

# Comparative Analysis of Two Automated Fat-processing Systems

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**Background:** Plastic surgeons desire more efficient methods of processing lipoaspirate when performing fat grafting procedures. We compared, in a preclinical study, the quantity and quality of lipoaspirate processed by a novel Poloxamer Wash, Absorption, mesh filtration System (PWAS) to a frequently used Ringer's Lactate wash, Decant, and mesh filtration System (RLDS).

**Methods:** Lipoaspirate from 10 patients was processed with the RLDS and PWAS systems. The processed lipoaspirate from each device was centrifuged to quantify the amount of fat, free oil, and aqueous components remaining in the fat graft. A trypan blue dye exclusion test assessed cell viability. The processing time for the lipoaspirate was also measured.

**Results:** The 10-patient average fat volume processed and available for grafting was similar using both systems. The adipose volume fraction of PWAS was greater ( $89\% \pm 3\%$ ) than RLDS ( $76\% \pm 10\%$ ,  $P = 0.02$ ). The trypan blue exclusion values and processing time were similar for both systems. Oil was efficiently removed from the lipoaspirate, and both systems processed fat efficiently.

**Conclusion:** The PWAS effectively cleans lipoaspirate with increased fat concentration. (*Plast Reconstr Surg Glob Open* 2020;8:e2587; doi: 10.1097/GOX.0000000000002587; Published online 17 January 2020.)

## INTRODUCTION

Autologous fat grafting has become a widespread technique for treatment of contour abnormalities in reconstructive and aesthetic surgery, and for soft tissue augmentation. A recent survey demonstrated that 80% of plastic surgeons have used fat grafting in their clinical practice.<sup>1</sup> Fat grafting is utilized for facial rejuvenation, facial contour abnormalities, secondary to trauma or tumor resection, breast augmentation, radiation damage, breast capsular contracture, posttraumatic disfigurement, congenital deformity, and burn injuries.<sup>2-6</sup> Autologous fat grafts are in many ways ideal fillers which have several beneficial characteristics, including lack of immunogenicity, simple surgical approach, low expenditure, and easy accessibility. More recently, large volume autologous fat grafting has gained popularity in both aesthetic and

reconstructive breast surgery, allowing for more natural feeling and aesthetically pleasing results.<sup>4,7-11</sup>

Although the procedure of autologous fat grafting has gained significant momentum in recent decades, the long-term outcomes remain variable. Fat graft retention and sustainability are inconsistent, which affect clinical outcomes and can lead to additional procedures. Variability in the harvesting technique and graft processing is thought to play an important role in fat grafting outcomes.<sup>12,13</sup> There are many techniques currently used to process lipoaspirate for fat grafting, but the most frequently used are simple decantation, mesh filtering, centrifugation, and liquid absorption (Telfa; Covidien, Mansfield, MA).<sup>12,14,15</sup> Centrifugation is commonly used and is effective at concentrating the graft by removing tumescence, blood, debris, and oil.<sup>16</sup> Removing these components diminishes the inflammatory response of the graft, reducing resorption.<sup>16</sup> The mesh and liquid absorption techniques have also become prevalent because of their relative ease of use compared to the "Coleman" centrifugation technique. The mesh technique involves placing lipoaspirate above a porous mesh and draining the aqueous portion by gravity.

Common reagents used to process adipose tissue are a tumescent solution, Ringer's lactate solution, and

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normal saline. AuraClens [active ingredient Poloxamer 188 (P188), 10mg/mL] is a detergent that removes unwanted components thus improving graft concentration and retention.<sup>17</sup> Several published studies conclude that using a filter mesh to concentrate the fat removes excess fluid and blood, thereby improving fat graft retention.<sup>18,19</sup>

Despite the ever-increasing application of fat grafting for soft tissue augmentation, the literature still lacks experimental evidence to optimize processing steps. An automated system using Ringer's lactate wash, decant, and mesh filtration system (RLDS) has been commercially produced (Revolve Advanced Adipose System; Allergan Corporation, PLC, Madison, NJ). It allows up to 350 mL of lipoaspirate to be processed with a single device.

A new P188-based system has been developed to further improve clinical outcomes. In this study, we compare the Poloxamer wash, adsorption, mesh filtration system (PWAS; AuraGen 123 Suction Lipoplasty System, AuraGen Aesthetics LLC, Weston, MA) to the RLDS system.

