





Optimizing Fat Grafting Using a Hydraulic System Technique for Fat Processing: A Time and **Cost Analysis**

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Abstract

Background Many authors have researched ways to optimize fat grafting by looking for a technique that offers safe and long-term fat survival rate. To date, there is no standardized protocol. We designed a "hydraulic system technique" optimizing the relationship among the quantity of injected fat, operative time, and material cost to establish fat volume cutoffs for a single procedure.

Methods Thirty-six patients underwent fat grafting surgery and were organized into three groups according to material used: standard, "1-track," and "2-tracks" systems. The amount of harvested and grafted fat as well as material used for each procedure was collected. Operating times were recorded and statistical analysis was performed to establish the relationship with the amount of treated fat.

Results In 15 cases the standard system was used (mean treated fat 72 [30–100] mL, mean cost 4.23 ± 0.27 euros), in 11 cases the "1-track" system (mean treated fat 183.3 [120–280] mL, mean cost 7.63 ± 0.6 euros), and in 10 cases the "2-tracks" one (mean treated fat 311[220–550] mL, mean cost 12.47 \pm 1 euros). The mean time difference between the standard system and the "1-track" system is statistically significant

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Keywords

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[►] lipofilling

[►] fat washing

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Joint first authors.

Conclusion Data analysis would indicate the use of the standard system, "1-track," and "2-tracks" to treat an amount of fat $< 90 \,\text{mL}$ of fat, $90 \div 240 \,\text{mL}$ of fat, and >240 mL of fat, respectively.

Introduction

Autologous fat grafting is applicable to many fields of plastic surgery ranging from reconstructive to cosmetic surgery.^{1,2} Since the introduction of this technique in the 1990s³⁻⁵ many authors have performed research into the ways in which fat grafting can be optimized by looking for a technique that offers safe and long-term fat survival rate. To date, there is no current standardized protocol or best procedure indicating how to treat aspirated fat. Indeed, the existing techniques state that it can be purified by gravity or by centrifugation, 6,7 combined with autologous adipose-derived stem cells (ADSCs), with platelet-rich-plasma, 8,9 or with adipose-derived mesenchymal stem cells following in vitro expansion.¹⁰ Thirty years after its introduction, many authors continue to carry out studies looking for new systems that are able to optimize both time and expenditure while reducing fat handling and material waste. Therefore, we designed a "hydraulic system technique" specifically for fat grafting. The aim of the study is to evaluate the relationship among the quantity of injected fat, operative time, and the cost of disposable material to delineate a time and costeffective procedure of fat processing.

Methods

Data were retrospectively reviewed for 36 female patients who underwent fat grafting revision surgery with our technique from March to July 2019 and who had previously undergone breast reconstruction in the 5 years prior. All patients underwent breast reconstruction following either minor or major oncological surgery, which was performed by the same surgical team. Patients were identified from the theater registers and electronic logbooks (>Table 1).

We obtained informed consent from the patients and conducted our study according the Declaration of Helsinki. We identified three groups in relation to the disposable materials used: the standard group, the "1-track" group, and the "2-tracks" group (►Table 2).

The amount of harvested and grafted fat, materials used, and fat washing time data (from the start of the first syringe washing to the end of the last syringe washing for each unit of treated fat) per hydraulic system were collected (>Table 3). The unit of treated fat corresponds to 30 mL, aspirated in one 60-mL syringe and the number of syringes needed per amount of fat were recorded and evaluated. The whole standard procedure requires the following materials: two

"3-way stopcocks," two "60-mL Luer-Lock syringes," one "10mL Luer-Lock syringe," and one "extension set" (►Fig. 1).

A tumescent solution is set up and a "3-way stopcock" (stopcock A) is joined to an infusion set as well as to a 60-mL Luer-Lock syringe (syringe A), making up Module A (Fig. 1A), and then to an extension set connected to a blunt infiltration cannula. The infusion set is connected to the tumescent solution bag and the solution is aspirated from the bag to fill syringe A. The following step consists in making a small cutaneous incision to insert the cannula for infiltration while turning the valve and the solution is then gradually released by the surgeon.

