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ARTICLE

Mechanical emulsification of lipoaspirate by different Luer-Lok connector changes the viability of adipose derived stem cells in Nanofat

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ABSTRACT

Nanofat grafting is a fat transfer procedure that uses a thin needle to smooth out wrinkles, thereby achieving the goal of skin rejuvenation. The Luer-Lok connector is one of the most common methods for obtaining Nanofat. In the present study, we compared three different Luer-Lok connectors (2.0 mm, 1.5 mm and 1.1 mm in diameter) in terms of their impact on the viability of adipose-derived stem cells (ADSCs) to determine the optimal size of the connector for efficient Nanofat grafting. We observed that a smaller diameter of the Luer-Lok connector created a higher mechanical shear force, which broke more fat cells during the emulsifying procedure, thereby reducing the viability of ADSCs from the stromal vascular fraction (SVF). Nanofat obtained from the 2-mm Luer-Lok connector had a better effect on skin rejuvenation than the 1.5-mm and 1.1-mm connectors. Therefore, this study presents an advance in the simple procedure of preparing Nanofat based on a previous technique and provides evidence that a procedure associated with less trauma may be a better choice.

Abbreviations: SVF: stromal vascular cell fraction; LA: lipoaspirate; ADSCs: adipose derived stem cells; VEGF: vascular endothelial growth factor

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KEYWORDS
Nanofat grafting; adipose derived stem cells; Luer-Lok connector; skin rejuvenation

Background

Autologous fat grafting has become a popular method to restore soft tissue volume since it was first reported by Coleman in the early twentieth century [1]. Studies have demonstrated that the stromal vascular fraction (SVF) of adipose tissue represents a rich component of regenerative precursor cells with proangiogenic capabilities [2,3]. The term 'Nanofat grafting' was first used by Tonnard et al., and constitutes a simple procedure to obtain the SVF for skin rejuvenation through intradermal and subdermal injections [4].

Nanofat grafting has been widely utilized as a superficial injection to improve skin quality in delicate areas [4]. Nanofat can be acquired by repeatedly shuffling lipoaspirate (LA) between two interconnected syringes. The extracted emulsion is filtered and then injected into appropriate anatomic areas using a small-bore needle. It has been confirmed that this technique is suitable for obtaining good clinical results, implying that cultured adipose-derived stem cells (ADSCs) derived from Nanofat are capable of adipocyte differentiation [5]. Many procedures for mechanically micronizing LA have been reported; one of the most common methods is the Luer-Lok connector [6]. By shifting between two 10-ml syringes that are connected to each other, large chunks of fat tissue can be shredded into smaller sizes while the ADSCs remain intact. However, Luer-Lok connectors are manufactured in different sizes and shapes. Whether the different sizes of Luer-Lok connectors produce the same shear force, yield the same number of ADSCs and/or change the surgical procedure and the final healing effect remains unknown. In this study, by comparing three different sizes of Luer-Lok connectors, we identified a range of Luer-Lok connector diameters that yielded different viabilities of ADSCs. More importantly, our data showed promising results for skin rejuvenation.

Materials and methods

Subjects

A total of 27 patients (ages 20–55 years) were enrolled in this study from December 2017 to October 2019. We obtained signed informed consent from all patients to perform the LA according to clinical procedures. All research protocols were approved by the ethical committee of Zhongshan Hospital, Xiamen University.

Fat grafting method and sample preparation

Fat tissue was harvested either from the abdominal region or front-lateral thigh region through a multiport 3-mm cannula with side holes (diameter, 2.5 mm) under low negative pressure through a handheld syringe aspiration according to the standard surgery procedure [7]. Approximately, 60 ml of standard LA from each subject was kept in separate sterile centrifuge tubes. The LA was centrifuged at 1200×*g* for 3 min. After removing the bottom liquid and top oil layer, one portion was taken as the unshredded fat sample. Three more equal portions of the same LA were used to prepare the 2.0-mm Luer-Lok connector-shredded fat, 1.5-mm Luer-Lok connector-shredded fat and 1.1-mm Luer-Lok connector-shredded fat, respectively (Figure 1(A)). The

