface marker levels: CD34+ (34.9%), CD31+ (28.9%), CD105+ (34.2%), and CD73+ (9.3%) (Fig. 3B).

Differentiation and Gene Expression Analysis

To examine the adipogenic differentiation-related gene expression, adiponectin, PPAR, C/EBP α, and C/EBP β levels were analyzed using the RT-PCR method. Results demonstrated that M-SVF cells resulted in around 2.5-fold increase in adiponectin gene expression, 2.1-fold increase in PPAR gene expression, 2.2 fold increase in C/EBPα, and 1.8 fold increase in C/EBP β gene expression levels with respect to E-SVF. On the other hand, the SVM group resulted in 3.4-fold increase in adiponectin gene expression, 2.4-fold increase in PPAR, 2.6-fold increase in C/EBP α, and finally, 2.1-fold increase in C/EBP β gene expression levels when compared with the E-SVF group. (**See figure, Supplemental Digital Content 2**, which shows phase-contrast microscopy images, **http://links.lww.com/PRSGO/C301**.) DL had very low cell yield (thus insufficient cellular attachment) and growth; therefore, gene expression and adipogenic differentiation potential analysis could not be performed. For the differentiation potential analysis, adipogenic differentiation was analyzed between three groups, and oil red staining was applied according to the method stated in the literature.[25] Results demonstrated that M-SVF and SVM showed higher adipogenic differentiation than the E-SVF based on oil red staining data.

Clinical Summary

We performed 88 cases of hybrid-SVF–enriched autologous fat grafting, with a mean age of 39.27±5.24 years and a mean BMI of 25.31±1.62 kg/m^2 either combined with otherwise traditional lipostructure techniques in a single session for aesthetic or reconstructive indications varying from soft tissue deficits (Fig. 4) to Parry Romberg syndrome (Fig. 5). Total fat graft injection volume ranged from 10 to 36 mL, and all the fat grafts were combined with the hybrid-SVF, which had been prepared from 80 mL of harvested fat tissue.

No infections, fat cysts, granulomas, skin reactions, or other unwanted side effects were observed, and wound closure scores were traced by the TIME-H system.[26] The only complication was subcutaneous ecchymosis in two cases, which resolved spontaneously in 2 weeks after the surgery. Back to theater rate was nil, and none of the patients required a secondary procedure.

DISCUSSION

Fat grafting has become an essential component of plastic and reconstructive surgery. Adipose tissue research is shifting away from volumisation toward regeneration in wound healing and angiogenesis, as well as rheumatoid diseases, osteoarthritis,[27] and fibrosis. Until now, the regulatory and scaling burdens associated with purification and concentration techniques have limited this translation. hybrid-SVF administration could allow us to harness the regenerative potential of adipose tissue, and it could have a much larger clinical impact than a marginal increase in volume augmentation. To our knowledge, this study is the first to describe hybrid-SVF as a cell maximization method for ASC isolation and the first to report its clinical application. Moreover, the data we obtained via 11 consecutive laboratory studies regarding the cell count and profile of hybrid-SVF confirmed that the enzymatic manipulation of SVF is not essential to achieve high numbers of viable and regenerative cells.

This study is focused on the preclinical data for comparison and optimization of mechanical isolation methods versus enzymatic digestion. To keep the article focused on this target, clinical data were completely excluded from the article except for safety and complication rates. A randomized double-blinded clinical study with more than 90 patients already enrolled is being conducted to investigate