## METHODS

### Study Subjects

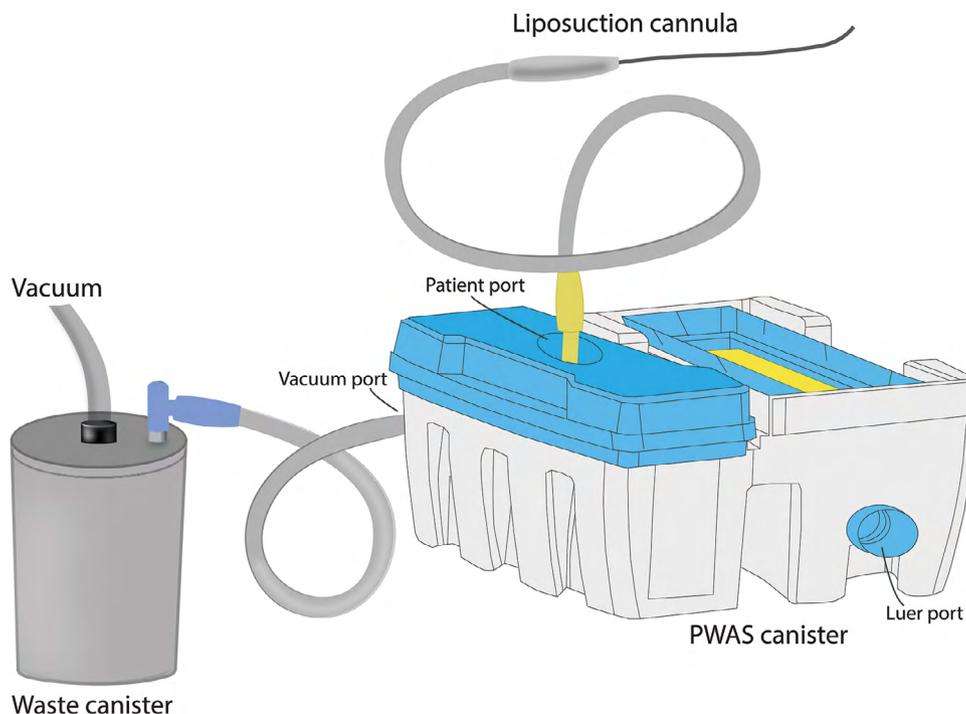
We collected 375–725 mL of discarded autologous adipose tissue from 10 patients undergoing elective liposuction. Lipoaspirate from each patient was transported to our laboratory and processed using 2 different methods: RLDS and PWAS. For both systems, 175 or 350 mL of each of the 10 lipoaspirate samples was processed following the directions on the package inserts. The experimental protocol was approved by the institutional review board of Brigham and Women's Hospital.

### PWAS

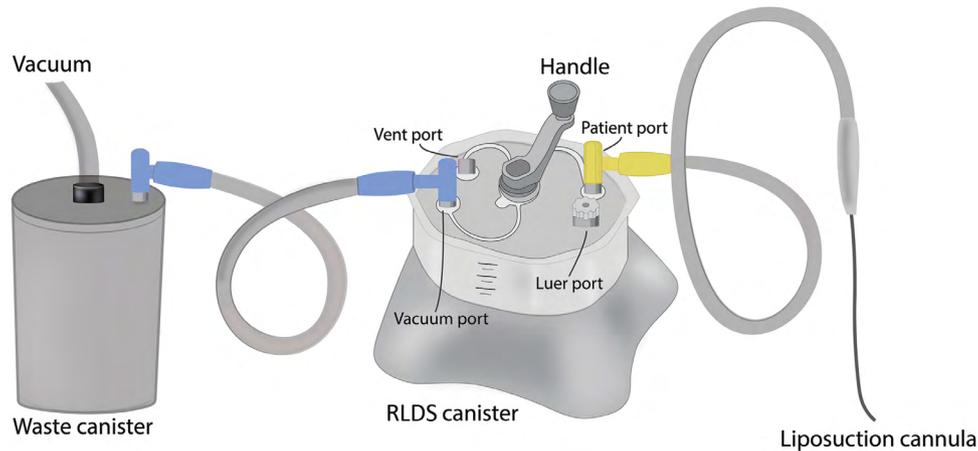
The PWAS functions as a 3-step suction lipoplasty system for (1) harvesting/collecting, (2) washing/filtering, and (3) concentrating/transferring autologous adipose tissue for fat grafting (Fig. 1).

