Influences of Centrifugation on Cells and Tissues in Liposuction Aspirates: Optimized Centrifugation for Lipotransfer and Cell Isolation

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Background: Although injective autologous fat transplantation is one of the most attractive options for soft-tissue augmentation, problems such as unpredictability and fibrosis resulting from fat necrosis limit its universal acceptance. Centrifugation is one of most common methods for overcoming these difficulties. This study was performed to investigate quantitatively the effects of centrifugation on liposuction aspirates to optimize centrifugal conditions for fat transplantation and isolation of adipose-derived stem cells.

Methods: Liposuction aspirates, obtained from eight healthy female donors, were either not centrifuged or centrifuged at 400, 700, 1200, 3000, or 4200 *g* for 3 minutes. The volumes of the oil, adipose, and fluid portions and numbers of blood cells and adipose-derived cells in each portion were examined. The processed adipose tissues (1 ml) were injected into athymic mice, and grafts were harvested and weighed at 4 weeks. Morphologic alterations were observed using light and scanning electron microscopy.

Results: Centrifugation concentrated adipose tissues and adipose-derived stem cells in the adipose portion and partly removed red blood cells from the adipose portion. Centrifugation at more than 3000 *g* significantly damaged adipose-derived stem cells. Centrifugation enhanced graft take per 1 ml centrifuged adipose but reduced calculated graft take per 1 ml adipose before centrifugation.

Conclusions: Excessive centrifugation can destroy adipocytes and adipose-derived stem cells, but appropriate centrifugation concentrates them, resulting in enhanced graft take. The authors tentatively recommend 1200 *g* as an optimized centrifugal force for obtaining good short- and long-term results in adipose transplantation. (*Plast. Reconstr. Surg.* 121: 1033, 2008.)

Soft-tissue augmentation by autologous fat transplantation is an attractive therapy, but some problems remain, including the low survival rate of transplanted adipose tissue and formation of calcifications. To overcome these difficulties, a number of technical improvements in harvesting, processing, and injection were reported.[1–9] Among these, we focused on centrifugation of liposuction aspirates. Centrifugation is also an important process for isolating adipose-derived stem cells from liposuction aspirates.

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Centrifugation, which has been used in processing lipoaspirates since the 1980s,[10] is now widely used to concentrate aspirated fat and to remove oil, fluid, and blood products. Various views and ideas regarding centrifugation of lipoaspirates have been reported,[1,3,5,7–9,11–13] and the current consensus is as follows. Centrifugation concentrates adipocytes[3,5,7,8] and separates them from substances that may degrade adipocytes, such as blood cells, lipids, proteases, and lipases,[3,5,8] but does not enhance immediate fat

Disclosures: *A centrifugation device (Lipokit; Medikan Corp., Seoul, Korea) and syringes made specific for the device (Medikan Corp.) were provided to authors for research purposes by the company.*

tissue viability.[9] Centrifugation may enhance the total amount of transplanted fat,[11] although excessive centrifugal force may damage intact adipocytes.[7,9,13,14]

Undifferentiated stromal cells present in adipose tissue recently became a focus. Stromal vascular fractions isolated from liposuction aspirates contain multiple cell populations,[15,16] one of which has been identified as adipose-derived stem cells.[17] Adipose-derived stem cells can differentiate into various lineages forming adipose, bone, cartilage, neuronal, and vasculature tissues. Our recent research[18] showed that both the fatty and fluid portions of liposuction aspirates contain a significant amount of adipose-derived stem cells, and we characterized freshly isolated adipose-derived stem cells as $CD31^{-}CD34^{+}CD45^{-}CD90^{+}CD105^{-}CD146^{-}$ cells. The presence of adipose-derived stem cells has clinical implications for autologous fat transfer because adipose-derived stem cells may contribute to neoangiogenesis in the acute phase by acting as endothelial progenitor cells[19] or angiogenic-factor-releasing cells.[20] Adipose-derived stem cells are known to secrete angiogenic factors such as vascular endothelial growth factor and hepatocyte growth factor under hypoxic conditions[20] and to contribute to increased capillary density and blood flow.[19] In addition, they can affect long-term survival of transplanted adipose by acting as preadipocytes.

We have recognized positive effects of centrifugation on fat transfer in our clinical experience, but the force-dependent influences of centrifugation on liposuction aspirates have not been well studied thus far. The purposes of this study were (1) to determine the anatomical and physiologic alterations of cell and tissue components in liposuction aspirates during the centrifugation process, and (2) to optimize centrifugation parameters for autologous lipotransfer and isolation of adipose-derived stem cells.

