

Supercharged Mechanical Stromal-cell Transfer (MEST)

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Summary: PRP and fat-derived stromal-cell applications are the 2 most commonly used methods in regenerative medicine. PRP has a wide spectrum of indications. Mechanical methods have become very popular recently in fat-derived stromal-cell applications due to the advantages they provide. Combining these 2 methods has produced more successful results. To date, this combination has been in the form of combining 2 products obtained separately just before they are administered to the patient. In this study, fat tissue and blood samples obtained from eight volunteers were mixed with PPP as a new idea not previously reported in the literature, and stromal cells were obtained mechanically with sharp blades (adinizing). Later, the obtained PRP was added to the final product and became “supercharged.” The results were tested by the dual fluoroscopy method for cell number and viability, and the results obtained were analyzed statistically. By adding the plasma to the oil before stromal cells were obtained and cutting with sharp blades by mechanical separation, twice the volume and 4.7 times more cells were obtained compared with that obtained in the saline group ($P < 0.001$). We believe that the reason for this is the “binding” effect of the proteins in the plasma. This approach provided a higher cell count by using PPP, which is a “waste product,” and in addition, the potential efficiency was increased by adding PRP. However, the clinical results of this innovative method should be evaluated with advanced clinical studies. (*Plast Reconstr Surg Glob Open* 2021;9:e3552; doi: 10.1097/GOX.0000000000003552; Published online 10 May 2021.)

INTRODUCTION

In many medical disciplines, regenerative medicine has recently been a fast-growing and popular trend. The use of fat-derived stromal-cells and blood-derived platelet-rich plasma (PRP) is one of the most common applications.¹ Stromal-cells are obtained mechanically rather than enzymatically, not only because of legal restrictions but also because such procedures are easier and are capable of obtaining more cells efficiently and economically.² Obtaining stromal cells from adipose tissue by enzymatic method has been described elsewhere in detail.³ To date, many devices have been applied in different ways, but consensus has yet to be reached on the definition of the final product or even the preparation protocols in mechanical ways.⁴ Copcu and Oztan, in their study published in 2020 on using sharp-knife systems, obtained a high number of stromal cells mechanically without creating blunt-force

pressure.² The name they gave to the procedure of cutting fat tissue with a sharp knife was “adinizing” and represents the first time indication-based protocols were established for the final product, its desired physical structure (solid, liquid, emulsified), and the required number of cells. Unlike enzymatic methods, they suggested that the term total stromal-cell (TOST) should be applied to the final product, instead of stromal vascular fraction (SVF).⁴

PRP, on the other hand, has a much longer history than stromal cells, and many methods are used successfully in terms of the effects of growth factors on wound healing and regeneration.⁵

In this study, as an innovative alternative to the saline solution used in the indication-based protocols, the process of cutting with sharp blades (adinizing) was performed by combining platelet-poor plasma (PPP) and condensed fat. Thus, by using plasma stromal as a “binder” for cells, the aim was to obtain more cells and greater volume.

MATERIAL AND METHODS

This study was conducted according to the standards of good medical practice (ICH-E6) and the principles

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of the Declaration of Helsinki. All patients were provided detailed information preoperatively, and they gave written consent for all surgical procedures, anesthesia, intraoperative video recording, and photography. In addition, a written consent form was obtained from the patients stating that they willingly donated their adipose tissue for laboratory analysis. In this study, a patented CE marking, and ISO 13485 certified blade system was used, and rules of minimal manipulation were followed. No enzymes and similar chemicals were used, and the structure of the fat tissue was not altered. A TriCell PRP kit (Rev-Med Inc, Korea) was used to obtain PPP. Twenty-seven cm³ of venous blood was mixed with 3 cm³ citrates. It was first centrifuged at 3200 rpm for 4 minutes, then at 3300 rpm for 3 minutes, and after the second centrifuge, the PPP in the second chamber of the kit was automatically obtained. Under local anesthesia, 15 cm³ of adipose tissue was harvested from the abdominal area with a 3-mm-diameter 4-hole cannula and then centrifuged at 500 G for 2 minutes, and condensed fat was obtained by discarding tumescent fluid and blood elements. An estimated 5 cm³ condensed fat was mixed with 5 cm³ PPP in the study group and 5 cm³ saline in the control group, and then the adinizing process was performed with 2400- μ m, 1200- μ m, and 600- μ m diameter ultra-sharp blades, respectively (Adinizer, BSL-rest, Korea) with 25 back-and-forth movements between the 2 injectors. Finally, stromal cells were obtained by centrifugation at 1200 G for 5 minutes. The final product, total stromal-cells (TOST), was obtained mainly in liquid form. (See Video [online], MEST preparation.) Total viable nucleated cell recovery and the viability percentage were determined using a LunaStem Automated Fluorescence Cell Counter device (Logos Biosystems, South Korea) with acridine orange/propidium iodide stain in each delivery method before and

after the process. After the process was completed, PRP was added to TOST. Thus, stromal cells were obtained from adipose tissue mechanically by using PPP simultaneously, and a much stronger effect was expected by adding PRP obtained from blood to TOST.

