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Research

Fat Juice: A Novel Approach on the Usage and Preparation of Adipose Tissue By-Products

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Abstract

Background: Adipose tissue is considered to be naturally rich in a range of bioactive substances that may be extracted directly for therapeutic use without the need for cell isolation or culture

Objectives: The aim of this study was to introduce a novel approach that utilizes stromal vascular fraction in conjunction with fat extract, termed "fat juice," and to perform a comprehensive biochemical analysis in relation to the potential clinical relevance of this new combination.

Methods: A total of 11 samples of fat juice from the abdominal lipoaspirate were extracted from 11 healthy patients and analyzed in terms of the quantity and viability of stem cells, the presence and quantification of connective tissue fibers on histopathologic examination, and the levels of interleukin-6, mannose receptor C type 1, and vascular endothelial growth factor measured by enzyme-linked immunosorbent assay.

Results: Total stem cell amounts ranged from 0.14×10^5 to 1.31×10^5, and cell viability rates varied between 20% and 67.9%. Interleukin-6 protein and vascular endothelial growth factor expressions were highest in Sample 3, while staining intensity was highest in Sample 4. For collagen I, collagen III, and elastin, the highest expressions were observed in Samples 4 and 8, in Sample 3, and in Samples 2 and 4, respectively.

Conclusions: Fat juice provides an easy-to-inject concentration of adipocyte/preadipocytes, red blood cells, adipose-derived stem cells, endothelial-derived cells, and cell residues. Prepared through an easy isolation process enabling abundant availability, fat juice seems to be an effective skin quality enhancer with potential for widespread use in the fields of plastic surgery, dermatology, and aesthetic/regenerative medicine.

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Tissue regeneration and tissue repair are sophisticated biological events that occur throughout a person's life, and have been linked to the activities of stem cells. Stem cells are abundant in the human body. Adult stem cells (ASCs) can be found in many organs and tissues. Mesenchymal stem cells (MSCs) are, for many reasons, one of the most useful and important types of ASC, but most importantly, they are readily accessible and pose few ethical issues because they can be harvested from bone marrow stroma and adipose tissue. Adipose tissue shows an advantage in this respect due to its higher MSC concentration, and easy and safe accessibility.[1] Because it is a rich source of adipose-derived stem cells (ADSCs), adipose tissue has received considerable attention. As a consequence, adipose tissue and its by-products, which were once mostly utilized to increase and volumize soft tissue, are now being used for regenerative and rejuvenative applications.

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Figure 1. Mechanic emulsification process showing the shifting between 20-mL syringes connected by an adinizer.

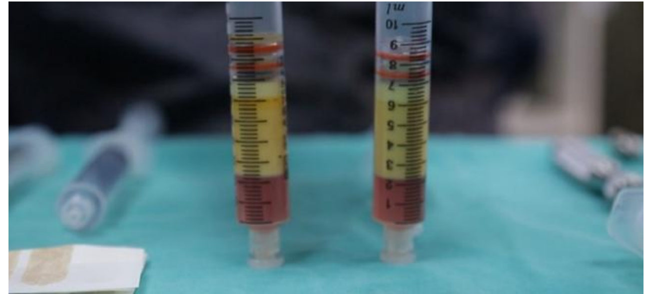

Figure 2. After the centrifugation, 4 layers are visible: oil, fat, fat extract, and stromal vascular fraction. The stromal vascular fraction layer is small but visible under the fat extract layer.

