SUPERCHARGED MECHANICAL STROMAL-CELL TRANSFER (MEST)

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SUMMARY

PRP and fat-derived stromal cell applications are the most commonly used regenerative medicine methods. PRP has a broad spectrum of indications. Due to their advantages, mechanical methods have recently become very popular in fat-derived stromal cell applications. Combining these two methods has produced more successful results, providing reassurance about the effectiveness of the MEST method. This combination combines two products obtained separately before they are administered to the patient. In this study, fat tissue and blood samples obtained from eight volunteers were mixed with PPP, 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 using PPP, a "waste product," and increased potential efficiency 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 May 10, 2021)

Keywords: PRP, fat-derived stromal cells, regenerative medicine, MEST method, PPP, cell viability, mechanical separation

INTRODUCTION

In many medical disciplines, regenerative medicine has recently been a fast-growing and popular trend. Using 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 more accessible 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. However, 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 regarding 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.

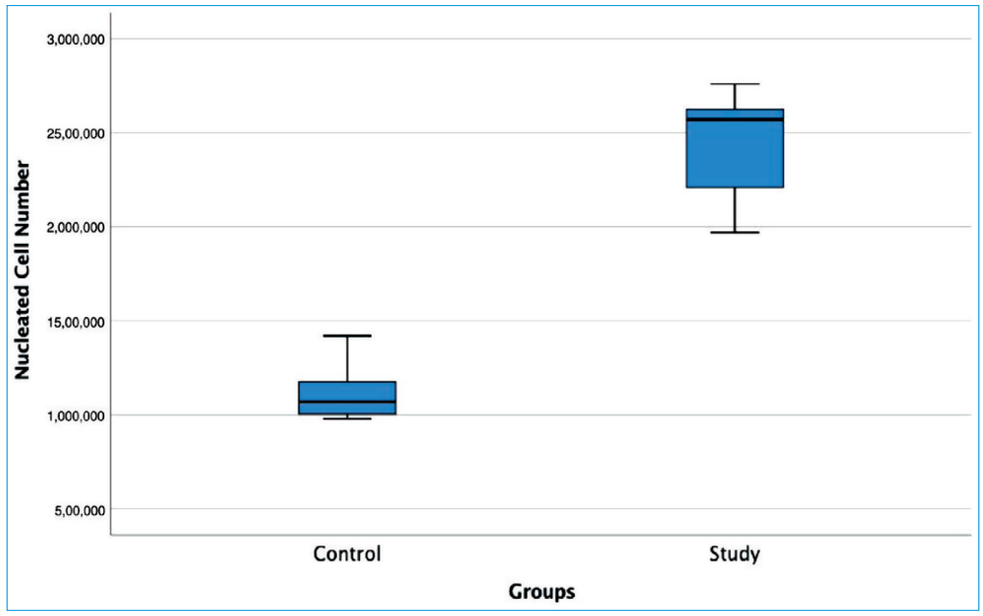
METHODS

This study was conducted according to the standards of good medical practice (ICH-E6) and the principles 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. This study used a patented CE marking and ISO 13485-certified blade system, 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-µm, 1200-µm, and 600- µm diameter ultra-sharp blades, respectively (Adinizer, BSL-rest, Korea) with 25 back-and-forth movements between the two injectors. Finally, stromal cells were obtained by centrifugation at 1200 G for 5 minutes. The final product, total stromal cells (TOST), was received 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.