RESULTS

Supercharged mechanical stromal-cell transfer (MEST) was tested in 8 cases, and results are presented in Figures 1–4. Components of whole blood and adinized fat after centrifugation are presented in Figure 5.

DISCUSSION

When PRP is obtained in conventional applications, the plasma part (called PPP) is discarded, and the PRP part is applied in a wide spectrum due to the growth factors it contains.⁵ The clinical application of PRP by combining it with stromal cells obtained from adipose tissue both enzymatically and mechanically is not a new concept.^{1,5–7} Stevens et al described this approach as platelet-rich stroma and reported that it would yield more successful results in androgenic alopecia and osteoarthritis than PRP alone or SVF alone.^{1,6} Similarly, Butt et al obtained stromal cells from adipose tissue mechanically and emphasized that in its combination with PRP, it provided results far superior to the sole use of PRP.⁷

Our study differs from all stromal cell PRP combinations in the literature.^{1,5–7} In our study, for the first time, we obtained stromal cells from adipose tissue by mixing 50% of the condensed adipose tissue with PPP before the procedure, mechanically using sharp blades. In the technique described previously by Copcu,² indication-based protocols were defined to obtain a higher number of stromal cells in liquid form (conventionally, they are in solid or emulsified fatty consistency) by mechanical stromal-cell

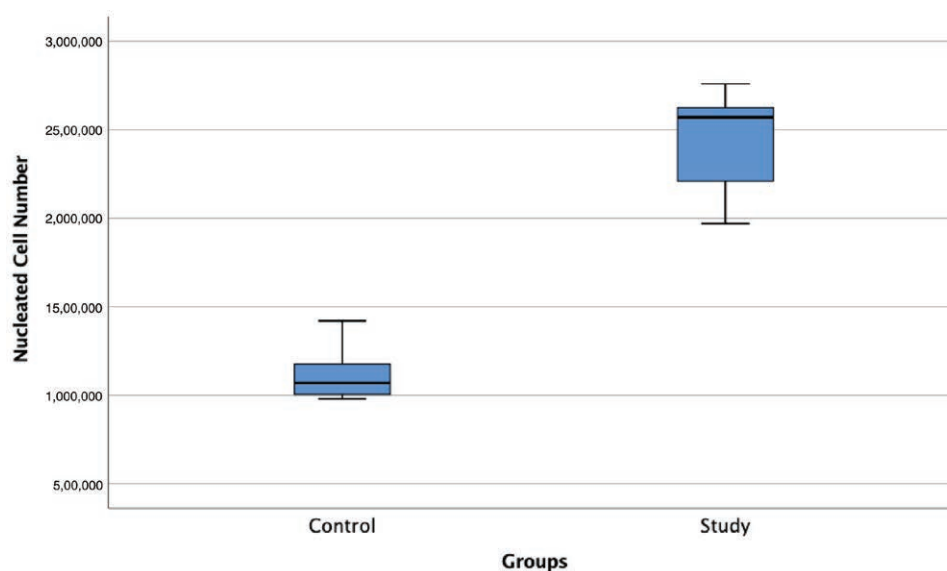


Fig. 1. Comparison of nucleated cells in milliliters. While an average of $1.11 \times 10^6 \pm 1.46 \times 10^5$ nucleated cells were obtained in the control group, this number was $2.44 \times 10^6 \pm 2.99 \times 10^5$ in the study group. The 2.2-fold difference between them was found to be statistically significant (<0.001).