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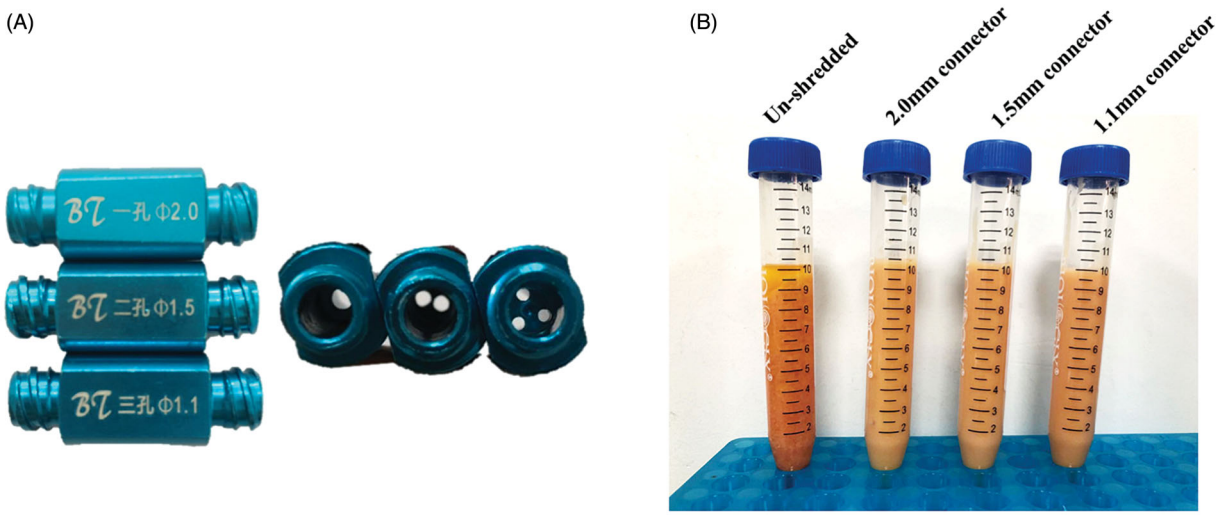

Figure 1. Extraction of fat samples using different Luer-Lok connectors. (A) The three types of Luer-Lok connectors from top to bottom and left to right were 2.0 mm, 1.5 mm and 1.1 mm in diameter. The 2.0-mm connector had one hole, the 1.5-mm connector had two holes and the 1.1-mm connector had three holes. (B) The centrifuge tubes containing equal volumes of fat samples from left to right were un-shredded fat, 2.0-mm Luer-Lok connector-shredded fat, 1.5-mm Luer-Lok connector-shredded fat and 1.1-mm Luer-Lok connector-shredded fat. All the emulsified fat samples had a homogeneous, white-colored appearance.

samples were mechanically emulsified by shifting between two 10-ml syringes, which were connected to each other by Luer-Lok connectors of specific sizes. After 30 passages in 2 min, the solid fat was liquefied and took on a white-colored appearance. Then, the fatty emulsions were subjected to either cell culture or clinical procedure.

Oil Red O and Trypan Blue staining

For Oil Red O staining, the fat samples were spread on a glass slide and placed in a 60 °C dryer for 30 min. Then, the samples were rinsed with 60% isopropanol and immersed in Oil Red O staining solution (Solarbio, Beijing, China) for 60 min. After rinsing with water, the fat samples were subjected to observation using a microscope (IX51; Olympus, Tokyo, Japan). For Trypan Blue staining, the emulsified fat from the four groups was stained using 0.4% Trypan Blue Solution (diluted 1:9 using the mixture of emulsified fat) (Solarbio, Beijing, China). The number of Trypan Blue-positive cells was observed and photographed for 3 min under a microscope (IX51; Olympus, Tokyo, Japan).

Crystal violet staining

Briefly, cells were cultured in a 60-mm plate for seven days before crystal violet staining. The cells were immediately fixed with cold methanol, and crystal violet staining solution (Solarbio, Beijing, China) was added to cover the whole plate. After incubation at 25 °C for 10 min, the cells were rinsed with water and subjected to observation.