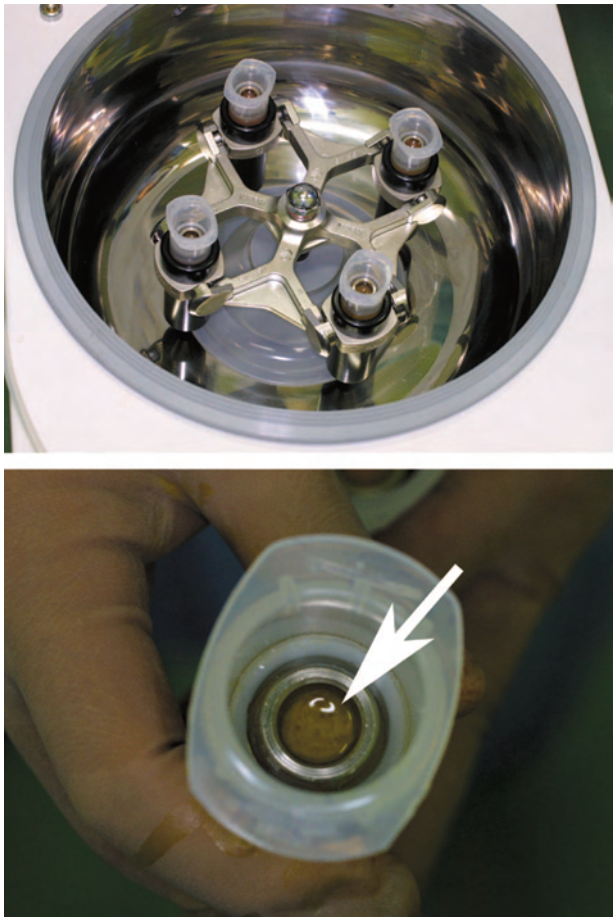

Fig. 1. Centrifugation of liposuction aspirates. (*Above*) A machine for centrifugation used in this study. (*Below*) A disposable sterilized syringe (50 ml) with a filter piston. By centrifugation, oil was shifted onto the piston (*arrow*).

MATERIALS AND METHODS

Centrifugation of Liposuction Aspirates

Liposuction aspirates were obtained from surgery performed on the abdomen or thigh regions of eight healthy female donors aged 21 to 38 years with informed consent approved by our institutional review board. Infiltration of saline (tumescent solution), liposuction, and subsequent centrifugation of syringes were conducted using a single combined machine (Lipokit; Medikan Corp., Seoul, Korea) (Fig. 1, *above*). Liposuction aspirates were divided and poured into disposable sterilized syringes (50 ml) with a filter piston (Medikan). The filter piston was specifically designed for separation of the oil from the other portions by centrifugation (Fig. 1, *below*). After being placed upright for 10 minutes, specimens were divided into two portions: a floating adipose portion and a denser fluid portion. Syringes were allotted to six groups (two syringes each) and centrifuged at 0, 400, 800, 1200, 3000, or 4200 *g* by means of the Lipokit for 3 minutes. After centrifugation, specimens were divided into three portions; oil (top), adipose (middle), and fluid (bottom) (Fig. 2). The volume and weight of each portion were measured before and after centrifugation, and the specific gravity [weight (in grams)/volume (in milliliters)] of the adipose portion was calculated. The volume of the oil portion was measured by aspirating the oil on the filter piston with a 10-ml syringe, and those

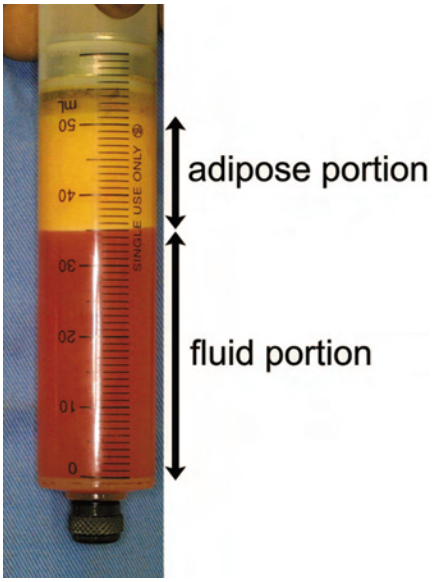

Fig. 2. The adipose and fluid portions of liposuction aspirates were clearly separated by 3 minutes of centrifugation and stayed below the piston after that.

of the adipose and fluid portions were measured by reading a scale on the centrifugation syringe (50 ml).

Counting of Blood Cells and Adipose-Derived Stem Cells

Although blood cells are thought to disturb engraftment of injected adipose and to be partly extracted by centrifugation, there are no reliable data on the extent of blood cell clearance by centrifugation. Adipose portion tissues before and after centrifugation were digested at 37°C for 30 minutes on a shaker with an equal volume of 0.075% collagenase. Mature adipocytes and connective tissues were separated from pellets by centrifugation (400 *g* for 10 minutes). The pellet was resuspended in phosphate-buffered saline and passed through a 100-μm mesh filter (Millipore, Billerica, Mass.). Fluid portions (3 ml each) before and after centrifugation were centrifuged (400 *g* for 10 minutes), and the pellets were resuspended and passed through a 100-μm mesh filter. Numbers of red blood cells and nucleated cells in adipose and fluid portions were counted separately with CellTec (Nihon Koden, Tokyo, Japan). Nucleated cells counted corresponded to the total of white blood cells, adipose-derived stem cells, and other adipose-derived cells such as endothelial cells.

Isolation, Culture, and Counting of Adipose-Derived Stem Cells from the Adipose and Fluid Portions

Adipose-derived stem cells were isolated separately from the adipose and fluid portions of liposuction aspirates as described before.[18] The cells were cultured on 100-mm gelatin-coated dishes with M-199 medium containing 10% fetal bovine serum, 100 IU penicillin, 100 mg/ml streptomycin, 5 ng/ml heparin, and 2 μg/ml acidic fibroblast growth factor, at 37°C, in a humid atmosphere of air and 5% carbon dioxide. After being cultured for 7 days, cell counts were performed using a NucleoCounter (Chemometec, Denmark).

