



Comparison of Three Fat Graft Preparation Methods: Centrifugation, the Lipokit™ System, and the Cytori Puregraft® System

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INTRODUCTION

As autologous fat grafting gains clinical prominence,^{1, 2, 3} there is a need to assess the quality of different graft preparation methods.⁴ Graft tissue is comprised of four components: adipose tissue, aqueous fluid (residual tumescent solution or wash solution), free lipids released from ruptured adipocytes, and a nonbuoyant component comprised of blood cells and tissue fragments. Free lipids, blood cells, and debris within the graft can promote the formation of oil cysts and negatively impact graft retention by exacerbating an inflammatory response.^{5, 6, 7}

An ideal graft preparation technique must remove unwanted contaminants and reduce the aqueous liquid component, while maximizing viability of the graft. In a peer-reviewed paper published in *Plastic and Reconstructive Surgery*,⁵ results were reported from a side-by-side comparison of three common adipose tissue graft preparation methods: graft washing and filtration using the Cytori Puregraft® 250/PURE System (Puregraft System), centrifugation, and separation by gravity sedimentation.⁵ In this paper, results are reported for a similar study comparing two of the three previously compared methods to the Lipokit™ System (Medi-Khan International, Korea). Each graft preparation method was evaluated for quality, composition, graft tissue viability, and processing time and cost using identical starting material from the same donors.

METHODS

Adipose tissue from six donors, one male and five females, was acquired from subcutaneous abdomen, flank, thigh, and back adipose tissue depots using vacuum-assisted suction (N=6). Tissue from each donor was mixed evenly to ensure that samples taken for each processing method were matched, and divided into four groups for processing by different methods: Control (equivalent to a surgeon aspirating and immediately using tissue for grafting), centrifugation, the Lipokit System, and the Puregraft System. Control samples were analyzed without further manipulation. Centrifugation samples were loaded into capped 10 mL syringes, placed into a fixed angle rotor centrifuge (Medilite 6, Thermo electron corporation, Asheville, NC), and centrifuged at ~1,200 x g for three minutes. Free lipids floating above the adipose tissue were removed by aspiration and the aqueous liquid below the tissue was drained. Samples in the Lipokit arm were loaded into 50 mL Fat Processing Units for processing in the Lipokit System for five minutes at 3,500 rpm in accordance with the manufacturer's instructions. Samples in the Puregraft arm were prepared using the Puregraft System with two washing steps in accordance with the manufacturer's instructions.

The volume of graft generated by each preparation method was measured and 5 mL samples were taken for assessment of adipose tissue viability using a lipolysis assay that has been validated



to show a close correlation with tissue viability.⁵ Grafts were then separated into their component parts (free lipids, adipose tissue, aqueous fluid, and a cell/debris pellet).⁵ Triplicate 10 mL samples of graft generated by each of the four methods for each donor tissue were transferred to 15 mL BD Falcon™ centrifuge tubes (BD Biosciense, San Jose, CA) and centrifuged at 400 x g for five minutes at room temperature. The volume of each component was determined and recorded as a percentage of the graft as a whole. The cellular pellet was then retrieved and resuspended for quantification of red blood cells content using a Bright-Line™ hemocytometer, improved Neubauer Ruling (Sigma-Aldrich, St. Louis, MO). Results of cell counts were averaged and expressed as a percentage of the Control group on the basis of cells per gram of graft. Statistical comparison was performed with a Mixed Effects

ANOVA and Tukey Test with a p-critical of 0.05. Results are reported in terms of mean ± standard error.

Reliability of the three graft preparation methods was gauged by calculating the systemic variance. An additional assessment of ease of use for each graft preparation process was made by calculating the number of steps a trained user needs to perform to prepare graft from a starting volume of 200 mL of lipoaspirate.