Lipoaspiration is performed after approximately 10 minutes with a 3-mm blunt aspiration cannula connected to a 60-mL Luer-Lock syringe: approximately 30 mL of fat (syringe B) is collected. The tumescent solution bag is replaced with a lactate Ringer 500 mL bag. A second "3way stopcock" (stopcock B), joined to syringe B, is then added between the first stopcock (A) and the extension set. The latter serves to drive away the fluids directly into a special waste container. At this stage, Module B (Fig. 1A) is considered as functional.

Thirty milliliters of Ringer solution are aspirated from the bag with syringe A, changing the stopcock position by turning it. The solution is then pumped from syringe A into syringe B by a simple valve regulation (we open the connection between the first and the second stopcock and close the second stopcock's third valve). Fat washing is performed by the "lulling" of the system until a yellow and heterogeneous lipoaspirate is obtained. The fluid component derived from washing is now drained out through the extension set. For large amounts of fat, instead, one ("1track") (►Fig. 2) or two ("2-tracks") (►Fig. 3) "50 cm polyvinyl chloride (PVC) extension line with a 5-way manifold" could be used, replacing the 3-way stopcocks, so that up to 120 or 240 mL of lipoaspirate can be treated at the same time.

The subsequent fat injection is performed as follows: stopcock B with the loaded syringe B is disconnected and joined both to a 10-mL syringe and to the blunt infiltration cannula (Fig. 1B). In one step, using a simple valve rotation, the operator can first switch the fat from syringe B to the 10mL syringe and then to the blunt cannula for injection.

The average of the time data was calculated and a statistical linear interpolation of nonmeasurable data (Microsoft Excel Linear Prevision) according to available samples per each system was performed. The relative 95% confidence intervals (CIs) of the mean values were then calculated and

Table 1 Summary collection of clinical and technical data

Number of patients	36 females
Mean age	52 (±12.5)
Comorbidities	Breast cancer (100%), hypertension (25%), smokers (25%), diabetes (12.5%), thyroiditis (12.5%)
Body mass index 30–35	11 (30.5%)
Body mass index 25–29	25 (69.5%)
Major complications	None
Minor complications	30% slight blood suffusion of the skin of the grafting area
Treated with standard system	15 (41.6%), mean of fat treated 72 mL \pm 28.08, mean time 17.6 min, mean costs 4.23 \pm 0.27 euros
Treated with "1-track"	11 (30.5%), mean of fat treated 183.3 mL \pm 56.26, mean time 6.87 min, mean cost of 7.63 \pm 0.6 euros
Treated with "2-tracks"	10 (27.7%), mean of fat treated 311 mL \pm 103, mean time 9.3 min, mean cost of 12.47 \pm 1 euros

Note: Data grouped according to the information of major clinical interest and their correlation with the data obtained from the use of hydraulic systems.