Fat Transplantation to Nude Mice

To examine the influences of centrifugation on engraftment of adipose tissue, lipoaspirates obtained from a single donor were divided into six graft materials using centrifugations at 0, 400, 700, 1200, 3000, and 4200 *g*. Six milliliters of each material was injected into the backs of six 5-week-old nude mice using an 18-gauge needle, with 1 ml injected per mouse. The same experiment was repeated twice using lipoaspirates from another donor. Consequently, a total of 72 mice were used as recipients of human fat transplantation. Animals were killed 4 weeks after fat transplantation, and transplanted grafts were measured for weight. Harvested samples were fixed and processed for histology (see below). To estimate the net efficacy of transplantation per volume of adipose portion before centrifugation, a calculation was performed with the following hypothetical equation: Putative graft take of 1 ml uncentrifuged adipose = (graft take of 1 ml centrifuged adipose) × (volume of adipose portion after centrifugation)/(volume of adipose portion before centrifugation).

Scanning Electron Microscopic Study

Adipose samples were fixed with paraformaldehyde and 2.5% glutaraldehyde in 0.2 M cacodylate buffer for 1 week at room temperature, and fixed in 1% osmium tetroxide. After dehydration, they were dried with a supercritical point carbon dioxide dryer (HCP-2; Hitachi, Tokyo, Japan), sputter-coated with platinum/palladium, and examined with a scanning electron microscope (S3500N; Hitachi). Light microscopic examination of hematoxylin and eosin–stained slides was also performed.

Statistical Analyses

Results were expressed as mean ± SE. Paired *t* tests were performed to evaluate the differences between centrifugal conditions, and no correction was made for multiple comparisons. Statistical significance was defined as $p < 0.05$.

RESULTS

Gross Effects of Centrifugation on the Adipose Portion of Liposuction Aspirates

A total of 78 syringes containing liposuction aspirates were centrifuged with six different centrifugal forces. With increased centrifugal force, the volumes of the oil and fluid portions increased, and the volume of the adipose portion decreased. Although there was no significant difference between 3000 *g* and 4200 *g*, volumes of oil, adipose, and fluids altered significantly with increased centrifugal forces (Fig. 3, *above*). For 48 samples, the specific gravity of the adipose portion was measured; it did not change significantly except for a decrease between control and 400 *g* (Fig. 3, *below*). On scanning electron microscopic observation, the adipose portion was observed as clusters of spherically shaped adipose cells. Adipocyte size was not remarkably altered with increased centrifugal forces. In all samples, including uncentrifuged controls, clusters of adipocytes were partially ruptured. The degree of ruptured adipocyte clusters seemed to vary among donor subjects, but no remarkable difference was seen between the samples processed with different centrifugal forces from the same subject (Fig. 4).

Effects of Centrifugation on Numbers of Red Blood Cells, Adipose-Derived Stem Cells, and Nucleated Cells

Numbers of red blood cells, adipose-derived stem cells, and nucleated cells in the adipose and fluid portions were counted separately using 48 samples. Although the total number of red blood cells in the adipose and fluid portions did not change significantly based on centrifugal forces (Fig. 5, *above*), red blood cells shifted significantly from the adipose portion to the fluid portion at all different centrifugal forces. In addition, a significant difference was seen between 400 *g* and 700 *g* but not between 700 *g* and more than 1200 *g* (Fig. 6, *above*). Total numbers of nucleated cells, which included white blood cells, adipose-derived stem cells, and other adipose-derived cells, did not change significantly by centrifugation; nor were there statistically significant shifts in numbers of nucleated cells detected for any centrifugal