RESULTS

Graft Appearance: An example of the grafts prepared by each approach is provided in **Figure 1**. The appearance of the tissue suggests that red blood cell content is lowest in the grafts prepared within the Puregraft System. This observation

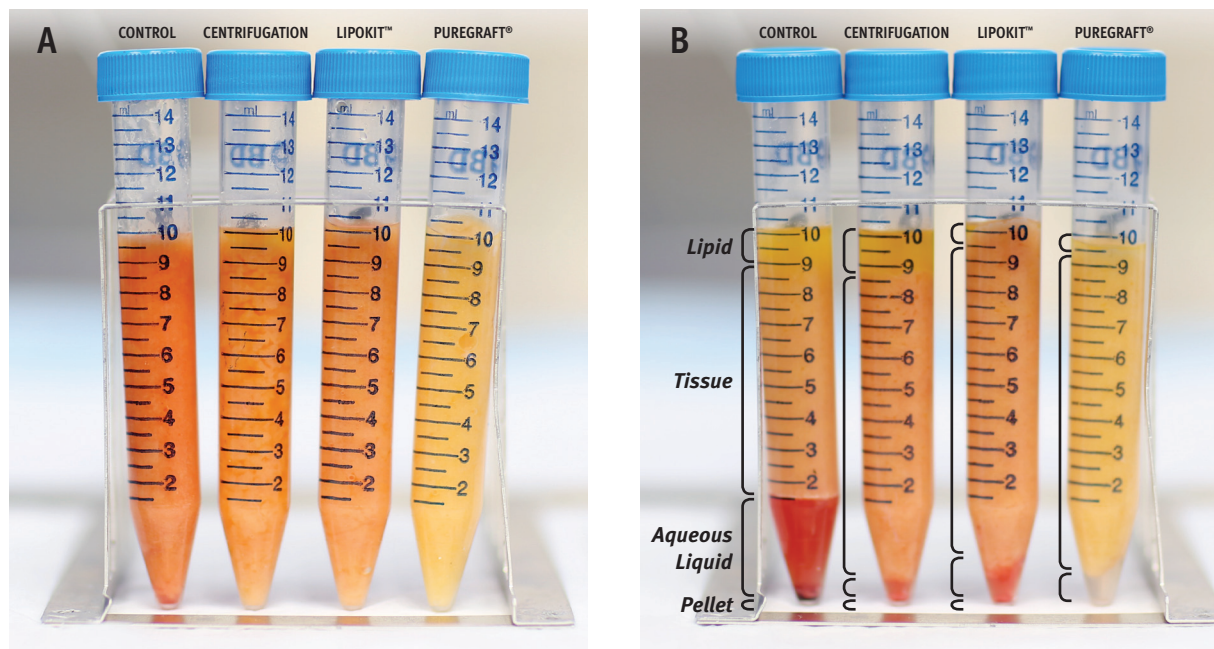


Figure 1. Graft Tissue Output Prepared by Different Methods. (A) Example grafts prepared by (from left to right): Control, centrifugation, the Lipokit System, and the Puregraft® System. (B) The same grafts are shown after separating the prepared grafts into four phase components: a pellet comprised mainly of blood cells and debris, aqueous liquid, adipose tissue, and free lipid (top).



is supported by the size of the cell pellet and the color of the aqueous component obtained following centrifugation (**Figure 1B**).

Graft Composition: The relative content of fluid, free lipids, and adipose tissue within grafts prepared by each method are shown in **Figure 2**. These data show that, on average, adipose tissue comprised only 66% of the volume of the Control grafts compared to 87–93% of the volume of processed grafts.

Aqueous Fluid Content: The aqueous liquid content of grafts prepared using the different preparation methods can be seen for one representative sample in **Figure 1**. All tissue preparation methods resulted in grafts with significantly lower aqueous content than the un-manipulated Control ($p < 0.0001$ for all comparisons). Statistical analysis found no significant difference in graft aqueous content among three processed groups (centrifugation, Lipokit, and Puregraft).