Lipoaspirate was collected from the operating room and transferred to the laboratory where it was processed through the devices. The liposuction cannula was attached to the port on the collection chamber lid, and the lipoaspirate was suctioned into the PWAS. Once the desired amount of lipoaspirate was collected into the collection basket, the vacuum was turned off. The lid on top of the collection chamber was removed, and the drain valve was closed. The contents of 1 P188 sachet (10g of P188) was fully dissolved in 1.0L of room temperature and sterile 0.9% normal saline solution and was poured onto the adipose tissue in the collection basket (approximate ratio of P188 solution:adipose tissue was 1:1). During washing, the spatula was used to facilitate mixing. After 2 minutes of washing, the drain valve was opened and the vacuum was turned on for 30 seconds to remove the P188 solution and other fluids from the adipose tissue.

The collection basket containing the washed adipose tissue was manually transferred to the concentration chamber. In this chamber, the collection basket contacts the surrounding proprietary fluid absorption pads. During the concentration step, the spatula was used to maximize the amount of fluid removed. The concentration time was set to 3 minutes. At the end of the concentration step, a 60-mL Toomey tip syringe was inserted into the collection port of the concentration chamber and loaded with processed adipose tissue.



**Fig. 1.** PWAS system is a sterile, disposable unit for single patient use.



**Fig. 2.** RLDS is a fat-processing device in which 350 mL of adipose tissue is collected directly into a closed system.

### RLDS

The RLDS is an inline fat-processing device in which lipoaspirate is immediately harvested into the system (Fig. 2). The RLDS is composed of an external canister and an internal filter basket (200- $\mu$ m pores) that permits adipose tissue to be isolated from the tumescent liquid. The RLDS was setup in the laboratory, and the desired amount of lipoaspirate, transferred from the operating room, was collected into the system. The adipose tissue was retained in the filter basket and was then washed with an equal volume of Ringer's lactate solution whereas agitation was accomplished by rotating the paddle for  $15 \pm 5$  seconds. The fluid was aspirated by vacuum, and the washing step repeated 3 times. Following the final wash, vacuum, with the device set on maximum suction, was applied for  $60 \pm 5$  seconds. The processed adipose tissue was aspirated through the patient port with a catheter tip syringe and transferred into conical tubes for further analysis.

### Fat Volume and Concentration

After fat processing 175 or 350 mL of lipoaspirate, the washed fat was transferred into 50-mL conical tubes. A volume of 25 mL of unprocessed lipoaspirate was also collected into a conical tube as a control. Each sample was centrifuged at 3,220g for 3 minutes to separate the oil, fat, and aqueous phases. The volume of oil, fat, and aqueous layers was recorded. The fat volume was defined as the total amount of fat in the "fat layer." Fat concentration was recorded as the ratio of the volume of the fat layer to the total volume. We calculated the ratio of volume of processed fat to the volume of unprocessed fat for each system.

### Trypan Blue Dye Exclusion Test (Cell Viability)

A volume of 6 mL of processed adipose tissue was transferred into 2-mL aliquots (3 PWAS samples and 3 RLDS samples from each patient). Red Cell Lysis Buffer, 8 mL, was added (Sigma-Aldrich R7757, St. Louis, MO, USA) to each conical tube, and the tubes were placed on a rocker in a 37°C incubator for 3 minutes. The tubes were centrifuged at 1,200g for 3 minutes and 1 g of fat was transferred into

a 15-mL conical tube for each sample. Collagenase, 1 mL (Sigma-Aldrich C6885), was added to 1 g of fat and placed on a rocker in 37°C incubator. After 30 minutes, 1 mL of the cell suspension was transferred to a new conical tube. The cell suspension, 50  $\mu$ L, was transferred to an Eppendorf tube with equal parts of 0.4% trypan blue dye, thoroughly mixed, loaded into a hemocytometer and examined under a microscope. The number of dead (stained) and live cells (unstained) was manually counted, and viability was calculated using the following formula: % viable cells = (number of unstained cells  $\div$  number of total cells)  $\times$  100.

### Processing Time

Processing time (PT) was defined as the time from the start of the washing step to the end of the transfer/extraction step.