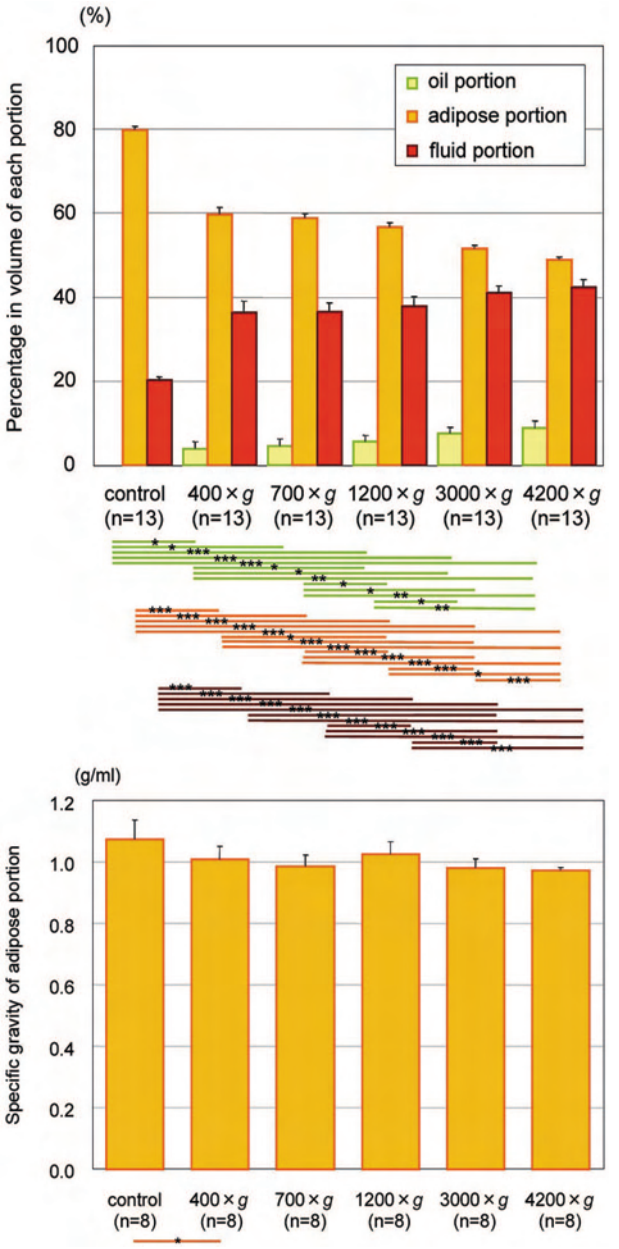

Fig. 3. (*Above*) Columns on the left, middle, and right demonstrate the volumetric proportions of the oil, adipose, and fluid portions, respectively, under each centrifugal condition. Statistical analysis was performed with paired *t* tests between groups. *Green*, *orange*, and *red bars* indicate statistical significances in the oil, adipose, and fluid portions, respectively. The significance levels are indicated with the number of asterisks (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). A significant volume reduction in the adipose portion was observed not only between uncentrifuged and centrifuged samples but also between different centrifugal forces. The fluid and oil portions significantly increased in volume in a centrifugal force–dependent manner. Data represent means ± SE. (*Below*) Specific gravity of the adipose portion tends to decrease with increased centrifugal force, but the differences were not statistically significant. Data represent means ± SE.

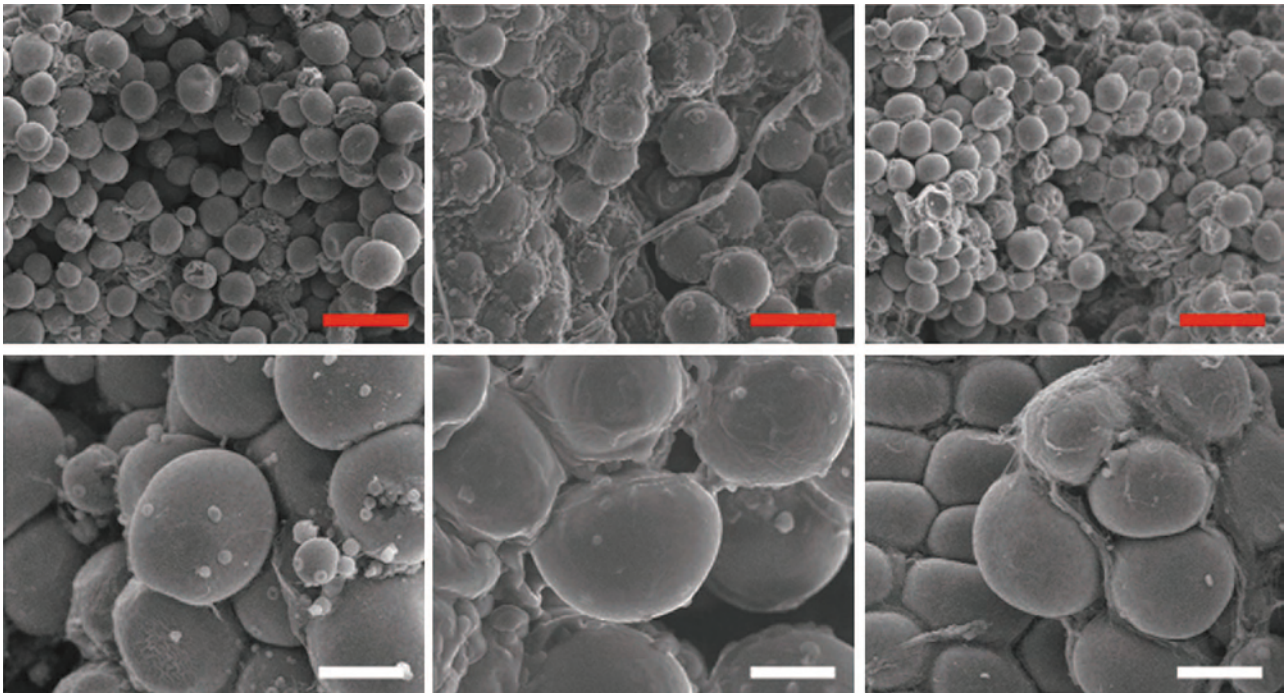

Fig. 4. Scanning electron microscopic photographs of representative uncentrifuged and centrifuged samples derived from a single donor. Clusters of spherically shaped adipocytes and intermittently dispersed ruptured cells were observed, regardless of centrifugal forces [*left*, control (uncentrifuged); *center*, 1200 *g*; *right*, 4200 *g*). Magnified photographs show that there are adipocytes that were not morphologically altered even in samples centrifuged at 4200 *g* (*below*, *right*). Scale bar = 200 μm (*above*) and 50 μm (*below*).

force (data not shown). The total number of adipose-derived stem cells remained consistent up to 1200 *g*, whereas the number of adipose-derived stem cells decreased significantly at more than 3000 *g* (Fig. 5, *below*). Unlike red blood cells, adipose-derived stem cells did not shift significantly between the adipose and fluid portions (Fig. 6, *below*).

Adipose Graft Survival of In Vivo Experimental Models

The results of the transplantation of uncentrifuged or centrifuged adipose using 72 nude mice revealed that centrifugation significantly enhanced the proportion of graft survival (Fig. 7, *above*). Significance was detected not only between control and all centrifugal forces, but also between increased centrifugal forces, although results from centrifugation at 3000 *g* were superior to those from 4200 *g* (Fig. 7, *above*).