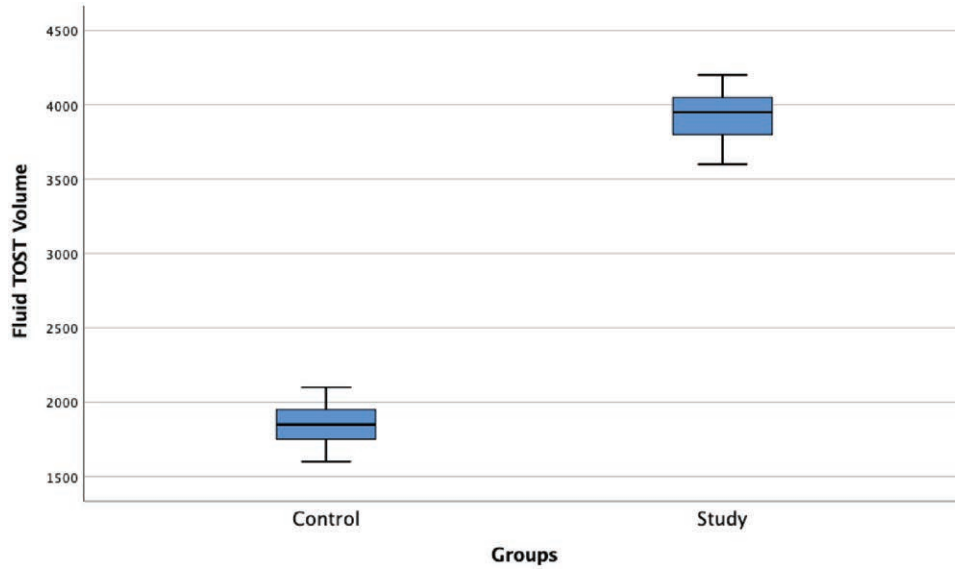


Fig. 2. Comparison of volumes of total stromal cells (TOST). While an average of 1.85 ± 0.16 mL TOST was obtained after the procedure in the control group, this volume was 3.92 ± 0.19 mL in the study group. The 2.1-fold difference between them was found to be statistically significant (<0.001).

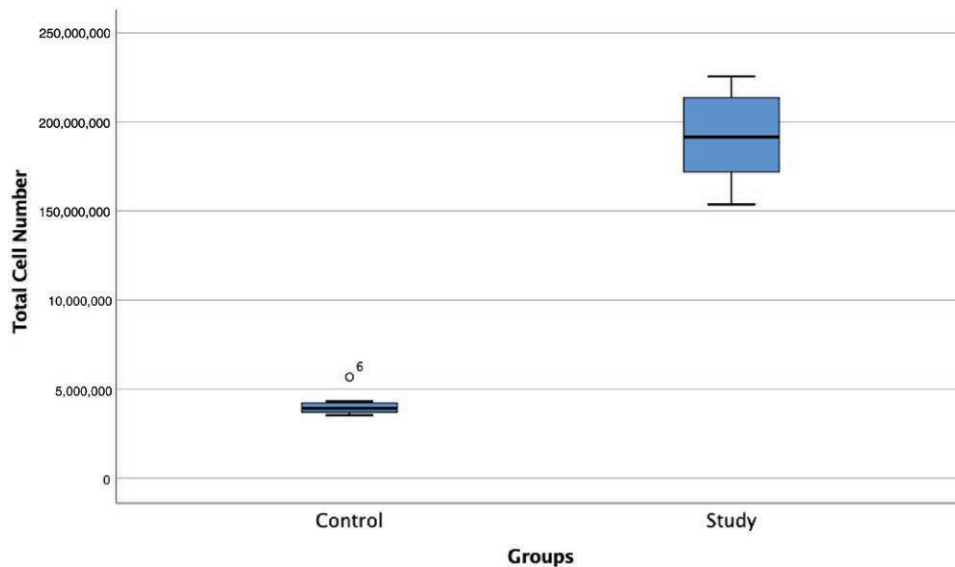


Fig. 3. Comparison of total nucleated cells in 10 mL condensed fat. When 10 cm^3 of condensed fat tissue was taken as reference in the control group, an average of $4.11 \times 10^6 \pm 6.78 \times 10^5$ stromal cells were obtained after all procedures, while this number was $19.16 \times 10^6 \pm 2.58 \times 10^5$ in the study group. The 4.7-fold difference between them was found to be statistically significant (<0.001).

recovery processes. In this approach, when the adipose tissue was mixed with saline at a rate of 50% before adinizing, more cells and total stromal cells were obtained in liquid form. It has been speculated that possible reasons for this may be polarity and density. Adipocytes have no positive and negative charged points—the charge distribution is equal, indicating that they are nonpolar. Molecules that are nonpolar do not dissolve well in polar structures such as water; they tend to repel each other and remain separated, even when shaken vigorously.⁸ However, mesenchymal stromal cells respond to superficial electric charges,

unlike adipocytes.⁹ With the back-and-forth movements described above, the stromal cells are released when the adipose tissue passes through the metal blades between the 2 injectors. However, the kinetic energy generated at this time affects the polarity of the cells. We believe that in pre-adinizing dilution, this electrical polarity affects the relationship between saline and stromal cells and helps separate stromal cells more successfully. Also Zimmerlin described intra-tracheal route of stromal cells combining with fibrin as a kind glue.¹⁰ In the innovative approach we are presenting in this study, plasma is used instead