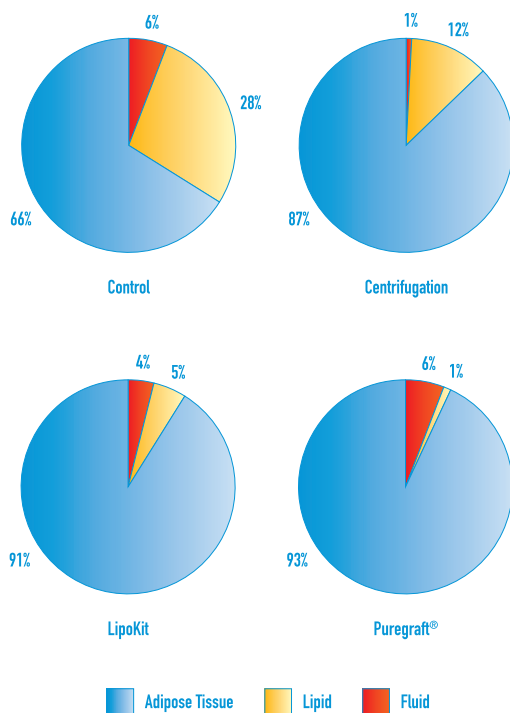


Figure 2. Relative Graft Composition (by volume).

Free Lipid Content: Free lipids are formed by disruption of adipocytes (fat cells) during tissue collection and processing and indirectly reflect the overall health of the processed tissue. Extracellular lipids can also contribute to formation of lipid cysts once the graft has been re-injected. As shown in **Figure 2** and **Figure 3**, graft tissue prepared using the Puregraft System had significantly lower free lipid content than grafts prepared by Control, centrifugation, or the Lipokit System ($p < 0.004$ for all comparisons). There was no significant difference in free lipid content between the un-manipulated Control and grafts prepared by centrifugation or the Lipokit System.

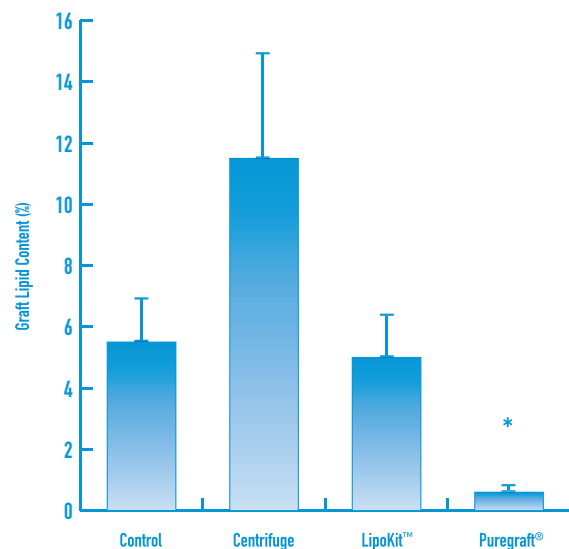


Figure 3. Comparison of Lipid Content of Graft Output. (*) $p < 0.004$ for all comparisons with Puregraft.

Red Blood Cell Content: As mentioned above, data shown in **Figure 1** suggests that grafts prepared using the Puregraft System contain lower contamination with red blood cells than grafts prepared using the other processing methods. This parameter was quantified by collecting the cell pellets and performing cell counts. The data shown in **Figure 4** confirms that grafts prepared with Puregraft contained the lowest red blood cell content of all groups with only $2.4 \pm 0.6\%$ of the red blood cell content observed in the

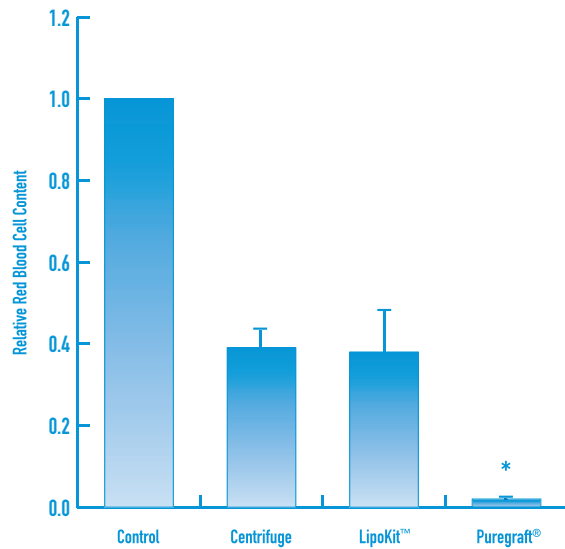


Figure 4. Relative Graft Red Blood Cell Content (Normalized to the Control). (*) Puregraft has the lowest red blood cell content, Tukey adjusted p-value <0.0001 for all comparisons. No statistically significant difference in graft red blood cell content was observed between centrifugation and the Lipokit System.

un-manipulated Control. Red blood cell debris was considerably higher in grafts prepared by centrifugation and the Lipokit System ($38.1 \pm 4.4\%$ and $38.0 \pm 8.4\%$ of Control respectively).