Centrifugation made the original volume of fat compact, and thus 1 ml of uncentrifuged fat and 1 ml of centrifuged fat differed originally in fat volume. If we have a sufficient volume of aspirated fat, we conclude that centrifugation can enhance the graft take. In contrast, if we tried to obtain the largest adipose graft by using 1 ml of uncentrifuged adipose alone, it could be concluded by virtual calculation that the uncentrifuged graft would be better than any centrifuged grafts (Fig. 7, *below*).

Under microscopic observation, no remarkable difference was observed in cell integrity or structure among samples centrifuged at different forces. Even in samples centrifuged with a maximum force of 4200 *g*, adipocytes survived well 4 weeks after transplantation.

DISCUSSION

Concentration of the Graft

Although various authors have recommended performing precentrifugation of fat grafts, many reports described centrifugal force using *rpm* (revolutions per minute) units.[1,3,5,8,21–24] Working centrifugal forces with the same *rpm* value can differ in terms of radius of centrifugation. For example, centrifugation at 3000 rpm with either a 12- or 18-cm radius of gyration is equivalent to 1207 or 1811 *g*, respectively. Thus, it is hard to compare our results with those from other previous reports that used *rpm*.

Our study showed that 3 minutes of centrifugation compacts aspirated fat and partly excludes oil, water, and blood cells, but not adipose-derived

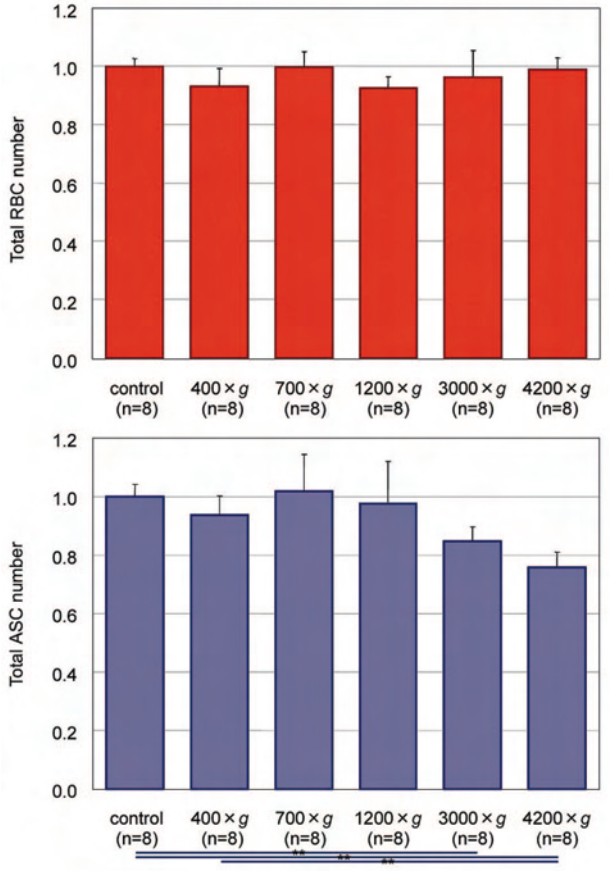

Fig. 5. Total numbers of red blood cells and adipose-derived stem cells in the adipose and fluid portions of liposuction aspirates before and after centrifugation. Proportions of total (adipose and fluid portions) cell count at each centrifugal condition compared to control are presented. Significance was analyzed using paired *t* tests for groups. Data represent means ± SE (*$p <$ 0.05; **$p < 0.01$; ***$p < 0.001$). (*Above*) Total numbers of red blood cells did not change significantly by centrifugation. (*Below*) Total number of adipose-derived stem cells showed no remarkable alteration after centrifugation up to 1200 *g*, but a significant decrease was observed between control and samples centrifuged at 3000 and 4200 *g*.