The regenerative effects of adipose tissue's by-products are thought to be primarily connected to their cellular component via growth factor secretion, as shown by Sarkanen et al.[2] Agreeing with those findings, Pallua et al found that fresh lipoaspirate contains proangiogenic substances such as basic fibroblast growth factor, vascular endothelial growth factor (VEGF), and platelet-derived growth factor.[3] Stromal vascular fraction (SVF), one of the most researched adipose tissue by-products, is a heterogeneous cell population commonly obtained via enzymatic fat digestion. SVF has been utilized for many diverse treatments.[4,5] Another by-product of the manipulation of adipose tissue, nanofat—a fat emulsion generated by mechanical forces—has recently been demonstrated to increase fat graft survival and skin rejuvenation and has been used to treat many different skin issues and wound-healing problems.[6,7]

Nanofat, in turn, has several by-products that have also been well researched in a number of studies. The principal by-product is SVF-gel, which can be created when nanofat is centrifuged, and an oil layer, a liquid layer, and a cellular/extracellular layer or fraction become visible. SVF-gel is the cellular/extracellular portion of the nanofat[8,9] and is composed of a high concentration of ADSCs and a functional extracellular matrix (ECM) that can promote angiogenesis in ischemic tissues.[10–12] The second by-product of nanofat is the aforementioned liquid layer, hereinafter called fat extract (FE), although sometimes referred to as adipose liquid extract. Extensive studies concerning the clinical applications and biomolecular properties of FE have been also conducted in recent years. These studies show that FE contains a variety of growth factors and possesses proangiogenic activity.[13–17]

These findings suggest that adipose tissue is naturally rich in a range of bioactive substances that may be extracted directly for therapeutic use without the need for cell isolation or culture. Based on these indicators, we separated the liquid fraction or FE, and combined it with the purified SVF component of the lipoaspirate, calling the resulting product "fat juice."

The primary goal of developing fat juice was to create a lipoaspirate by-product that could be used to treat and improve a variety of skin quality issues without having the volumizing effects of nanofat, allowing it to be applied more widely into the dermis. We also aimed for a product that was simple to make and reproduce, making it an accessible therapy for a variety of medical fields. In the current study, we devised and described a novel mechanical technique to isolate fat juice, then examined the cellular and acellular components of fat juice extracted from 11 healthy patients.

METHODS

Fat Harvesting and Fat Juice Preparation

Human abdominal lipoaspirate was obtained between August 2020 and August 2021 from 11 healthy patients with no systemic diseases or significant health comorbidities at the Plastic Surgery Department of Estetik İnternational Hospital, İstanbul, Turkey, after informed consent and approval of the Committee of Human Research of Verona University. Tumescent infiltration was made with 1000 mL of Ringer's lactate, 2 mg of adrenalin, and 10 mL of marcaine; the lower abdomen was infiltrated with 300 to 500 mL of the tumescent solution for each case. Liposuction was performed with a 3 mm multiport cannula, which contained several sharp-sided holes of 1 mm in diameter. The harvested fat was allowed to decant for 15 minutes. The liquid portion was then discarded and the fat (the starting volume of adipose was consistent between samples from the available methods) was then mechanically emulsified by shifting between two 20-mL syringes connected by 4 different sizes of adinizer (BSL Co. Ltd., Gyeongsangnam-do, South Korea) with internal cutting diameters of 2400, 1200, 600, and 400 µm successively (Figure 1).

The shifting speed remained stable with a passage number of 20 times per adinizer. After processing, the fat turned into an emulsion. The emulsified fat was then processed by centrifugation at 1700 relative centrifugal force for 4 minutes. Finally, the SVF layer and the FE layer

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were extracted as the fat juice, and the remaining fat and oil layers were discarded (Figure 2). The before-mentioned method is how 11 fat juice samples from the abdominal lipoaspirate of 11 healthy patients were extracted for analysis.