ADSC isolation and culture

Fat samples were digested with 0.1% collagenase I (Sigma-Aldrich, St. Louis, MO) and incubated at 37 °C for 40 min until the mixture was cloudy. An equal volume of control medium (Dulbecco's Modified Eagle medium (DMEM); Hyclone, Logan, UT) with 10% fetal bovine serum (FBS, 10099-141, Gibco, Grand Island, NY) was added to stop the digestion reaction. After standing for 5 min, the top layer containing oil was aspirated and filtered through a 100-μm Falcon cell strainer (Corning, NY). The samples were centrifuged at 400×*g* for 3 min, and the cell pellets were resuspended in red blood cell lysis buffer (Tiangen, Beijing, China). After 3 min of reaction time, a control medium was added to stop the reaction. Next, the cells were centrifuged again and resuspended in DMEM with low glucose (Hyclone, Logan, UT) supplemented with 10% FBS and 1% penicillin/streptomycin (Hyclone, Logan, UT) and kept in a CO_2 incubator.

Flow cytometry

Flow cytometry was performed on ADSCs obtained from different Nanofat procedures. The antibodies were monoclonal anti-human against HLA-ABC-PE, STRO-1-PE, CD45-FITC and HLA-DR-PE (Invitrogen, Carlsbad, CA). In brief, the cells were digested using trypsin and washed once in cold phosphate-buffered saline (PBS) containing 1% FBS. Then, a volume of 100 μl cell suspension (approximately 5×10^5 cells) was aliquoted per tube and incubated with monoclonal antibodies at 4 °C in darkness for 30 min. At least 10,000 events for each antibody were acquired on a Beckman Coulter Gallios flow cytometer using Kaluza Analysis software (Beckman Coulter, Brea, CA). Positive cell populations (percentages) are presented as the *x*-means ± standard errors (SEs) obtained from three different patients.

Cell proliferation curve

Cells were incubated in 96-well microplates with 100 μl of medium for 24 h. Then, 10 μl of Cell Counting Kit-8 (CCK-8) solution (Dojindo Laboratories, Kumamoto, Japan) was added to the first row of the 96-well plate. After incubation for 1 h, the absorbance was measured at 450 nm. Then, the cells were put back to the incubator, and the absorbance was detected on days 2, 3 and 4. Results were obtained from three independent experiments.

SAβ-galactosidase staining

The positive blue staining of β-galactosidase is commonly used as a biomarker of cellular senescence. To detect SAβ-gal staining, cells were cultured in six-well plates on the day before the experiment. The cells were washed with PBS, fixed for 3–5 min with 3% formaldehyde, and washed again with PBS. The cells were then incubated overnight at 37 °C in a CO_2-free atmosphere with

freshly prepared SAβ-gal stain solution (1 mg/ml X-gal; 40 mM citric acid/sodium phosphate, pH 6.0; 5 mM potassium ferrocyanide; 5 mM potassium ferricyanide; 150 mM NaCl; and 2 mM $MgCl_2$). Positive staining was evaluated after 12–14 h. Blue staining-positive cells were scored under a phase-contrast microscope (CPH-200, Techlab, Shenzhen, China). Five random fields were taken from each sample.

Clinical procedure

We used 2.0-mm, 1.5-mm and 1.1-mm Luer-Lok connectors to shred fat in the clinical procedure. After emulsification, the fatty liquid was injected under dark eye areas, filling lines or the glabella using a 1 ml syringe with a 30 G needle into the subdermal layer for rejuvenation. Post-treatment results were evaluated half a year later. Briefly, at 6 months post-treatment, analysis of preoperative and postoperative photographs was used to evaluate the defect correction and skin quality improvement. Two methods for the outcome evaluation were used, including doctor evaluation and patient self-evaluation. Both evaluation methods were based on a scale of six scores (excellent, 5; good, 4; discreet, 3; enough, 2; poor, 1; inadequate, 0). The clinical features of these subjects are presented in Table 1.

Statistical analysis

All data are expressed as the means ± standard deviations (SDs) of three independent experiments. Data analysis was performed using SPSS 25.0 (IBM, Armonk, NY) and GraphPad Prism 7.0 software (GraphPad Software, San Diego, CA) with a two-tailed Student's *t*-test and two-way analysis of variance (ANOVA). Statistical analysis of outcome evaluation was performed using GraphPad Prism 7.0 software (GraphPad Software, La Jolla, CA) using chi-squared tests. All comparisons were made relative to the control group or positive control group, and significant differences were indicated by $^{*}p<.05$.