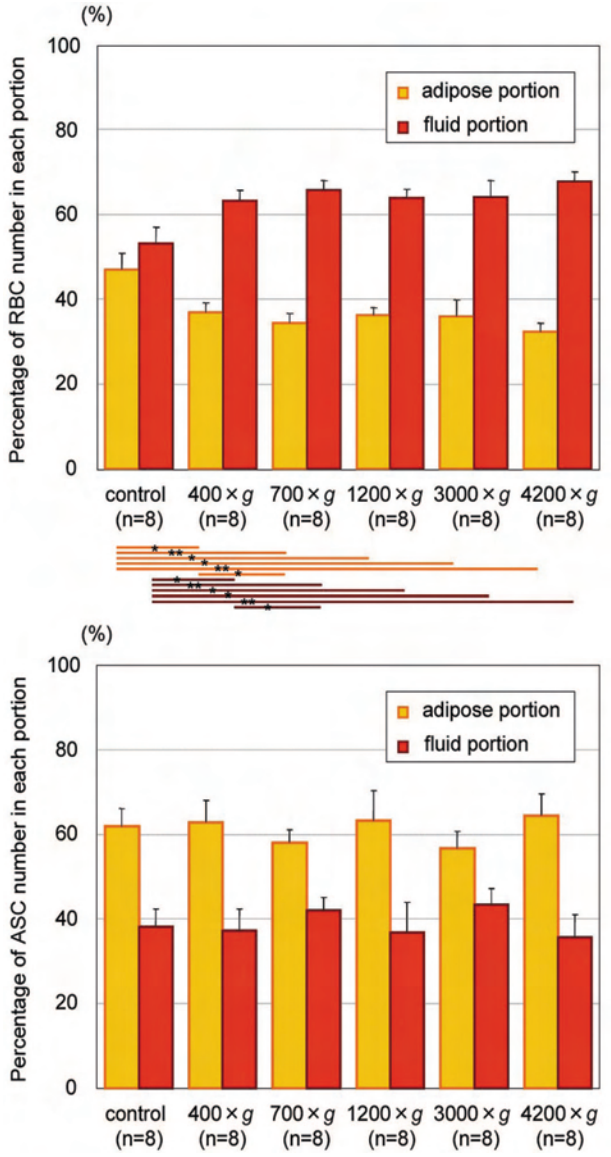

Fig. 6. Shift of red blood cells and adipose-derived stem cells between the adipose and fluid portions by centrifugation. Proportions of cell numbers contained in adipose and fluid portions before and after centrifugation are presented. Significance was analyzed using paired *t* tests between groups. Data represent means ± SE (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). (*Above*) Percentages of red blood cell count contained in the adipose and fluid portions. Red blood cells shifted significantly from the adipose portion to the fluid portion at all different centrifugal forces compared with control. In addition, significance was seen between 400 *g* and 700 *g*, but not between 700 *g* and more than 1200 *g*, suggesting that centrifugation at 700 *g* is enough and that more than 700 *g* may not be necessary for red blood cell extraction from aspirated adipose. (*Below*) Percentage of adipose-derived stem cell count contained in the adipose and fluid portions. Adipose-derived stem cells did not shift significantly between the adipose and fluid portions at any level of centrifugal force.

stem cells. Consequently, adipose tissue, extracellular matrix, and adipose-derived stem cells are concentrated by centrifugation, likely contributing to a boost in the graft take.[25,26] The degrees of concentration and exclusion tended to be elevated with increased centrifugal force.

Damage to Adipocytes

Boschert et al.[7] reported that the quantity of oil increased because of adipocyte destruction when specimens were centrifuged at more than 100 *g* and thus concluded that centrifugation at greater than 100 *g* was not appropriate for autol-

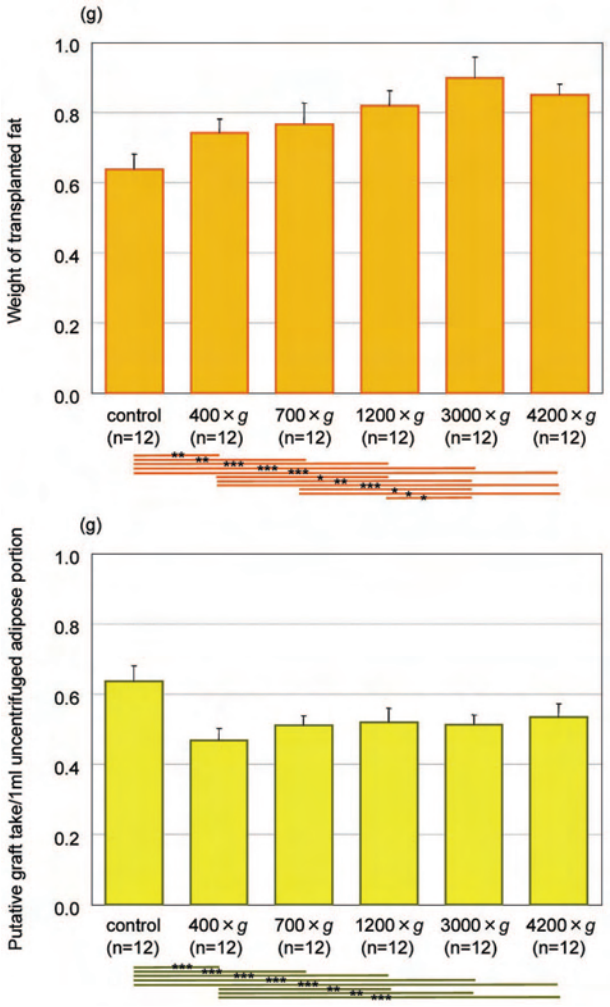

Fig. 7. Transplantation of uncentrifuged and centrifuged adipose tissue lipoaspirates obtained from a single donor were divided into six graft materials using centrifugation at 0, 400, 700, 1200, 3000, and 4200 *g*. Six milliliters of each material was injected into the backs of six 5-week-old nude mice using an 18-gauge needle, with 1 ml injected per mouse. The same experiment was repeated twice using lipoaspirates from another donor. The surviving adipose tissues were harvested 4 weeks later. Significance was analyzed with paired *t* tests between groups. Data represent means ± SE (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). (*Above*) Weights of transplanted adipose tissues. With centrifugation at 1200 *g* or more, transplanted adipose tissue was significantly greater in weight than uncentrifuged control. Centrifugation significantly contributed to obtaining a better graft take at least in short-term observations, although centrifugation at 4200 *g* might be excessive compared with 3000 *g*. (*Below*) Calculated putative graft take per volume of uncentrifuged adipose. Values were calculated as follows. Putative graft take of 1 ml uncentrifuged adipose = (graft take of 1 ml centrifuged adipose) × (volume of adipose portion after centrifugation)/(volume of adipose portion before centrifugation). Based on these putative calculations, if there was a limited volume of aspirated adipose, graft take would be best when it was not centrifuged before transplantation.

ogous fat transplantation. However, the results of our fat graft experiments indicate that centrifugation with more than 100 *g* centrifugal force can surely be used in aspirated fat transplantation.