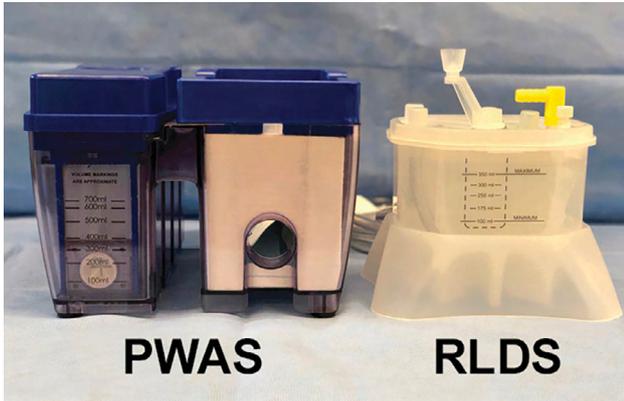
### Statistical Analysis

The mean and SD were calculated for all quantitative methods. The comparative SD of adipose tissue retention was evaluated (mean/SD) to measure predictability in each test arms. A 1-way analysis of variance followed by a post hoc test (Bonferroni) across groups was determined with  $\alpha$  set at 0.05.

## RESULTS

### Gross Observations and Fat Volume Test

The 2 systems are of different design but similar size (Fig. 3). Fat processed by the 2 techniques was photographed before centrifugation (Fig. 4). The volume of free oil in the adipose tissue collected from the PWAS was similar to that collected from RLDS (0% versus  $3\% \pm 3\%$ ;  $P = 0.38$ ) and statistically lower than unprocessed lipoaspirate (0% versus  $13\% \pm 8\%$ ;  $P < 0.001$ ; Fig. 5). Processed tissue from the PWAS had significantly higher volume fraction of adipose tissue in the "fat" layer ( $89\% \pm 3\%$ ) than both the RLDS ( $76\% \pm 10\%$ ;  $P = 0.02$ ) and unprocessed lipoaspirate ( $37\% \pm 12\%$ ;  $P < 0.001$ ). Additionally, the aqueous layer in the



**Fig. 3.** Comparison photograph of the 2 systems, PWAS and RLDS, showing differences in design and size.

fat processed with PWAS was similar to that processed with RLDS ( $11\% \pm 3\%$  versus  $20\% \pm 8\%$ ;  $P = 0.14$ ), but statistically lower than unprocessed lipoaspirate ( $51\% \pm 13\%$ ;  $P < 0.001$ ). The PWAS yielded on average 71-mL fat volume for graft processing, similar to the 57 mL from RLDS ( $P = 0.35$ ). The ratio of volume of processed fat to the volume of unprocessed fat did not differ between the PWAS and RLDS ( $23\% \pm 8\%$  versus  $17\% \pm 8\%$ ;  $P = 0.12$ ; [Table 1](#)).

**Trypan Blue Dye Exclusion Test (Cell Viability)**

Average adipocyte viability from PWAS was similar to RLDS ( $95\% \pm 5\%$  versus  $86\% \pm 11\%$ ;  $P = 0.05$ ; [Table 1](#)).

**PT**

There was no statistical difference in PT of 350 mL of lipoaspirate between the PWAS and the RLDS ( $8.4 \pm 1.1$  versus  $9.9 \pm 8.1$  minutes,  $P = 0.57$ ; [Table 1](#)).

**DISCUSSION**

Fat grafting for soft tissue defect reconstruction is a widely used treatment modality. Many surgeons have noted a loss of adipose volume over time.<sup>12</sup> Fat-processing techniques have been shown to play a role in outcome variability.<sup>20</sup> This study compares fat graft quality, volume, and concentration in a novel PWAS to the established RLDS.

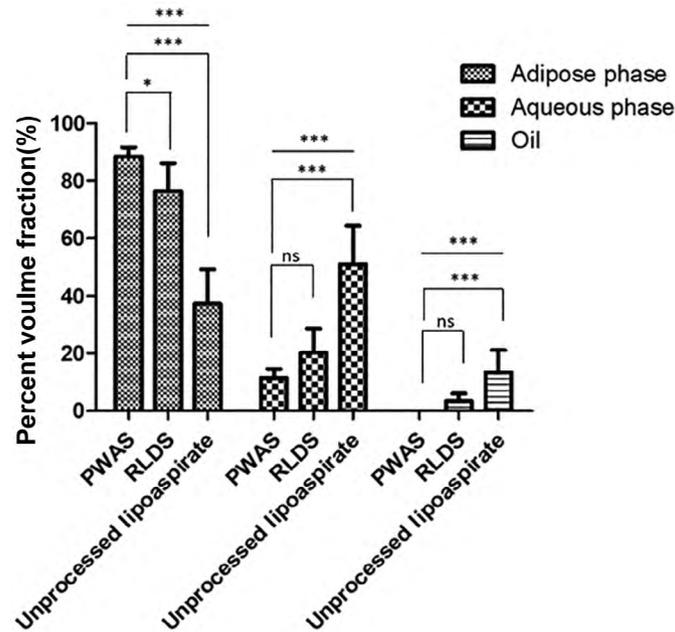
In-line collection and processing systems are gaining popularity as large volume fat grafting increases. RLDS is frequently used clinically and was first described by Ansgore et al where they compared RLDS with simple decanting and centrifugation.<sup>18</sup> They determined a statistically lower free oil phase and lower debris level in the RLDS samples.<sup>18</sup>