Table 2 Hydraulic systems

Hydraulic systems	Disposable materials	Ref.	Cost (euro)	No.	Total cost (euro)
Standard	3-way stopcock	BD 9400198	0.46	2	0.92
	60 mL Luer-Lock syringe	RAYS 6400048	0.31	2	0.62
	10 mL Luer-Lock syringe	RAYS 6400128	0.09	1	0.09
	50 cm Luer-Lock extension set	DELTAMED 9400185	0.53	1	0.53
	500 mL Ringer's lactate solution	GALENICA SENESE 102322118	1.66	1	1.66
					3.82
"1-track"	50 cm PVC extension 5-way manifold	ARIES SRL IN045915	2.64	1	2.64
	60 mL Luer-Lock syringe	RAYS 6400048	0.31	5	1.55
	10 mL Luer-Lock syringe	RAYS 6400128	0.09	1	0.09
	3-way stopcock	BD 9400198	0.46	1	0.46
	50 cm Luer-Lock extension set	DELTAMED 9400185	0.53	1	0.53
	500 mL Ringer's lactate solution	GALENICA SENESE 102322118	1.66	1	1.66
					6.93
"2-tracks"	50 cm PVC extension 5-way manifold	ARIES SRL IN045915	2.64	2	5.28
	60 mL Luer-Lock syringe	RAYS 6400048	0.31	10	3.10
	10 mL Luer-Lock syringe	RAYS 6400128	0.09	1	0.09
	3-way stopcock	BD 9400198	0.46	2	0.92
	50 cm Luer-Lock extension set	DELTAMED 9400185	0.53	1	0.53
	500 mL Ringer's lactate solution	GALENICA SENESE 102322118	1.66	1	1.66
					11.58

Abbreviation: PVC, polyvinyl chloride. Note: Material, reference code, and costs.

Table 3 Age, kind of previous breast surgery, amount of harvested and grafted fat, cost, and operating time

Age (y)	Surgery	Harvested fat (mL)	Grafted fat (mL)	Materials	Cost (euro)	Time (min)	Syringes with fat
28	r Lumpectomy	30	20	Standard	3.82	4	1
35	r Lumpectomy	30	22	Standard	3.82	5	-
51	r Lumpectomy	30	19	Standard	3.82	4	-
48	rl Lumpectomy	40	30	Standard	3.82	12	2
54	r Lumpectomy	09	47	Standard + 1 "60 mL Luer-Lock syringes"	4.13	13	2
45	r Implant contracture	09	50	Standard + 1 "60 mL Luer-Lock syringes"	4.13	10	2
53	r Mastectomy + implant	08	55	Standard + 2 "60 mL Luer-Lock syringes"	4.44	17	3
70	I Mastectomy+ expander + RT	08	65	Standard + 2 "60 mL Luer-Lock syringes"	4.44	18	3
54	rl Implant contracture	08	70	Standard + 2 "60 mL Luer-lock syringes"	4.44	18	3
53	r Mastectomy + expander	06	80	Standard + 2 "60 mL Luer-Lock syringes"	4.44	25	4
54	r Quadrantectomy + RT	100	84	Standard + 2 "60 mL Luer-Lock syringes"	4.44	23	4
57	r Mastectomy + implant	100	78	Standard + 2 "60 mL Luer-Lock syringes"	4.44	25	4
61	rl Quadrantectomy + RT	100	80	Standard + 2 "60 mL Luer-Lock syringes"	4.44	23	4
52	r Mastectomy + expander	100	70	Standard + 2 "60 mL Luer-Lock syringes"	4.44	23	4
52	r Mastectomy + implant	100	70	Standard + 2 "60 mL Luer-Lock syringes"	4.44	24	4
63	/ Implant contracture	120	91	"1-track"	6.93	7	4
48	/ Latissimus dorsi flap + implant	120	100	"1-track"	6.93	7	4
55	r Mastectomy + implant	120	100	"1-track"	6.93	7	4
48	r Latissimus dorsi flap + implant	150	130	"1-track" + 1 "60 mL Luer-Lock syringes"	7.24	13	2
46	rl Quadrantectomy + RT	150	132	"1-track" + 1 "60 mL Luer-Lock syringes"	7.24	14	2
49	/ Mastectomy + implant	700	170	"1-track" + 3 "60 mL Luer-Lock syringes"	7.86	14	7
20	rl Mastectomy + implants	200	170	"1-track" + 3 "60 mL Luer-Lock syringes"	7.86	12	7
62	r Mastectomy	700	160	"1-track" + 3 "60 mL Luer-Lock syringes"	7.86	14	7
45	rl Mastectomy + implant	220	192	"1-track" + 3 "60 mL Luer-Lock syringes"	7.86	15	8
45	rl Mastectomy + implant	260	210	"1-track" + 5 "60 mL Luer-Lock syringes"	8.48	22	6
52	rl Mastectomy + implant	280	235	"1-track" + 6 "60 mL Luer-Lock syringes"	8.79	23	10
44	rl Mastectomy	220	185	"2-tracks"	11.58	8	8
25	rl Mastectomy	230	190	"2-tracks"	11.58	10	8
51	rl Mastectomy	230	185	"2-tracks"	11.58	10	8
09	/ Mastectomy	250	200	"2-tracks" + 1 "60 mL Luer-Lock syringes"	11.89	13	6
							(Continued)