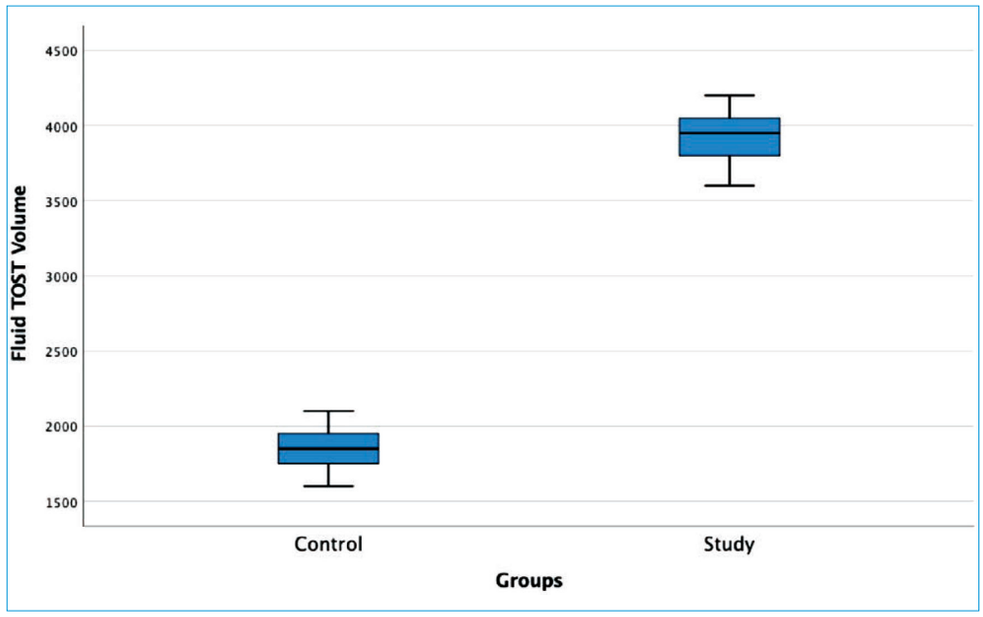
Thus, stromal cells were obtained mechanically from adipose tissue using PPP simultaneously, and a much stronger effect was expected by adding PRP obtained from blood to TOST.

Figure 1. ***Comparison of nucleated cells in milliliters.***



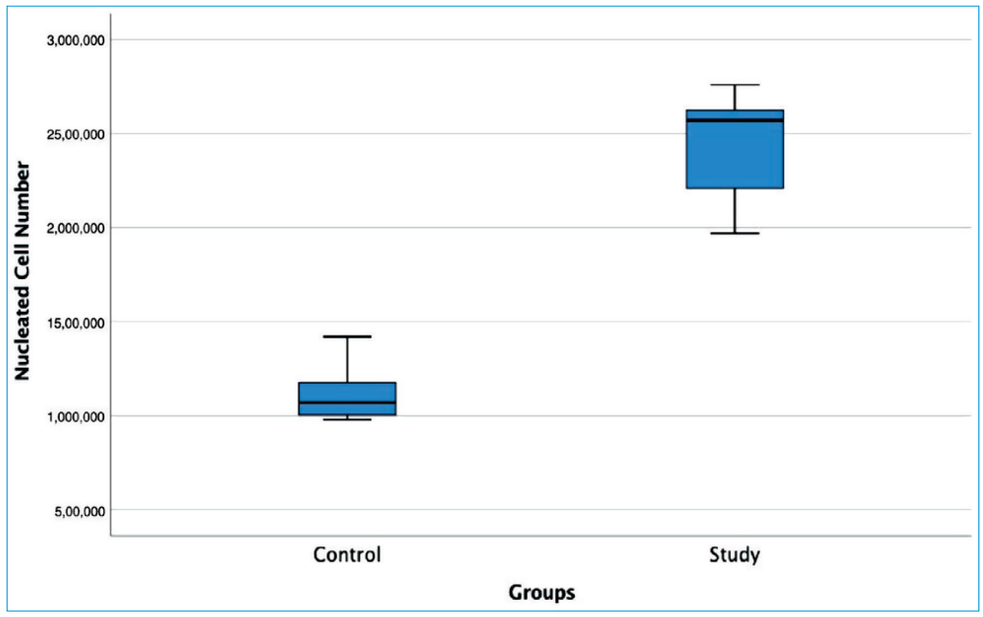
While an average of 1.11 x 106 ± 1.46 × 105 nucleated cells were obtained in the control group, this number was 2.44 x 106 ± 2.99 × 105 in the study group. The 2.2-fold difference between them was statistically significant (<0.001).

Figure 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 statistically significant (<0.001).

Figure 3. ***Comparison of total nucleated cells in 10 mL condensed fat.***



When 10 cm3 of condensed fat tissue was taken as reference in the control group, an average of 4.11 × 106 ± 6.78 × 105 stromal cells were obtained after all procedures, while this number was 19.16 x 106 ± 2.58 × 105 in the study group. The 4.7-fold difference between them was statistically significant (<0.001).