Graft Viability: Graft viability was assessed using an assay for hormone-induced lipolysis activity, a key measure of global adipose tissue function. In order to account for differences in adrenergic stimulation of lipolysis between donors, lipolysis data for each sample were normalized to that of tissue in the Control arm for each donor. As shown in **Figure 5**, grafts prepared using the Puregraft System exhibited significantly higher lipolytic activity than those prepared by centrifugation or the Lipokit System. Indeed, on average, tissue prepared using the Puregraft System exhibited approximately twice the lipolytic activity of Control tissue and approximately 60% greater activity than grafts prepared using centrifugation or the Lipokit System ($p < 0.05$). There was no statistically significant difference in lipolysis activity between centrifugation and the Lipokit System, both having lipolysis activity that was only 40% greater than the Control (**Figure 5**). The lower viability seen in

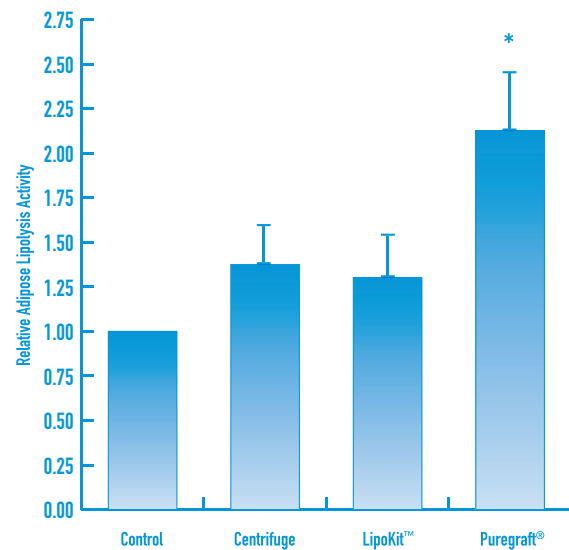


Figure 5. Adipose Lipolysis. (*) Puregraft exhibited the highest lipolysis activity, $p < 0.001$ for all comparisons. No statistically significant difference was observed between grafts prepared by centrifugation and the Lipokit System.

grafts prepared by centrifugation and the Lipokit System suggest that these grafts will exhibit greater variability and loss of volume following implantation.

Processing Parameters: Graft preparation time, not including set up, for each approach to prepare a standard 200 mL graft is reported in **Table 1**. The Lipokit System had the shortest processing time and centrifugation had the longest. However, all approaches other than the Control were able to complete processing within five minutes of each other.

Table 1. Processing Parameters for Graft Preparation Methods.

DEVICE	Processing Time for 200 mL of Tissue (Minutes)
Control	1
Centrifugation	42
LipoKit™	11
Puregraft®	15



DISCUSSION

Presence of Contaminants: Side effects such as graft reabsorption and formation of oil cysts are commonly reported for fat transfer procedures.⁶ These phenomena are due, at least in part, to inflammatory processes initiated in response to graft contaminants and non-viable adipose tissue. The relatively high and variable content of these graft components using certain graft preparation methods are a key reason for the difficulty in predicting outcomes. Thus, there is a need to reduce the content of these contaminants and create a standardized, more reliable graft.

Early loss of volume following implantation is largely due to absorption of aqueous fluid present within the graft tissue. Hence, management of volume loss also involves management of the amount of such fluid in the graft at the time of implantation. Centrifugation, the Lipokit System, and the Puregraft System all successfully reduced the amount of aqueous fluid in grafts compared to the un-manipulated Control. No significant difference in graft aqueous content was detected, yet the Puregraft System offers physicians the flexibility of controlling the graft hydration to meet their individual surgical preferences, by shortening or extending drainage time in order to prepare a more wet or dry graft, respectively. This feature is not offered by the other methods.