Age (y) Surgery	Surgery	Harvested Grafted fat (mL)		Materials	Cost (euro)	Cost (euro) Time (min)	Syringes with fat
69	/ Mastectomy	250	215	"2-tracks" + 1 "60 mL Luer-Lock syringes" 11.89	11.89	14	6
59	$oldsymbol{r}$ Quadrantectomy $+I$ mastectomy and implant	310	280	"2-tracks" + 3 "60 mL Luer-Lock syringes" 12.51	12.51	13	11
54	$\it r$ Mastectomy $+\it l$ quadrantectomy and RT	320	280	"2-tracks" + 3 "60 mL Luer-Lock syringes" 12.51	12.51	14	11
57	$oldsymbol{r}$ Quadrantectomy $+I$ mastectomy and implant	350	295	"2-tracks" + 4 "60 mL Luer-Lock syringes" 12.82	12.82	15	12
62	/ Mastectomy + implant	400	350	"2-tracks" + 6 "60 mL Luer-Lock syringes" 13.44	13.44	15	14
44	rl Prophylactic mastectomy	550	460	"2-tracks" + 11 "60 mL Luer-Lock syringes" 14.99	14.99	22	19

Note: Data grouped for each patient, according to the type of hydraulic system materials (respectively the standard system, "1-track," and "2-tracks" ones) Abbreviations: I, left; r, right; RT, radiotherapy

recorded. The time data (and relative CIs) between the various systems for each unit of volume were compared. Whenever there was a statistically significant difference for the average time values according to the relative CIs, the lowest time value for the system selection was chosen as the cutoff.

Results

A total of 36 patients, with an average age of 52 (28–70) years, were included in this study and recorded in **Table 1** according to their main pathologies, comorbidities, body mass index, and surgical complications. Each patient underwent lipofilling as the final or intermediate step of their reconstructive procedure following breast cancer surgery (**Table 3**).

In 15 cases, with a mean age of 51 (28–70) years, the standard materials were used (\succ **Fig. 1**; \succ **Table 3**], in some cases with the addition of "60 mL Luer-Lock syringes" (\succ **Table 2**): the average amount of 72 (30–100) mL of fat was treated to obtain 56 (19–84) mL of fat to graft, with a mean cost of 4.23 \pm 0.27 euros.

In 11 cases, with a mean age of 51 (45–63) years, the "1-track" materials were utilized (**>Fig. 2**; **>Table 3**), in some cases with the addition of "60 mL Luer-Lock syringes": a mean of 183.3 (120–280) mL of fat was harvested and treated, obtaining 153.3 (91–235) mL of fat to graft, with a mean cost of 7.63 ± 0.6 euros.

In 10 cases, the "2-tracks" materials were used (\succ Fig. 3; \succ Table 3), in some cases with the addition of "60-mL Luer-Lock syringes" (\succ Table 2): an average of 311 (220–550) mL of fat was treated, obtaining a mean of 264 (185–460) mL of fat, with a cost of 12.47 \pm 1 euros.