Stem Cell Isolation and Quantitative Analysis

Isolation and quantitative analyses of stem cells were performed at the laboratory of the Faculty of Pharmacy, Erciyes University, Kayseri, Turkey. Frozen fat juice samples were sent to the laboratory packed with dry ice and left to thaw to room temperature. After the cell solution was dissolved, 1 mL was taken from each sample and transferred to Eppendorf tubes. Eppendorf tubes were centrifuged at 1200*g* at 25°C for 10 minutes in a microcentrifuge. Supernatant portions were discarded. Then, 3 mL of red blood cell lysis buffer (160 mM NH_4Cl) was added to the precipitate which was incubated for 10 minutes at room temperature. After 10 minutes of incubation, the sample was centrifuged at 1200*g* for 10 minutes at 25°C. The supernatant was discarded. The precipitate was suspended in 2 mL of Dulbecco's Modified Eagle's Medium and seeded in 6-well plates. Plates were incubated in the appropriate environment (37°C, 5% CO_2) for 48 hours. Fat juice and SVF contain heterogeneous cell populations—including mesenchymal progenitor/stem cells, preadipocytes, endothelial cells, pericytes, T cells, and M2 macrophages—and are rich in stem cells. Therefore, we aimed to only count the stem cell numbers after eliminating other cells by incubation for 48 h. During this time, stem cells adhered to the plate bottom and other cells remained in the medium. After 48 hours, the plates were washed 3 times with phosphate-buffered saline (PBS) and non–stem cells were removed from the medium. After washing, 0.5 mL of trypsin-ethylenediaminetetraacetic acid was added to each well and the mixture was incubated for 5 minutes in a suitable environment (37°C, 5% CO_2). After the cells had been lifted from the bottom of the plate with trypsin, 3 mL of medium was added and the sample was centrifuged at 1200*g* at 25°C for 10 minutes. The supernatant was discarded. Cells were suspended in 1 mL of medium and 20 µL of sample was mixed with 20 µL of trypan blue in an Eppendorf tube. Sample-specific analyses such as total cell number, live cell number, dead cell number, viability rate, and average cell sizes were performed with a Cedex XS analyzer (Roche, Penzberg, Germany).

Enzyme-Linked Immunosorbent Assay

In our study, interleukin-6 (IL-6) and VEGF protein expressions were determined with enzyme-linked immunosorbent assay (ELISA) kits (FineTest, Wuhan, China). The test procedure was carried out in accordance with the manufacturer's instructions. Briefly, the cells in the fat juice samples were treated with radioimmunoprecipitation assay lysis buffer at 2×10^6 cells/0.5 mL radioimmunoprecipitation assay solution, and protein extraction was performed. Total protein amounts were determined with a BCA kit (Thermo Fisher Scientific, Waltham, MA). The ELISA test was performed on the combined aqueous fraction + cell pellet, ie, fat juice. We used the BCA protocol to calculate the total amount of protein in a sample, and normalized the target protein level to the total amount of protein. We aimed thereby to eliminate the effect of any potential difference coming from the aqueous fraction. Having determined the total protein amounts, the ELISA test was started. In this test, each sample was run in 4 replicates. To perform the test, 100 µL of sample was applied to each well and incubated at 37°C for 90 minutes. After incubation, each well was washed twice with the washing solution. The plate was incubated for 60 minutes at 37°C after applying 100 µL of biotin-labeled antibodies to each well. Following incubation, the plate was washed 3 times with washing solution: 0.05M Tris, 0.14M NaCl, 0.0027M KCl, and 0.05% Tween 20 solution, 0.2M H_2SO_4 (Wuhan Fine Biotech, Wuhan, China). Then, 100 µL of horseradish peroxidase–streptavidin conjugate was applied to each well and the plate was incubated at 37°C for 30 minutes. The plate was then washed 5 times with washing solution. Then, 90 µL of 3,3′,5,5′-tetramethylbenzidine substrate was added to each well followed by incubation at 37°C for 20 minutes in the dark. After incubation, 50 µL of stop solution was added to all wells and the absorbance of the wells was measured at 450 nm in the ELISA reader. By measuring the absorbances, the amount of target protein in the samples was calculated according to the standard concentration.