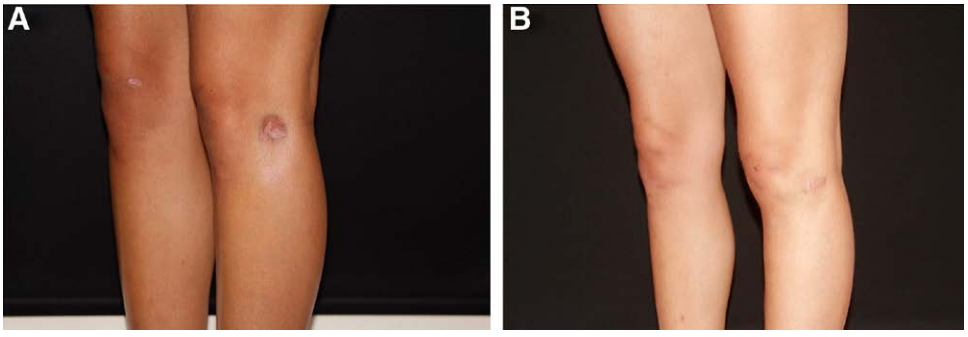

Fig. 4. Clinical case of hybrid-SVF technology: a 38-year-old female patient presented with a complaint of a depressed area on her left knee that happened after an intra-articular steroid injection. 10 mL fat grafting combined with hybrid-SVF injection was applied to the defect area. During her follow-up visits, it was observed that the contour of the fat grafted area remained unchanged even after 6 months postoperative, and the skin quality improved over time. The patient did not require an additional fat grafting session. A, Contour deformity on her left knee after intra-articular injection. B, Postoperative 6-month appearance after 10-mL fat grafting combined with hybrid-SVF.