Time data analysis (\neg **Table 4**; \neg **Fig. 4**) shows that at three fat syringes (90 mL), the mean value by the standard system is 17.66 minutes (95% CI = 16.23, 19.10); while for the "1-track" system it is 6.87 minutes (95% CI = 3.15, 10.58), hence the difference is statistically significant. The mean time difference between the "1-track" system and the "2-tracks" system becomes significant from 240 mL of fat onwards: 15 minutes for the "1-track" system versus 9.3 minutes (95% CI = 6.99, 11.67) for the "2-tracks" system.

Discussion

Studies into fat grafting infection^{11,12} and antibiotic resistance mean that surgeons perform lipofilling according to strict sterile regulations. To reduce fat contamination, fat should be manipulated as little as possible, and treated in a closed system without enzymatic elements, thus increasing the technique's costs.¹³ Our initial purpose was to use systems already present in clinical practice making it possible to construct a simple module.

Indeed, we used material available in every operating room guaranteeing the closed circuit in each phase of the procedure. Tedde's system, described by Rubino et al¹⁴ in 2014, was used as the first step of our technique (tumescent solution's infiltration) and then for fat washing. The Lull

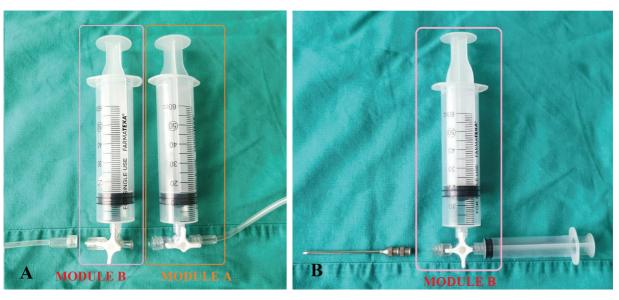


Fig. 1 The standard system. (A) Module A is joined to Module B through a "3-way stopcock." Ringer solution is introduced into the system drawn from syringe A and then transferred to syringe B. After fat-washing in syringe B, the liquids are expelled through the extension set. (B) Module B is reused to set up the fat injection.



Fig. 2 "1-track" system. "50 cm polyvinyl chloride (PVC) extension line with a 5-way manifold" replacing the "3-way stopcock." Here, up to 5 syringes of fat can be simultaneously processed.

System by Morselli et al, 15 represented the main principle for our fat washing, the second step of the standard system (Fig. 1A) and our Module B. Finally, the "stopcock technique" described by Paolini et al¹⁶ was used to perform fat infiltration through a closed and manageable circuit. Our new contribution is of most relevance when higher quantities of fat are needed.

Time analysis reveals at which amount of fat processing the use of the hydraulic system results in significantly shorter mean time. Data from -Table 4 show that for 1 or 2 fat syringes (30-60 mL of fat) there is not any significant difference between the standard and the "1-track" system, whereas from three syringes onwards (treated fat from 90 mL and above), the difference is statistically significant and, therefore, the use of the "1-track" system is more time efficient than the standard system (standard system,

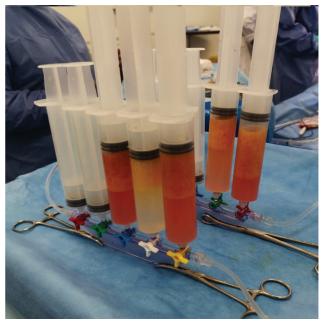


Fig. 3 "2-tracks" system. Intraoperative photo of two "50 cm polyvinyl chloride (PVC) extension lines with 5-way manifolds" working in parallel.

17.66 minutes [95% CI = 16.23, 19.10]; "1-track" system, 6.87 minutes [95% CI = 3.15, 10.58]). The first cutoff was established at 90 mL for the use of the "1-track" system. For this amount of treated fat there is a cost difference of 2.49 euros between the two systems (>Table 4), which we believe to be suitable when considering the 10 minutes saved.