Immunohistochemical Analysis

The fat juice samples in fixation solution (Cell Solutions BD C-101) were placed in centrifuge tubes and centrifuged at 1650 rpm for 10 minutes. The supernatant was discarded, and 2 mL of erythrocyte disrupter (SytoRed, Thermo Fisher Scientific, Waltham, MA) was added to the pellet, which was vortexed and left for 10 minutes before being centrifuged at 1650 rpm for 5 minutes to give the final pellet. A Mega Funnel + Positively Charged Slide (Thermo Fisher Scientific) was placed in the cytocentrifuge and 0.1 mL of this pellet was placed in the funnel. The cytocentrifuge was operated at 1250 rpm for 5 minutes, which spreads the slides. The slides were fixed in 96% alcohol for 15 minutes, and then cleaned of alcohol with distilled water. Citrate buffer diluted 1/10 was applied to unmask the antigen. In the immunohistochemistry stainer, slides were attached to the rack slots together with the cover plate, and then washed with PBS for 5 minutes. Endogenous peroxidase activity was blocked with 3%

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Table 1. Analysis Information of Stem Cells Isolated From Samples

Sample no.	Total cells (cells/mL)	Live cells (cells/mL)	Live cells (%)
1	0.43×10^5	0.28×10^5	65.2
2	0.74×10^5	0.24×10^5	32.5
3	0.29×10^5	0.16×10^5	55.2
4	1.31×10^5	0.31×10^5	23.4
5	0.36×10^5	0.18×10^5	50.0
6	0.19×10^5	0.11×10^5	42.9
7	0.74×10^5	0.17×10^5	22.5
8	0.78×10^5	0.53×10^5	67.9
9	0.14×10^5	0.03×10^5	20
10	0.25×10^5	0.11×10^5	44.4
11	0.56×10^5	0.14×10^5	25

Table 2. IL-6 and VEGF Protein Levels in Fat Juice Samples of Patients

Sample No	IL-6 protein levels (pg/mg protein)	VEGF protein levels (pg/mg protein)
1	2.3 [0.51]	12.24 [0.62]
2	1.62 [0.02]	11.55 [0.15]
3	48.87 [1.64]	110.39 [8.60]
4	2.95 [0.10]	14.98 [0.01]
5	2.05 [0.34]	8.26 [0.28]
6	15.25 [1.95]	27.21 [2.78]
7	2.56 [0.32]	10.21 [0.09]
8	6.71 [0.25]	14.10 [1.26]
9	2.84 [0.24]	13.67 [0.22]
10	1.66 [0.13]	11.24 [0.02]
11	1.90 [0.17]	11.52 [0.23]

Protein levels are normalized to the total protein and are presented as mean [standard deviation]. IL-6, interleukin 6; VEGF, vascular endothelial growth factor.

hydrogen peroxide for 5 minutes before washing with PBS. Protein block was applied for 5 minutes and the primary antibody then incubated for 60 minutes. Amplifier Quanto (Thermo Fisher Scientific) was left for 20 minutes and then horseradish peroxidase Polymer Quanto was applied for 30 minutes. Washing was done with PBS at each step. Staining was done with DAB Chromogen (Thermo Fisher Scientific) to identify positive cells. For floor painting, hematoxylin (HHS32, Sigma) was applied for 30 seconds. The stained cells were passed through a series of increasing alcohol concentrations, and after dewatering, were kept in xylol for 5 minutes to render them transparent. The samples were then sealed with entellan (C1795, Merck) and examined. The intensities of the protein staining were relatively quantitated with equal intervals between +/–, +, ++, +++, and ++++, respectively. The lowest protein staining was considered as +/–, while the densest protein staining was ++++ for each protein.

Statistical Analysis

All statistical analyses used in this study were conducted in GraphPad Prism 8 software (San Diego, CA). One-way analysis of variance was used to investigate the potential interpatient difference of the IL-6 and VEGF protein expressions. Tukey's post hoc tests were used for statistical comparisons. n = 4 for the ELISA assay. Data are presented as mean [standard error of the mean] in all experiments in order to measure how much discrepancy is likely in a sample's mean. An α value of 0.05 was considered to represent statistical significance ($P < 0.05$). The n value defines the technical quadruplicate from each patient. Therefore, it means that we applied the same protein extraction from a patient to 4 different wells to eliminate any potential technical mismeasurements in the ELISA test.