RESULTS

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

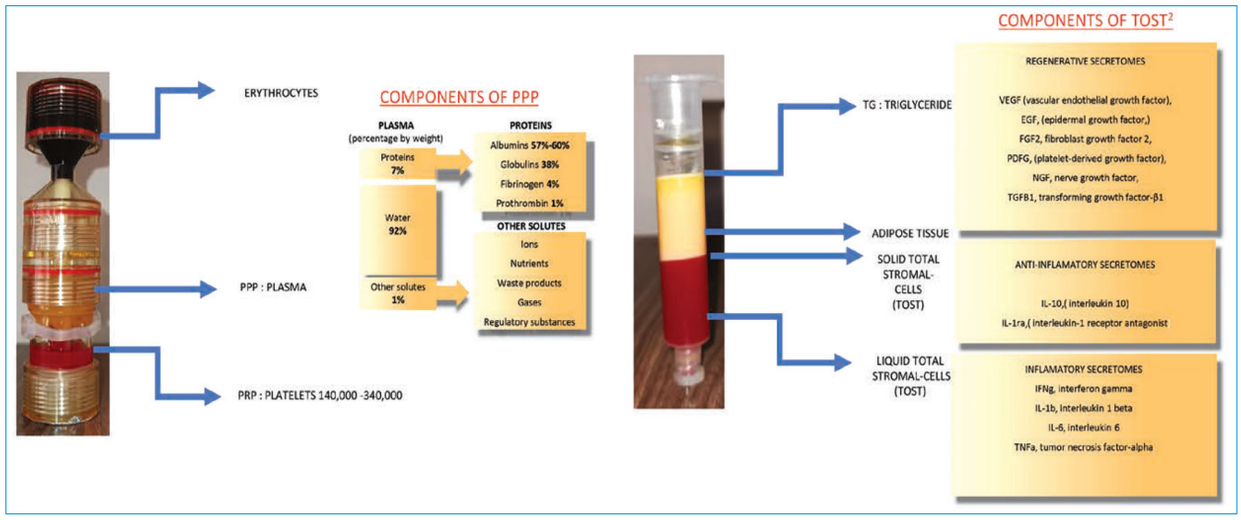
Figure 4. ***Comparison of results of control and study group.***

	Control group	Study group	P
Nucliated Cell Number in ml.	1,11 x 10^6 ± 1,46 x 10^5	2,44 x 10^6 ± 2,99 x 10^5	<0.001
Fluid TOST Volume ml.	1,85 ± 0,16	3,92 ± 0,19	<0.001
Total Nucliated Cell Number in 10 cc Adipose Tissue	4,11 x 10^6 ± 6,78 x 10^5	19,16 x 10^6 ± 2,58 x 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

(The data analysis was done using IBM SPSS Statistics for Windows (version 21.0; IBM Corp., Armonk, NY). The descriptive statistics were given as mean ± SD. The normal distribution of the numerical variables was determined 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. Mann-Whitney U tests were used if the data did not comply with a normal distribution. A P value of <0.05 was considered to be statistically significant.) The study group found 2.2 times more nucleated cells 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 a 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).

Figure 5. ***Components of whole blood and adinized condensed adipose tissue after centrifugation.***



DISCUSSION

When PRP is obtained in conventional applications, the plasma part (called PPP) is discarded, and the PRP part is applied in a broad 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 a concept that has been introduced previously.[1,5-7] Stevens et al. described this approach as plate let-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. They emphasized that its combination with PRP 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 research, 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 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. This may be due to polarity and density. Adipocytes have no positive and negative charged points – the charge

distribution is equal, indicating that they are nonpolar. Nonpolar molecules do not dissolve well in polar structures like 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] The back-and-forth movements described above release the stromal cells when the adipose tissue passes through the metal blades between the two injectors. However, the kinetic energy generated at this time affects the polarity of the cells. In pre-adinizing dilution, this electrical polarity affects the relationship between saline and stromal cells and helps separate stromal cells more successfully. Zimmerlin also described the intra-tracheal route of stromal cells combined with fibrin as a glue.[10] In the innovative approach we are presenting in this study, plasma is used instead of saline. The content of plasma is 7% protein and 4% fibrinogen. 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.

CONCLUSION

At the same time, adding 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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