From 90 to 210 mL of treated fat there is no statistically significant difference between the "1-track" and "2-tracks" systems. The difference between the "1-track" and "2tracks" systems became significant from 240 mL (8

Table 4 Values of the treated fat with relative average times per procedure, 95% confidence intervals (CI) and related costs

		Standard system		"1-track" system		"2-tracks" system	
No. of syringes	Fat (mL)	Mean time (min)	Cost (euro)	Mean time (min) CI	Cost (euro)	Mean time (min)	Cost (euro)
1	30	4.33333 2.90-5.77	3.82		6.93		
2	09	11.66667 7.87–15.46	4.13	5.17 1.44-8.9	6.93		
3	06	17.6667 16.23–19.10	4.44	6.87 3.15–10.58	6.93		
4	120	23.83333 22.80–24.87	4.44	7	6.93		
5	150	30.336391 29.42–31.25	4.44	13.5 7.15–19.85	7.24	8.7 6.11–11.33	11.58
9	180	36.771962 35.83–37.72	4.44	13.3 /	7.86	9.5 6.96–12.03	11.58
	210	43.207533 42.24–44.18	4.44	13.33333 10.46-16.20	7.86	10.2 7.8–12.7	11.58
8	240	49.643105 48.64–50.64	4.44	15	7.86	9.3 6.99–11.67	11.58
6	270	56.078676 55.05–57.10	4.44	- 23	8.48	13.5 7.15–19.85	11.89
10	300	62.514247 61.46–63.57	4.44	22	8.79	13.5	11.89
11	330	68.949819 67.87–70.03	4.44	24.176071 19.48–28.87	8.79	13.5 7.15–19.85	12.51
12	360	75.38539 74.28–76.49	4.44	26.525058 21.81–31.24	8.79	15 -	12.82
13	390	81.820961 80.69–82.95	4.44	28.952345 24.21–33.70	8.79	15 -	13.13
14	420	88.256532 87.10–89.41	4.44	31.379631 26.61–36.15	8.79	15 -	13.44
15	450	94.692104 93.51–95.87	4.44	33.572019 28.78–38.36	8.79	16 -	13.75
16	480	101.12768 99.93–102.33	4.44	35.999306 31.18–40.82	8.79	18	14.06
17	510	107.56325 106.34–108.79	4.44	38.348293 33.50–43.19	8.79	20	14.37
18	540	113.99882 112.75–115.25	4.44	40.775579 35.90–45.65	8.79	21	14.68
19	570	119.14727 117.88–120.41	4.44	42.889667 37.99–47.78	8.79	22	14.99

Note: Values of the treated fat with relative average times per procedure and 95% confidence intervals. All values obtained by linear interpolation are highlighted in gray. The time cutoffs which are useful for defining the hydraulic system of choice are highlighted in green. For each quantity of fat to be purified, both the average time and the planned expenditure for each of the three systems is reported.

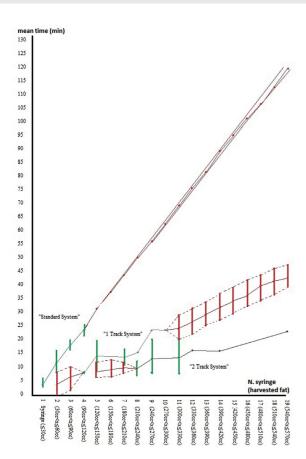


Fig. 4 Time data analysis diagram and related systems. On the x-axis, the amount of fat contained in a progressive number of syringes is reported $(30 \, \text{mL} = 1 \, \text{syringe}; 60 \, \text{mL} = 2 \, \text{syringes}; etc.)$, while on the y-axis, the time in minutes is reported. The blue lines represent the trend of the average times of the procedures for number of syringes needed, in green the relative 95% confidence interval (CI). The red lines, instead, represent the calculated predictions, with relative CI. For fat values treated with 2 syringes (up to 90 mL), the CI of the standard system and "1-track" are not overlapped. For fat values treated with 8 syringes ($> 210 \, \text{mL}$), the average time with the "1-track" is outside the CI of the "2-tracks" system.