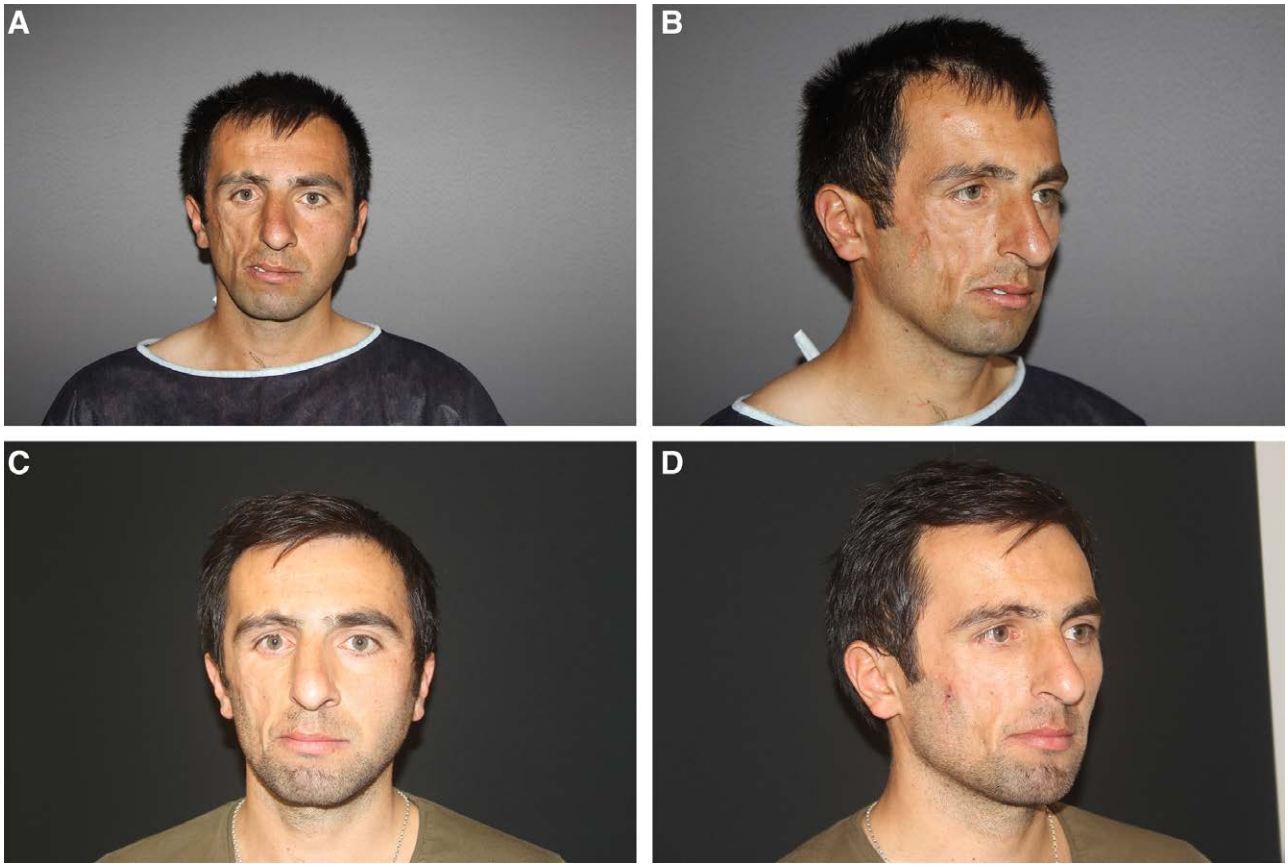

Fig. 5. Clinical case of hybrid-SVF technology. A 42-year-old male patient with a history of trauma-induced Romberg disease presented with a contour deformity on his right hemifacial area. The extensively scarred recipient bed was loosened via rigotomies, making it suitable for fat grafting; 34-mL fat grafting combined with hybrid-SVF injection was performed. The scarred and atrophic appearance of the right hemifacial area improved significantly. At the postoperative 1-year follow-up visit, it was noted that the fat grafted area preserved its form completely. A and B, History of trauma-induced Romberg disease presented with extensive contour deformity on his right hemifacial area. C and D, Postoperative first-year appearance of the patient after 34 mL of fat grafting combined with hybrid-SVF.

the clinical efficiency of hybrid-SVF. This technique was particularly successful in six secondary cases that had been previously treated with traditional fat grafting techniques without any significant improvement in skin quality, patient satisfaction, and long-term live implantation. It can be speculated that previous attempts of fat transfers might have prepared the recipient bed for increased graft uptake, but the outcomes of the hybrid-SVF injections seemed significantly better than those of the previous procedures.[3,16]

Our novel approach seems to have three potential advantages. First and foremost, the approach increases the cell yield in the final injectable SVF. In this study, we have shown that the cells that cannot be gathered at the M-SVF precipitate stay concentrated mostly in the SVM part. We have observed that the DL part contains low numbers of nucleated active cells. The total cell number of hybrid-SVF (mechanically digested SVF and SVM together) was noted to be almost the same as those produced by enzymatic digestion.

Second, the process increases cellular functionality. Potential phenotypic change of SVF cell fractions by mechanical forces was demonstrated by Banyard et al,[28] who found altered marker expression by mechanical digestion processing. According to Banyard et al and Tiryaki et al,[16,24] the mechanical stress increases both the cellular phenotypic activity and the differentiation potential of the cells. In this study, we also induced mechanical stress on SVM and M-SVF groups during isolation, which resulted in an increase in adipogenesis along with the adipogenic gene expression markers.

Third, hybrid-SVF preserves the ECM backbone rich in collagen, elastin, and other structural proteins, which have significant roles in cell migration, adhesion, cell signaling, and tissue elasticity. Similarly, the elasticity of hybrid-SVF might have yet undefined indications in orthopedic and joint surgery.[29–32]

The gold standard of SVF isolation was initially enzymatic digestion of ECM from the lipoaspirate; however, many practical and regulatory restrictions emerged.[24,33,34] The disadvantage of alternative mechanical SVF isolation methods was their low cell yield (30% to 50%) compared with that yielded via enzyme-based digestion.[16] To improve the quantity and quality of the M-SVF, new methods have emerged, in which the extracellular backbone is not totally discarded, but instead, utilized as SVM. According to

Tiryaki et al,[24] SVM resulted in 75% cell yields compared with the yields via enzyme-based digestion. SVM appears to be sufficient for substandard recipient circumstances and skin regeneration needs in clinical applications.[16,24] We mixed the mechanically isolated SVF and SVM together to further optimize the cellular yield, viability, and functional quality of our cell suspension and created hybrid-SVF.

There are some limitations to the present study. First, for focusing on the first ever usage of hybrid-SVF, both the preclinical and clinical outcomes of 88 patients are not included into this study. Second, because hybrid-SVF and non-hybrid-SVF grafts comparison studies are being performed by controlled groups, related results could not be attached to this study. Having said that, both quantitative and qualitative clinical results and comparisons will be shared in our ongoing prospective study.