The Puregraft System was also significantly more effective at removing contaminants such as free lipids and red blood cells than either centrifugation or the Lipokit System. Grafts prepared using Lipokit had, on average, 9-fold more free lipids than grafts prepared using the Puregraft System. The higher level of free lipids in grafts prepared with the Lipokit System suggests that this approach could result in a higher risk of lipid cyst following implantation. Grafts prepared by centrifugation and using the Lipokit System also contained, on average, 15-fold higher contamination with red blood cells than grafts prepared using Puregraft. Contaminating blood cells and debris have the potential to elicit an

inflammatory response that could negatively impact graft retention.

Graft Viability: The primary function of adipose tissue is the storage of energy in the form of triglycerides and the mobilization of this energy in response to physiologic stimuli. The lipolysis assay used in this study provides a global view of the health of the cascade of events occurring within adipose tissue between stimulation with an adrenergic agonist and release of fatty acids and glycerol following hormone-induced lipolysis. As such, this assay is an excellent measure of global tissue health and viability. In the current study, grafts prepared with the Puregraft System exhibited significantly higher lipolytic activity than those prepared using the other graft-preparation methods ($p < 0.05$). There was no significant difference in lipolysis activity between the Control, grafts prepared by centrifugation, and grafts prepared with the Lipokit System. These data indicate that preparation of grafts using the Puregraft System selectively removes adipose tissue that is less viable, presumably because such fragments, being smaller as a result of adipocyte damage, pass through the filter system contained within Puregraft and segregate with waste, as seen in **Figure 5**.

Ease of Use: In addition to graft quality parameters, each graft preparation system was assessed for the ease of use. Usability was determined from the number of steps the user needs to perform in order to process 200 mL of lipoaspirate (removing caps, making connections, transferring tissue, etc.) These data are shown in **Figure 6**. While centrifugation involves the greatest number of steps, the Lipokit System also requires considerably more steps than with the Puregraft System due to the need to adjust small screws and loosen caps on the consumable. The Puregraft System can be used to process 200 mL of lipoaspirate with only 12 user steps. In contrast, the same lipoaspirate volume requires 156 user steps to prepare utilizing centrifugation due to the multiple steps required for each of the twenty 10 mL syringes used to process the tissue.

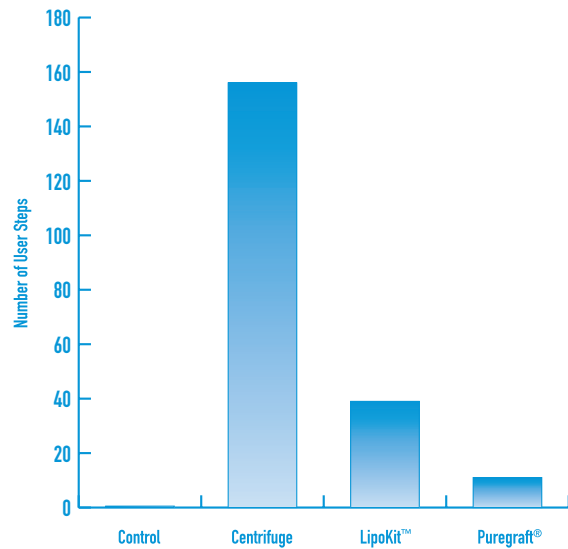


Figure 6. Graft Preparation Complexity. The number of user steps associated with each graft preparation process.

The devices and product needed for the three methods are shown in **Figure 7**.

Safety: Aside from the number of steps the user needs to perform during graft preparation, two critical areas of concern when evaluating the different graft preparation methods are the exposure of the graft tissue to environmental contaminants and exposure of operating room staff and users to potentially biohazardous agents within the tissue. Both Puregraft and Lipokit have minimal exposure to the environment compared to the open gravity and traditional centrifugation methods, thus minimizing the risk of bacterial contamination. Puregraft further protects the

user and graft by avoiding the need for the user to manually remove the waste fluid thereby eliminating this risk of exposure of the user to potential biohazards. This also enables the entire graft processing procedure to be performed within the sterile field.