There are 2 main technological differences between the 2 fat-processing techniques compared in our study. The PWAS uses a P188 detergent solution to wash the lipoaspirate, whereas the RLDS uses Ringer’s lactate. P188 is a Food and Drug Administration (FDA)-approved material with an excellent safety profile and has been used in a wide variety of pharmaceutical applications as a solubilizer, wetting agent, colloidal stabilizer, and emulsifier. The second technological difference is that the RLDS uses simple decantation, whereas PWAS also incorporates fluid-absorbing pads to further remove undesired fluids before fat reinjection. The exact composition of these pads was not known for this study, but in our experience they efficiently and successfully absorbed fluid.

To determine the efficiency of the systems, we quantified the resulting components of processed lipoaspirate. The fat fraction volume from PWAS was found to be significantly greater than RLDS. Our data add to evidence from Salinas et al who demonstrated that the mesh/gauze technique was just as effective as centrifugation at removing



**Fig. 4.** Characteristic samples of lipoaspirate processed by 3 techniques: RLDS method, PWAS method, and unprocessed fat control. R = RLDS, A = PWAS, and C = unprocessed fat control.



**Fig. 5.** Composition of processed lipoaspirate. The lipoaspirate was treated by 2 techniques: RLDS method and PWAS method, as described in the “METHODS” section. The percentage of major lipoaspirate elements was evaluated. \* $P < 0.05$ ; \*\*\* $P < 0.001$ . R = RLDS, A = PWAS, and C = unprocessed fat control. ns, not significant.

undesirable components and concentrating lipoaspirate without requirement of cumbersome centrifugation.<sup>19</sup>

Trypan blue dye exclusion test demonstrated that average viability of adipocytes from PWAS was similar to RLDS ( $P = 0.05$ ). Medina et al reported that fat washed with P188 had significant improvement in graft survival in terms of weight, cell viability, DNA content, and on histology.<sup>17</sup>

The PWAS processed samples were the most concentrated samples with the highest viability, indicating that the PWAS is as effective at cleaning lipoaspirate as RLDS. A further advantage of PWAS is that it is designed to process a large volume (700 mL) of lipoaspirate in a single harvest, lowering the overall surgical time required.

It should be noted that although some studies have shown that distinct harvesting techniques result in different outcomes,<sup>20-22</sup> other studies support that the harvesting procedure does not affect future graft survival.<sup>23,24</sup> Consequently, although we have shown that both systems process lipoaspirate equally efficiently, the clinical significance of this study cannot be determined. The fat processed from these systems was not grafted to assess outcomes in terms of volume retention and fat graft survival, and, hence, warrants a future study.

**Table 1. Assessed Parameters (Average  $\pm$  SD)**

	PWAS	RLDS	P
VPF/VUF ratio (%)	23 $\pm$ 8	17 $\pm$ 8	0.12 (ns)
Live cells (%)	95 $\pm$ 5	86 $\pm$ 11	0.05 (ns)
PT (min)	8.4 $\pm$ 1.1	9.9 $\pm$ 8.1	0.57 (ns)

ns, not significant; VPF/VUF ratio, the ratio of volume of processed fat to the volume of unprocessed fat.

As this system is not yet commercially available, information on its clinical experience has not yet been reported. This bench top experiment was designed to simulate clinical use. We found both systems easy to use, but felt that PWAS was more stable on a table and easier to use in terms of removing the processed fat.

This is reported as a preclinical study and is limited by not being able to blind the 2 systems and the potential bias due to funding of the study. Future clinical trials will be needed to quantify the final results of these systems including volume retention and tissue elasticity. In clinical practice, these less labor-intensive devices may be more efficient for large-volume fat grafting procedures.

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