RESULTS

The ages of the study participants ranged from 24 to 53 years (mean [standard deviation], 39.09 [8.67] years); there were 10 female patients and 1 male patient. Patients' BMIs ranged from 21.4 to 34.3 kg/m^2 (mean, 27.14 [3.79] kg/m^2). In order to assess the composition of the fat juice obtained from the patients, the samples were examined under a light microscope. As a result of these examinations, it was observed that the samples contained adipocyte/preadipocytes, red blood cells, ADSCs and endothelial-derived cells, and some cell residues. We also noted that adipocytes were in the form of elongated and thin residues rather than their usual rounded appearance due to the possible serial filtration process. In addition, varying levels of red blood cells were found in patient samples. Most cells, especially MSCs, were generally seen adjacent to adipocytes. In the current study, stem cell isolation and enumeration were performed in the samples of fat juice obtained from patients whose adipose tissue was removed by liposuction. In addition, the properties of stem cells such as total cells per mL and viability rates were analyzed in detail. Table 1 shows descriptive information of total cells and stem cells isolated from 11 samples for each sample separately. Our

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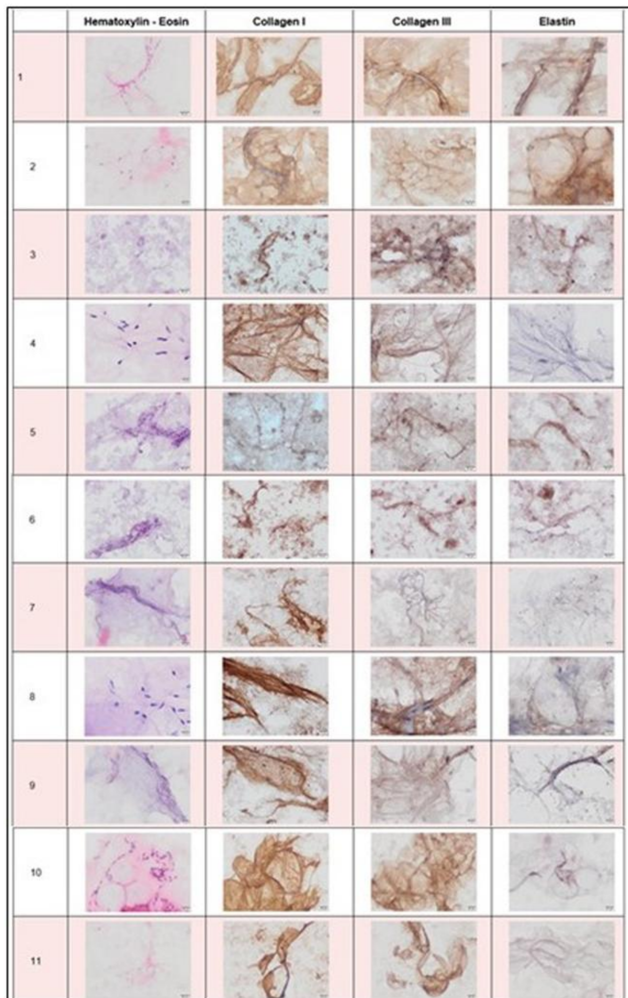

Figure 3. Example images of hematoxylin-eosin staining of collagen I, collagen III, and elastin immunostainings. Original magnification, ×40. Bars, 10-50 µm for each sample.

findings indicate that total stem cell quantity range from 0.14 to 1.31×10^5, while cell viability rates vary between 20% and 67.9%.