Our results showed that the oil volume increased with increased centrifugal forces, but histologic findings did not clearly demonstrate destruction of adipocytes. Based on our microscopic observations, the degree of adipocyte destruction differed among patients but showed only minor differences between different centrifugal forces. Scanning electron microscopic observation showed that remnant oil was seen in the adipose portion even after 3 minutes of centrifugation. Thus, we suggest that the increase in the oil portion does not necessarily mean an increase of adipocyte destruction by centrifugation but may rather mean an increase in separation of oil from the adipose portion.

Damage and Distribution of Blood Cells and Adipose-Derived Stem Cells

Our results are mostly in accordance with the view previously reported that centrifugation separates fat cells from lipid, blood cells, water, and water-soluble ingredients such as proteases and lipases.[3,5,7,8] To our knowledge, there have been no reports examining quantitatively the effects of centrifugation on blood cells and adipose-derived stem cells in liposuction aspirates. Although centrifugation induced a slight shift of red blood cells from the adipose portion to the fluid portion without change in total numbers of red blood cells, the volume of the adipose portion was compacted to a greater extent than the shift of blood cells, and thus these blood cells were slightly concentrated in the adipose portion. Although a previous author indicated that the presence of blood in the injected fat stimulates macrophage activity to remove the fat cells,[5] the actual effect of the blood in the graft has not clearly been elucidated. Thus far, we cannot determine whether a decrease of number and increase of concentration of red blood cells and white blood cells is advantageous or disadvantageous in fat grafting.

In contrast, the results indicated that adipose-derived stem cell yield after 1 week of culture was almost consistent up to 3000 *g* and decreased at more than 3000 *g*. In addition, it was shown that adipose-derived stem cells did not shift between the adipose and fluid portions by centrifugation, likely because adipose-derived stem cells contained in the adipose portion are resident in or strongly adhered to the adipose tissues. Accord-

ingly, centrifugation simply enhanced the density of adipose-derived stem cells as a result of compaction of the adipose portion. Condensation of adipose-derived stem cells in fat graft may be beneficial for enhancing the fat graft survival rate for the reasons discussed below.

Graft Survival

We propose that the aspirated fat graft takes were grossly influenced by the balance of the negative effects of destruction and the positive effects of condensation by centrifugation. Our results revealed that the short-term survival rate of aspirated adipose graft per volumetric unit after centrifugation increased with centrifugal forces up to 3000 *g*. Condensation of the graft material and adipose-derived stem cells is thought to dominantly contribute to this enhancement.

However, it was also shown by a virtual calculation that surviving fat graft per volumetric unit before centrifugation decreased by intervention with centrifugation. Surviving fat graft decreased at 400 *g* but did not decrease further with increased centrifugal forces; this outcome may imply that the decrease at 400 *g* could have resulted from destruction of adipocytes located especially in the superficial layers of adipose fragments and that adipocytes in the inner layers may be protected from mechanical injuries by the destroyed superficially located adipocytes.

Histologic findings of transplanted fat were consistent with previous reports,[5,27] which found that centrifuged graft samples were similar to uncentrifuged ones. Even samples centrifuged at 4200 *g* showed no remarkable differences in histology from controls after transplantation. It is thus suggested that once adipocytes succeed in avoiding critical damage during centrifugation, there will be no difference in the structural quality of adipocytes between centrifuged and uncentrifuged samples.

Our results of graft take with or without centrifugation indicate a clinical application for selective use of centrifugation. If there has only been a restricted amount of adipose (though it would be very rare), centrifugation might not have been the best approach from the standpoint of the most effective use of restricted graft material. For example, by using 10 ml of uncentrifuged adipose tissue, we would have a 6.4-ml augmentation (64 percent survival) if we transplanted the adipose without centrifugation. However, if we centrifuged the 10 ml of adipose at 1200 *g*, we would obtain centrifuged and condensed adipose, with a volume of 7.2 ml. After transplanting the 7.2 ml of centrifuged fat, we would have a 5.9-ml augmentation (82 percent survival). However, in most clinical cases, we can harvest a sufficient volume of aspirated fat, and in such cases, we should centrifuge aspirated fat before grafting to obtain the best augmentation effects. For example, by harvesting 13.8 ml of adipose tissue, we could transplant 10 ml of adipose centrifuged at 1200 *g* and achieve an 8.2-ml augmentation.

We suggest that adipose-derived stem cells and other adipose-derived cells are crucial for graft survivability in both the short and long term. Our recent report[26] revealed that aspirated fat is relatively adipose-derived stem cell deficient compared with excised whole fat, which contains large vessels and nerves, unlike aspirated fat, and that adipose-derived stem cells can survive and reside between adipocytes or in the connective tissues of surviving adipose tissue after transplantation. The relative deficiency of adipose-derived stem cells may be the reason for the lessened survival rate of aspirated fat after transplantation compared with excised fat reported by two previous experimental studies.[28,29] Condensation of adipose-derived stem cells by centrifugation may mean conversion of relatively stem-cell–deficient adipose to relatively stem-cell–rich adipose. This adipose-derived stem cell condensation may enhance the fat graft take[26] and prevent long-term atrophy of transplanted adipose by working as tissue-specific progenitors.[25,26]

Excessive centrifugation can destroy adipocytes and adipose-derived stem cells. Centrifugation, however, plays a beneficial role in concentrating adipocytes, extracellular matrix, and adipose-derived stem cells, and in partially excluding red blood cells. Extracellular matrix should maintain its volume after transplantation, at least in the short term, and exclusion of red blood cells from graft materials may contribute to a better survival rate of transplanted adipose.