	Control group	Study group	P
Nucleated Cell Number in mL	$1,11 \times 10^6 \pm 1,46 \times 10^5$	$2,44 \times 10^6 \pm 2,99 \times 10^5$	<0.001
Fluid TOST Volume mL	$1,85 \pm 0,16$	$3,92 \pm 0,19$	<0.001
Total Nucleated Cell Number in 10 cc Adipose Tissue	$4,11 \times 10^6 \pm 6,78 \times 10^5$	$19,16 \times 10^6 \pm 2,58 \times 10^5$	<0.001
Viability (%)	92.25 ± 3.19	92.13 ± 1.56	0.922
Average Nucleated Cell Size (μm)	9 ± 2	8 ± 3	0.896

Fig. 4. Comparison of results of control and study group (The data analysis was carried out using IBM SPSS Statistics for Windows (version 21.0; IBM Corp., Armonk, N.Y.). The descriptive statistics were given as mean \pm SD. The normal distribution of the numerical variables was determined by using the Shapiro-Wilk normality test. If the data complied with a normal distribution, the statistical differences between the groups were evaluated using the 1-way analysis of variance and post hoc tests. If the data did not comply with a normal distribution, Mann-Whitney U tests were used. A P value of <0.05 was considered to be statistically significant.) In the study group, 2.2 times more nucleated cells were found in 1 mL (<0.001). As a result of the process, TOST was obtained at 2.1 times higher volume (<0.001). When 10 cm³ of condensed adipose tissue was taken as reference, a total of 4.7 times more stromal cells was obtained (<0.001). There was no statistically significant difference in viability and average cell size in the study and control groups (0.922, 0.896).

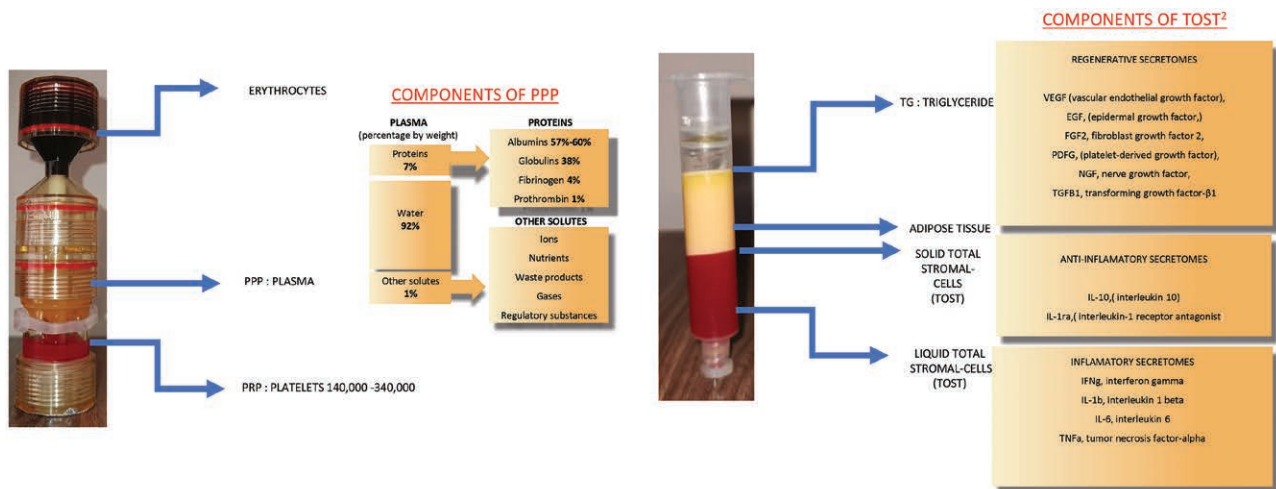


Fig. 5. Components of whole blood and adipized condensed adipose tissue after centrifugation.

of saline. The content of plasma is 7% protein and 4% fibrinogen. We argue that thanks to these structures in the plasma acting as a binder for stromal cells, it is possible to obtain both twice the volume and 4.7 times more stromal cells.

CONCLUSIONS

We think that at the same time, the addition of the obtained PRP to this final product will allow the application of “supercharged” cells in a much stronger sense, as described in many studies in the literature. However,

advanced clinical studies are required to prove this hypothesis.

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