Table 2 shows the mean average value of IL-6 and VEGF protein expressions measured by ELISA test studies performed on the 11 samples separately. IL-6 protein expression was detected the most on Sample 3 and the least on Samples 2 and 10. VEGF was expressed most in Sample 3 and least on Sample 5.

Collagen I, collagen III, and elastin protein levels were quantified in samples by histologic examination, and additionally, hematoxylin-eosin staining was performed to assess the general appearance as shown on Figure 3. As a result of these analyses, when staining intensities in fibers as a result of hematoxylin-eosin staining are compared, the highest concentrations were observed on Sample 4, and the lowest on Samples 11 and 3 (Table 3). When collagen I fibers and their expression in cells were examined, the highest expression was observed on Samples 4 and 8, and the lowest expression was observed on Sample 5 (Table 3). When collagen III protein levels were examined, it was determined that this protein was found the most on Sample 3 and the least on Sample 7 (Table 3). When elastin protein expressions were examined, it was seen that this protein was found mostly on Samples 2 and 4 and the least on Samples 7, 8, and 9 (Table 3). All histologic images were semiquantified with ImageJ software by a blind reviewer.

Table 3. Relative Densities of Hematoxylin-Eosin Staining and Collagen I, Collagen III, and Elastin Immunostainings

Sample No.	Total fiber intensity	Collagen I	Collagen III	Elastin
1	+++	+++	++	+++
2	+++	+++	+++	++++
3	+	+	++++	++
4	++++	++++	++	++++
5	++	+/–	++	++
6	++	+	++	++
7	+++	++	+/–	+/–
8	+++	++++	++	+/–
9	+++	++	+++	+/–
10	+++	+++	+++	+++
11	+	+++	+++	+

++++, very severe; +++, severe; ++, moderate; +, mild; +/–, very slight.

DISCUSSION

Among the most serious issues with the clinical usage of SVF as a standalone procedure are the poor survival and retention rates following injection.[17,18] Native adipose tissue produces ECM, which provides a native environment and ensures that ADSCs and other associated cells retain optimum cell growth and tissue regeneration, and is currently considered to be the best delivery method for SVF. With this in mind, the idea of combining SVF with FE was born.

The current study examined the SVF portion of fat juice by analyzing the total cell count, viable cell count, and the cell viability rate. The ECM portion of fat juice was examined by quantifying some of the ECM's most important fibers (collagen I, collagen III, and elastin). In order to demonstrate some of the best-known effects of SVF, the current study also examined the presence and quantity of VEGF,

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IL-6, and mannose receptor C type 1 proteins in all of the samples.

The highest viable cell count was found on Sample 8 and the lowest viable cell count was determined on Sample 9. It is thought that changes in the total number of cells obtained after the procedure may be due to various factors, especially individual factors, whereas changes in viable cell count and cell viability rates are thought to be caused by changes in storage time and environment after fat juice extraction. Because the process is simple to replicate and because it is an autologous implantation, changes in storage time and environment will be minor as the injection will be performed immediately after the extraction and preparation of fat juice. Changes in individual factors on total cell count, on the other hand, will require more investigation in future research.

ADSCs express a broad spectrum of paracrine factors that are known to be angiogenic, such as VEGF and IL-6.[19] The proangiogenic effect of those proteins is very important to the regenerative and rejuvenative clinical effect of fat juice. When the expressions of target proteins were examined among the samples, the values were close to each other in general terms, although some samples, eg, Sample 3, had higher IL-6 and VEGF expressions than others.