The oil portion increased only after centrifugation at 400 *g*. Red blood cells were shifted at 400 *g*. The adipose-derived stem cell-to-adipocyte ratio, which may influence long-term atrophy of grafted adipose, was suggested to be relatively improved by centrifugation, but adipose-derived stem cells were damaged at 3000 *g*. Graft takes of centrifuged adipose were best at 3000 *g*. Considered together, these data lead us tentatively to recommend 1200 *g* as an optimized centrifugal force among the tested centrifugal forces for obtaining good short- and long-term results in adi-

pose transplantation. Although our conclusion that selective centrifugation with relatively high centrifugal force may be beneficial for graft survival contradicts the views or recommendations of recent authors,[7,9,12] it is similar to the 1286 *g* recommended by Coleman based on abundant clinical experience.[13] Further investigation is necessary to elucidate the net efficacy of centrifugation on graft survival in clinical settings because the gross take of transplanted tissue will be clinically influenced by various factors associated with harvesting, processing, and transplanting adipose tissues.

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DISCUSSION

Influences of Centrifugation on Cells and Tissues in Liposuction Aspirates: Optimized Centrifugation for Lipotransfer and Cell Isolation

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The authors present a sophisticated and somewhat complex analysis of the role of centrifugation in fat grafting. This study addresses a number of aspects regarding the value of centrifugation and probably needs to be read more than once for one to extract and evaluate all of the information. The methods used are sound and the discussion is particularly germane to the reader's interpretation.

As with any good research, for each answered question several additional ones are raised, but this study does provide several interesting answers. Optimizing fat transfer has been an ongoing interest of ours, and clearly this article sheds additional light on the subject. The authors have analyzed cell viability after centrifugation at a variety of *g* forces. These data have been compared with ours and with those of other contributors.[1–3] Recent interest in the role of adipose-derived stem cells and their potentially positive effects on fat graft take are explored and some important points are made. Since our work was published and presented, the interest in adipose-derived stem cells has clearly increased. This has been fostered by the theoretical potential of adipose-derived stem cells to enhance neoangiogenesis and, by inference, also enhance adipocyte viability and graft take.

The authors used fat from female liposuction patients and indicated that the sources were abdominal and thigh fatty deposits. No subsequent analysis as to any differences in these sources was made and therefore no information can be gleaned in terms of site designation and its potential role in fat graft survival.

Beyond the identification of viable cells, the authors have pursued short-term cell culture survival and proliferation and longer term survival in transplantation to the athymic nude mouse model, assessed at 4 weeks. The authors conclude that although excessive centrifugation can destroy adipocytes and adipose-derived stem cells, appropriate centrifugation (lower *g* forces) increased the population of not only viable adipocytes but also adipose-derived stem cells, and resulted in enhanced *early* graft take. They have settled on an optimum centrifugation of 1200 *g*.

The authors also paid attention to the presence of red blood cells in the concentrated adipocyte portion of their specimens. They make the theoretical point that the presence of red blood cells may negatively affect cell take and viability, as their presence may stimulate macrophage activity that could destroy fat cells. They report the interesting finding that centrifugation did not decrease the number of adipose-derived stem cells but did result in the removal of some red blood cells, presumably into the fluid layer. They specifically note that the short-term survival rate of aspirated adipose graft (per volumetric unit after centrifugation) increased with centrifugation. They conclude that selective use of centrifugation has probable value in enhancing the viable adipocyte population, with no diminution in the number of adipose-derived stem cells. They mention, referencing one of their earlier studies, that the presence of reduced numbers of adipose-derived stem cells in aspirated fat grafts compared with whole fat transplants may be the explanation for the relatively lower survival rate of aspirated fat after transplantation.

Our personal conclusions from this study would be in concert. Appropriate centrifugation (50 to 1200 *g*) can provide a more generous concentration of viable fat cells without sacrifice of adipose-derived stem cells while reducing the concentration of potentially detrimental red blood cells. The authors made no mention of the sampling area of the adipose layer, but our work

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clearly demonstrated a higher concentration of viable adipocytes in the lowest segment of the fat layer.[2] Of parallel interest, Rohrich et al.[4] showed no increase in adipocyte viability after centrifugation at 500 *g* when compared with uncentrifuged specimens. This would be countered by their (Rohrich et al.) lack of indication of the layer from where the sample was taken—that is, top, middle, or lower layer—and the use of higher *g* force (500 *g*) in contrast to our recommendation of 50 *g*.

Another point made by the authors might benefit from additional interpretation. They observed good cell viability even with higher *g* centrifugation (4200 *g*). Our interpretation of this observation would be that although centrifugation may result in more lysis of cells, it does not appear to compromise the viability of the remaining intact cells in the aliquot of the centrifuged specimen.

The authors make the subtle point that centrifugation may not be appropriate in patients with impoverished sources of transplantable fat cells. However, this is a problem rarely encountered in Americans.

We believe that the authors have made their points well and have analyzed these data accurately and in detail. We also believe that the appreciation of the valuable role of adipose-derived stem cells in graft survival is at play here. It continues to be apparent to us that appropriate centrifugation, which we interpret to be lower *g* forces and for short periods of time, can contribute to improved fat graft transfer success and ultimately to its reliable use as the best (potentially permanent) method of soft-tissue augmentation. We applaud the authors' efforts to further define the role of centrifugation in aspirated fat grafts and feel that there are several valuable "take-home" messages in this article.

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