Procedure Time: The three systems completed tissue processing within five minutes of each other, as seen in **Table 1**. However when a larger volume of tissue is processed, the processing times lengthen. While both the Puregraft System and the Lipokit System can process 200 mL of lipoaspirate within 15 minutes, centrifugation would require 42 minutes.

Procedure Cost: Graft preparation costs were assessed based on disposable cost alone, labor was not included. As might be expected, preparing grafts by centrifugation had the lowest disposable cost, though, in the light of the data shown in Figure 6 and Figure 7, this financial saving is very likely lost in increased labor cost. The consumable cost for the Puregraft System was slightly higher than that for the Lipokit System (USD\$0.33 per mL of graft). This is also likely balanced by the increased labor cost associated with the added complexity shown in Figure 7.

Reproducibility: Reliability of the three graft-preparation methods was gauged by calculating the systemic variance. For example, covariance parameter estimation shows that the variance in residual lipid content seen in grafts prepared with Puregraft is almost 10-fold smaller than that

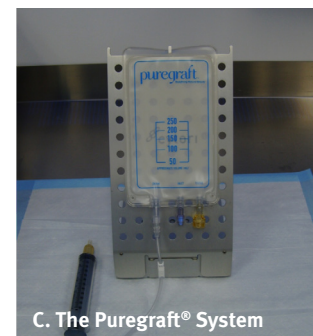
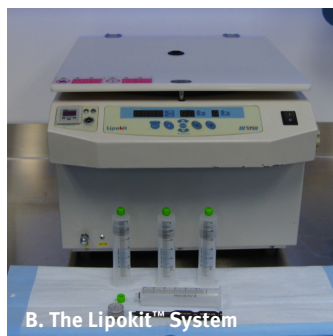
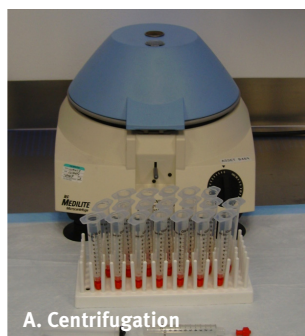


Figure 7. Products Needed for Three Graft Preparation Methods. A: Products needed to process 200 mL of tissue with the traditional centrifugation method. B: Products needed to process 200 mL of lipoaspirate with the Lipokit. C: Products needed to process 200 mL of tissue with the Puregraft System.



of Control, 35-fold smaller than Lipokit ($p < 0.014$) and almost 100-fold smaller than centrifugation ($p < 0.001$), so centrifugation has the greatest variability.

CONCLUSIONS

From the perspective of both the surgeon and the patient, optimal outcome following fat grafting requires limited and predictable volume loss over time. This demands the application of graft preparation methods that maximally and reproducibly remove contaminants that can negatively impact graft retention. The processing method used to attain an optimal graft must also meet practical requirements related to cost, operating room time, and risk of exposure of the graft material or operating room staff to potentially infectious agents. The current study assessed both quality and practical considerations for three graft-preparation techniques: centrifugation, the Lipokit System, and the Puregraft System in comparison with an un-processed graft.

All three systems tested in this study were able to reduce aqueous fluid content in the prepared graft. However, the Puregraft System consistently and significantly outperformed all other approaches in terms of reducing free-lipids and red blood cell content, while simultaneously producing a graft with significantly higher adipose tissue viability. The integrated design of the Puregraft System makes it the only system that allowed graft preparation entirely within the sterile field without exposing the graft to the open environment or users to potentially biohazardous material. This integration also led to simpler operation, as shown in **Figure 6**.

These results of this study are completely consistent with those in a prior published study⁵ evaluating different graft preparation approaches. The combination of these two reports shows that the Puregraft System consistently outperforms the other approaches evaluated in all key parameters. Confirmation that this increased performance leads to improved clinical performance is being evaluated in ongoing clinical studies.

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