Collagen is the most abundant fibrous protein in the ECM. The dermal matrix is largely made up of type I (80%-85%) and type III (8%-11%) collagen, and a network of elastin fibers that together give tensile strength, resilience to recoil, control cell adhesion, promote chemotaxis and migration, and guide tissue formation.[20] When hematoxylin-eosin staining intensities in fibers are compared between the samples; the highest concentrations were observed on Sample 4, and the lowest on Samples 3 and 11. When collagen I fibers and their expression in cells were examined, the highest expression was observed on Samples 4 and 8, and the lowest on Sample 5. The highest expression of collagen III was observed on Sample 3 and the lowest on Sample 7. When elastin expressions are examined, it is seen that this protein is found mostly on Samples 2 and 4, and the least on Samples 7, 8, and 9. The aforementioned differences in the values of both proteins and fibers found between the samples are in our opinion quite understandable based on our clinical experience, considering the variables present in the isolation of fat juice, the age of the patients, their BMIs, individual factors, and so on. We are currently testing and investigating these differences in other clinical research and studies in order to further develop the subject.

Most cells in the histologic analysis carried out in this study, especially MSCs, were generally seen adjacent to adipocytes. In this case, nonenzymatic production of fat juice and not using enzymes such as collagenase are thought to be the main reasons for this. The enzymatic digestion of lipoaspirate by collagenase, and the presence of collagenase in the injectable final product, does not bode well with regulatory authorities such as the US FDA.[21]

We agree that it is advantageous to perform mechanical emulsification rather than enzymatic digestion by collagenase to produce and isolate fat juice.

We believe that further clinical studies are needed to better understand the range of clinical applications of both MSCs and connective tissue fibers found in fat juice, and we aim to perform such clinical trials soon. The aim of this study was to analyze the content of fat juice, and we did not include comparative analysis of the fat juice content, which we admit is a limitation of the study.

CONCLUSIONS

This study described a detailed protocol for the production of a novel mixture of SVF and FE, named fat juice. Laboratory analysis of the samples makes clear the many clinical possible applications of the preparation, especially taking into account that we advise fat juice should not be used for augmentation or volumization but for rejuvenation and revitalization of the skin by means of intradermal injection. Further clinical trials are required to show the results of this dermal protocol. Based on the current findings, our personal experience leads us to be very optimistic because of the good results obtained so far with the clinical use of fat juice. We believe that the use of fat juice can be extended to many medical specialties other than plastic surgery, including aesthetics, dermatology, and regenerative medicine, but also gynecology, orthopedy, urology, and many more.

Disclosures

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REFERENCES

1. Alexander RW. Understanding adipose-derived stromal vascular fraction (AD-SVF) cell biology and use on the basis of cellular, chemical, structural and paracrine components: a concise review. *Prolotherapy*. 2012;4: e855-e869. doi:10.13140/2.1.2452.9924
2. Sarkanen JR, Kaila V, Mannerstrom B, et al. Human adipose tissue extract induces angiogenesis and adipogenesis in vitro. *Tissue Eng A*. 2012;18(1-2):17-25. doi:10.1089/ten.TEA.2010.0712