CONCLUSIONS

In this study, we present comparisons of E-SVF, M-SVF, and hybrid-SVF, a novel mixture of ECM-rich adipose tissue matrix, SVM, and M-SVF together. Hybrid-SVF was found to have regenerative potential due to high cell yield and increased cellular potency due to mechanical rather than enzymatic digestion along with preserved ECM support. To our knowledge, this is the first publication showing a mechanical isolation method achieving a head-to-head cell yield in comparison with enzymatic SVF isolation and hybrid-SVF concept in the literature. Although there are ongoing studies focusing on optimizing cell counts and validating our clinical findings as well as establishing further applications, we believe that this new hybrid-SVF concept might create a paradigm shift in regenerative fat grafting.

Tunc Tiryaki, MD
Department of Plastic and Reconstructive Surgery
Cadogan Clinic
120 Sloane St
London, UK
E-mail: tunc@tunctiryaki.com

PATIENT CONSENT

The patients provided written consent for the use of their images.

REFERENCES

1. Simonacci F, Bertozzi N, Grieco MP, et al. Procedure, applications, and outcomes of autologous fat grafting. *Ann Med Surg (2012).* 2017;20:49–60.
2. Landau MJ, Birnbaum ZE, Kurtz LG, et al. Review: proposed methods to improve the survival of adipose tissue in autologous fat grafting. *Plast Reconstr Surg.* 2018;6:e1870.
3. Tiryaki T, Findikli N, Tiryaki D. Staged stem cell-enriched tissue (SET) injections for soft tissue augmentation in hostile recipient areas: a preliminary report. *Aesth Plast Surg.* 2011;35:965–971.
4. Sinno S, Wilson SC, Brownstone ND, et al. Techniques and outcomes in fat grafting: using evidence to determine fact or fiction. *Plast Reconstr Surg.* 2015;136:131–132.
5. Zhu, M, Zhou, Z, Chen, Y, et al. Supplementation of fat grafts with adipose-derived regenerative cells (ADRCs) improves long-term graft retention. *Ann Plast Surg.* 2009;63:222–228.
6. Hu C, Li L. Preconditioning influences mesenchymal stem cell properties in vitro and in vivo. *J Cell Mol Med.* 2018;22:1428–1442.
7. Calabrese C, Tiryaki T, Findikli N, et al. Aesthetic regenerative surgery. *International Textbook of Aesthetic Surgery.* Berlin, Heidelberg: Springer; 2016: 1239–1251.
8. Eto H, Kato H, Suga H, et al. The fate of adipocytes after nonvascularized fat grafting: evidence of early death and replacement of adipocytes. *Plast Reconstr Surg.* 2012;129:1081–1092.
9. Seo Y, Shin TH, Kim HS. Current strategies to enhance adipose stem cell function: an update. *Int J Mol Sci.* 2019;20:3827.
10. Gentile P, Calabrese C, De Angelis B, et al. Impact of the different preparation methods to obtain human adipose-derived stromal vascular fraction cells (AD-SVFs) and human adipose-derived mesenchymal stem cells (AD-MSCs): enzymatic digestion versus mechanical centrifugation. *Int J Mol Sci.* 2019;20:5471.
11. Palumbo P, Lombardi F, Siragusa G, et al. Methods of isolation, characterization and expansion of human adipose-derived stem cells (ASCs): an overview. *Int J Mol Sci.* 2018;19:1897.
12. Ramakrishnan VM, Boyd NL. The adipose stromal vascular fraction as a complex cellular source for tissue engineering applications. *Tissue Eng.* 2018;24:289–299.
13. Bajek A, Gurtowska N, Olkowska J, et al. Adipose-derived stem cells as a tool in cell-based therapies. *Arch Immunol Ther Exp.* 2016;64:443–454.
14. Aronowitz JA, Lockhart RA, Hakakian CS. Mechanical versus enzymatic isolation of stromal vascular fraction cells from adipose tissue. *SpringerPlus.* 2015;4:713.
15. Senesi L, De Francesco F, Farinelli L, et al. Mechanical and enzymatic procedures to isolate the stromal vascular fraction from adipose tissue: preliminary results. *Front Cell Dev Biol.* 2019;7:88.
16. Tiryaki T, Condé-Green A, Cohen SR, et al. A 3-step mechanical digestion method to harvest adipose-derived stromal vascular fraction. *Plast Reconstr Surg Global Open.* 2020;8:e2652.
17. Raposio E, Caruana G, Bonomini S, et al. A novel and effective strategy for the isolation of adiposederived stem cells: minimally manipulated adipose-derived stem cells for more rapid and safe stem cell therapy. *Plast Reconstr Surg.* 2014;133:1406–1409.
18. Lu P, Takai K, Weaver VM, et al. Extracellular matrix degradation and remodeling in development and disease. *Cold Spring Harbor Perspect Biol.* 2011;3:a005058–a005058.
19. Pallua N, Grasys J, Kim BS. Enhancement of progenitor cells by two-step centrifugation of emulsified lipoaspirates. *Plast Reconstr Surg.* 2018;142:99–109.
20. Lindroos B, Suuronen R, Miettinen S. The potential of adipose stem cells in regenerative medicine. *Stem Cell Rev Rep.* 2011;7:269–291.
21. Trivisonno, A, Alexander, RW, Baldari, S, et al. Intraoperative strategies for minimal manipulation of autologous adipose tissue for cell-and tissue-based therapies: concise review. *Stem Cells Transl Med.* 2019;8:1265–1271.
22. Casadei A, Epis R, Ferroni L, et al. Adipose tissue regeneration: a state of the art. *J Biomed Biotechnol.* 2012;2012:462543.
23. Aronowitz JA, Lockhart RA, Hakakian CS, et al. Clinical safety of stromal vascular fraction separation at the point of care. *Ann Plast Surg.* 2015;75:666–671.
24. Tiryaki T, Canikyan S, Koçak P, et al. Adipose-derived stromal vascular matrix (SVM): a new paradigm in regenerative medicine. *CellR4.* 2021;9:e3060.
25. Sudo K, Kanno M, Miharada K, et al. Mesenchymal progenitors able to differentiate into osteogenic, chondrogenic, and/or adipogenic cells in vitro are present in most primary fibroblast-like cell populations. *Stem Cells.* 2007;25:1610–1617.
26. Guarro G, Cozzani F, Rossini M, et al. The modified TIME-H scoring system, a versatile tool in wound management practice: a preliminary report. *Acta Biomed.* 2021;92:e2021226.
27. Gadelkarim M, Elmegeed AA, Hafez A, et al. Safety and efficacy of adipose-derived mesenchymal stem cells for knee