syringes) upwards, meaning that from that point the "2-tracks" system is more time efficient than the "1-track" system, on the basis of our data. Hence, we set the second cutoff for the choice of the "2-tracks" system ("1-track," 15 minutes; "2-tracks," 9.3 minutes [95% CI = 6.99, 11.67]) (**Fig. 4**). For this amount of treated fat there is a cost difference of only 7.14 euros which saves around 6 minutes (**Table 4**). The increase in treated fat leads to further savings with the "2-tracks" system. Indeed, these systems make it possible for us to adapt materials and costs to clinical necessity (**Table 4**).

The staminality and the reabsorption of grafted fat have been widely debated for years. ^{7–10,17} Gupta et al¹⁸ in a noteworthy review in 2015, stated that literature was insufficient to clearly establish any fat graft processing method as superior to another one in long-term retention of transplanted graft volume. He stated that decanted fat graft contains the highest number of contaminants and the highest number of viable adipocytes while centrifugated fat graft contains the lowest amounts of the former. Rigotti et al¹⁰

demonstrated that the addition of platelets-rich plasma did not have significant advantages for regeneration over the use of expanded ADSCs or stromal vascular fraction (SVF)-enriched fat in skin rejuvenation. Therefore, the graft should be gently washed and/or contaminates should be further removed through centrifugation. Recently Morselli et al^{15,19} and Zhu et al²⁰ reported two closed systems for fat washing with decantation and centrifugation, showing that their procedures achieved the best balance between purification and amount of stem cells.

Since our study is an evolution of the fat washing technique proposed by Morselli et al, it was deemed unnecessary to demonstrate the quality and survival rate of the transferred fat. Indeed, Morselli et al^{15,19} has already outlined that his technique rendered good fat survival rates. Decantation caused no harm to mature adipocytes and preserved an elevated amount of ADSCs among all the SVF. The endpoints on which he focused were chosen to assess the in vitro regenerative potential of the processed tissue. He showed the highest amount of SVF cells isolated with his technique in terms of decantation and centrifugation samples, with a difference of around four and sixfold higher, respectively.

Furthermore, automated closed-system isolation devices allow clinicians to isolate a patient's cells and readminister the cells back to the patient within the same surgery. Ambient air exposure and transfer between syringes significantly decreased viability. New methods for fat washing in a closed system have been proposed by different companies. However, some of these are expensive and time consuming, in fact, Rodriguez et al²⁴ showed the possibility of treating 250 mL of fat in 110 minutes with a cost of 250 dollars with their technique.

Despite its simplicity, in our experience, it is important to note that our technique needs a certain level of practical training during its first uses. Moreover, we do not recommend fat transfer through the stopcocks before washing as this operation could result in the pressure being too high and an obstruction of the system. Disposable materials are commonly available in any operating room but, should "a 50-cm PVC extension 5-way manifold" be unavailable, several "3-way stopcocks" in a series can be used, despite system stability possibly being reduced. Another limitation of our study consists in having obtained some data through linear interpolation. However, in this case, the use of CIs allowed us to simulate the forecast more efficiently.

The costs of the hydraulic systems in our study range from 3.82 to 14.99 euros and we treated up to 550 mL of fat in less than 30 minutes.

Conclusion

Data analysis would indicate the use of the standard system, "1-track," and "2-tracks" to treat an amount of fat $< 90 \, \text{mL}$ of fat, $90 \div 240 \, \text{mL}$ of fat, and $\geq 240 \, \text{mL}$ of fat, respectively. Our technique is straightforward and requires a very low-cost device, particularly since

expensive lipofilling machines may not be available in many countries. The hydraulic systems guarantee time saving and contribute to saving resources in a time of international spending reviews.

Ethical Approval

The study was approved by the University of Salerno (IRB No. ORSA185920) and performed in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained.

Conflict of Interest None declared.

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