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3. Pallua N, Pulsfort AK, Suschek C, Wolter TP. Content of the growth factors bFGF, IGF-1, VEGF, and PDGF-BB in freshly harvested lipoaspirate after centrifugation and incubation. *Plast Reconstr Surg*. 2009;123(3):826-833. doi:10.1097/PRS.0b013e318199ef31
4. Atalay S, Coruh A, Deniz K. Stromal vascular fraction improves deep partial thickness burn wound healing. *Burns*. 2014;40(7):1375-1383. doi:10.1016/j.burns.2014.01.023
5. Mohammadi R, Sanaei N, Ahsan S, Masoumi-Verki M, Khadir F, Mokarizadeh A. Stromal vascular fraction combined with silicone rubber chamber improves sciatic nerve regeneration in diabetes. *Chinese Traumatol*. 2015;18(4):212-218. doi:10.1016/j.cjtee.2014.10.005
6. Batel FM, Bertrand B, Magalon J, et al. Treatment of wrinkles of the upper lip by emulsified fat or “nanofat”: biological and clinical study about 4 cases. *Ann Chir Plast Esthet*. 2018;63(1):31-40. doi:10.1016/j.anplas.2017.10.004
7. Tonnard P, Verpaele A, Peeters G, Hamdi M, Cornelissen M, Declercq H. Nanofat grafting: basic research and clinical applications. *Plast Reconstr Surg*. 2013;132(4):1017-1026. doi:10.1097/PRS.0b013e31829fe1b0
8. Mashiko T, Wu SH, Feng J, et al. Mechanical micronization of lipoaspirates: squeeze and emulsification techniques. *Plast Reconstr Surg*. 2017;139(1):79-90. doi:10.1097/PRS.0000000000002920
9. Cai Y, Yu Z, Yu Q, et al. Fat extract improves random pattern skin flap survival in a rat model. *Aesthet Surg J*. 2019;39(12):NP504-NP514. doi:10.1093/asj/sjz112
10. Sun M, He Y, Zhou T, Zhang P, Gao J, Lu F. Adipose extracellular matrix/stromal vascular fraction gel secretes angiogenic factors and enhances skin wound healing in a murine model. *Biomed Res Int*. 2017;2017:3105780. doi:10.1155/2017/3105780
11. Yao Y, Dong Z, Liao Y, et al. Adipose extracellular matrix/stromal vascular fraction gel: a novel adipose tissue-derived injectable for stem cell therapy. *Plast Reconstr Surg*. 2017;139(4):867-879. doi:10.1097/PRS.0000000000003214
12. Zhang P, Feng J, Liao Y, et al. Ischemic flap survival improvement by composition-selective fat grafting with novel adipose tissue derived product—stromal vascular fraction gel. *Biochem Biophys Res Commun*. 2018;495(3):2249-2256. doi:10.1016/j.bbrc.2017.11.196
13. Deng M, Wang X, Yu Z, et al. Cell-free fat extract promotes tissue regeneration in a tissue expansion model. *Stem Cell Res Ther*. 2020;11(1):50. doi:10.1186/s13287-020-1564-7
14. Deng M, Xu Y, Yu Z, et al. Protective effect of fat extract on UVB-induced photoaging in vitro and in vivo. *Oxid Med Cell Longev*. 2019;2019:6146942. doi:10.1155/2019/6146942.
15. He Y, Xia J, Chen H, Wang L, Deng C, Lu F. Human adipose liquid extract induces angiogenesis and adipogenesis: a novel cell-free therapeutic agent. *Stem Cell Res Ther*. 2019;10(1):252. doi:10.1186/s13287-019-1356-0
16. Yu Z, Cai Y, Deng M, et al. Fat extract promotes angiogenesis in a murine model of limb ischemia: a novel cell-free therapeutic strategy. *Stem Cell Res Ther*. 2018;9(1):294. doi:10.1186/s13287-018-1014-y
17. Tobita M, Orbay H, Mizuno H. Adipose-derived stem cells: current findings and future perspectives. *Discov Med*. 2011;11(57):160-170.
18. Zhang M, Methot D, Poppa V, Fujio Y, Walsh K, Murry CE. Cardiomyocyte grafting for cardiac repair: graft cell death and anti-death strategies. *J Mol Cell Cardiol*. 2001;33(5):907-921. doi:10.1006/jmcc.2001.1367
19. Bayes-Genis A, Soler-Botija C, Farré J, et al. Human progenitor cells derived from cardiac adipose tissue ameliorate myocardial infarction in rodents. *J Mol Cell Cardiol*. 2010;49(5):771-780. doi:10.1016/j.yjmcc.2010.08.010
20. Rozario T, DeSimone DW. The extracellular matrix in development and morphogenesis: a dynamic view. *Dev Biol*. 2010; 341(1):126-140. doi:10.1016/j.ydbio.2009.10.026
21. Nguyen A, Guo J, Banyard DA, et al. Stromal vascular fraction: a regenerative reality? Part 1: current concepts and review of the literature. *J Plast Reconstr Aesthet Surg*. 2016;69(2):170-179. doi:10.1016/j.bjps.2015.10.015

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