osteo-arthritis: a systematic review and meta- analysis. *Joint Bone Spine*. 2022;89:105404.
28. Banyard DA, Sarantopoulos CN, Borovikova AA, et al. Phenotypic analysis of stromal vascular fraction after mechanical shear reveals stress-induced progenitor populations. *Plast Reconstr Surg*. 2016;138:237e.
29. Rigotti G, Marchi A, Galie M, et al. Clinical treatment of radiotherapy tissue damage by lipoaspirate transplant: a healing process mediated by adipose-derived adult stem cells. *Plast Reconstr Surg*. 2007;119:1409–1422.
30. Lockhart RA, Hakakian CS, Aronowitz JA. Tissue dissociation enzymes for adipose stromal vascular fraction cell isolation: a review. *J Stem Cell Res Ther*. 2015;5:321.
31. Alexander RW. Understanding mechanical emulsification (nanofat) versus enzymatic isolation of tissue stromal vascular fraction (tSVF) cells from adipose tissue: potential uses in biocellular regenerative medicine. *J Prolother*. 2016;8:e947–e960.
32. Tonnard P, Verpaele A, Peeters G, et al. Nanofat grafting: basic research and clinical applications. *Plast Reconstr Surg*. 2013;132:1017–1026.
33. Yi J, Zhang J, Zhang Q, et al. Chemical-empowered human adipose-derived stem cells with lower immunogenicity and enhanced pro-angiogenic ability promote fast tissue re-generation. *Stem Cells Transl Med*. 2022; 11:552–565.
34. Frantz C, Stewart KM, Weaver VM. The extracellular matrix at a glance. *J Cell Sci*. 2010;123:4195–4200.