Results

Smaller diameter of the Luer-Lok connector creates more mechanical shear force, along with more broken fat cells during the emulsifying procedure

One of the most important steps in preparing Nanofat was to mechanically emulsify LA by shifting between two 10-ml syringes connected to each other through a Luer-Lok connector. Under the same fluid velocity with the same type of fluid, the shear force magnitude was determined by the wall shear stress (τ_w), which was calculated as follows [5]:

$$\tau_w = \frac{4\mu Q}{\pi R^3}$$

where the larger the radius of the Luer-Lok connector, the smaller the wall shear stress, and vice-versa. Connectors with diameters of 1.1, 1.5 and 2.0 mm were used to emulsify the LA. All samples were shredded for a total of 30 passages over 2 min. According to the equation above, the 1.1-mm connector created the highest level of shear force compared to the 1.5-mm and 2.0-mm connectors, while the 2.0-mm connector created the lowest level of shear force (Figure 1(B)). Subsequently, the fatty emulsions were subjected to Oil Red O staining. The results showed that adipose lobes and lobules were loosened, and connective tissue septa were damaged to various extents during treatment with different connector sizes. More loosened adipose lobules and damaged connective tissue septa appeared in the 1.1-mm connector group relative to the 1.5-mm and 2.0-mm connector groups (Figures 1(B) and 2(A)), the fourth image vs. the second and third images. Next, we determined the number of damaged cells in the aforementioned four groups. Based on Trypan Blue staining, we found that there were a few damaged cells (Trypan Blue-positive cells) in emulsified fat from the un-shredded group while the number of Trypan Blue-positive cells increased continuously with the reduction in the size of the connector. More damaged cells appeared in the 1.5- and 1.1-mm Luer-Lok connector groups

Table 1. Case summary.

Size of Luer-Lok connector (mm)	Gender	Age	Donor site	Area involved	Nano fat volume (ml)	Doctor evaluation	Patient evaluation
2.0	Female	23	Front-lateral thigh	Lower eyelid and tear trough	4	5	4
2.0	Female	20	Front-lateral thigh	Forearm scar tissue	2	4	4
2.0	Male	42	Lower abdomen	Glabella	2.5	5	5
2.0	Female	27	Lower abdomen	Lower eyelid	4	4	3
2.0	Female	24	Front-lateral thigh	Glabella	2	5	4
2.0	Male	36	Lower abdomen	Lower eyelid	4	3	4
2.0	Female	27	Lower abdomen	Glabella	3	5	5
2.0	Female	26	Front-lateral thigh	Lower eyelid	4	4	5
2.0	Female	38	Lower abdomen	Cheeks	4	4	5
2.0	Male	48	Front-lateral thigh	Full face wrinkle	4	3	3
2.0	Female	28	Lower abdomen	Cheeks	4	4	4
2.0	Female	31	Lower abdomen	Left lower eyelid	2	3	4
1.5	Female	33	Front-lateral thigh	Lower eyelid	4	3	2
1.5	Female	28	Lower abdomen	Lower eyelid	4	2	1
1.5	Female	31	Lower abdomen	Lower eyelid	4	2	2
1.5	Female	35	Front-lateral thigh	Glabella	2.5	3	2
1.5	Female	55	Front-lateral thigh	Full face	4	2	2
1.5	Female	38	Lower abdomen	Full face	4	2	3
1.5	Female	36	Lower abdomen	Full face	4	3	3
1.1	Female	24	Lower abdomen	Lower eyelid	4	1	0
1.1	Female	32	Front-lateral thigh	Lower eyelid	4	2	1
1.1	Female	36	Lower abdomen	Glabella	2.5	1	1
1.1	Female	30	Front-lateral thigh	Lower eyelid	4	1	0
1.1	Female	29	Lower abdomen	Lower eyelid	4	0	1
1.1	Male	25	Front-lateral thigh	Left lower lip scar	2.5	1	2
1.1	Male	24	Front-lateral thigh	Right lower eyelid	2.5	1	1
1.1	Female	32	Lower abdomen	Left forehead scar	2.5	1	0

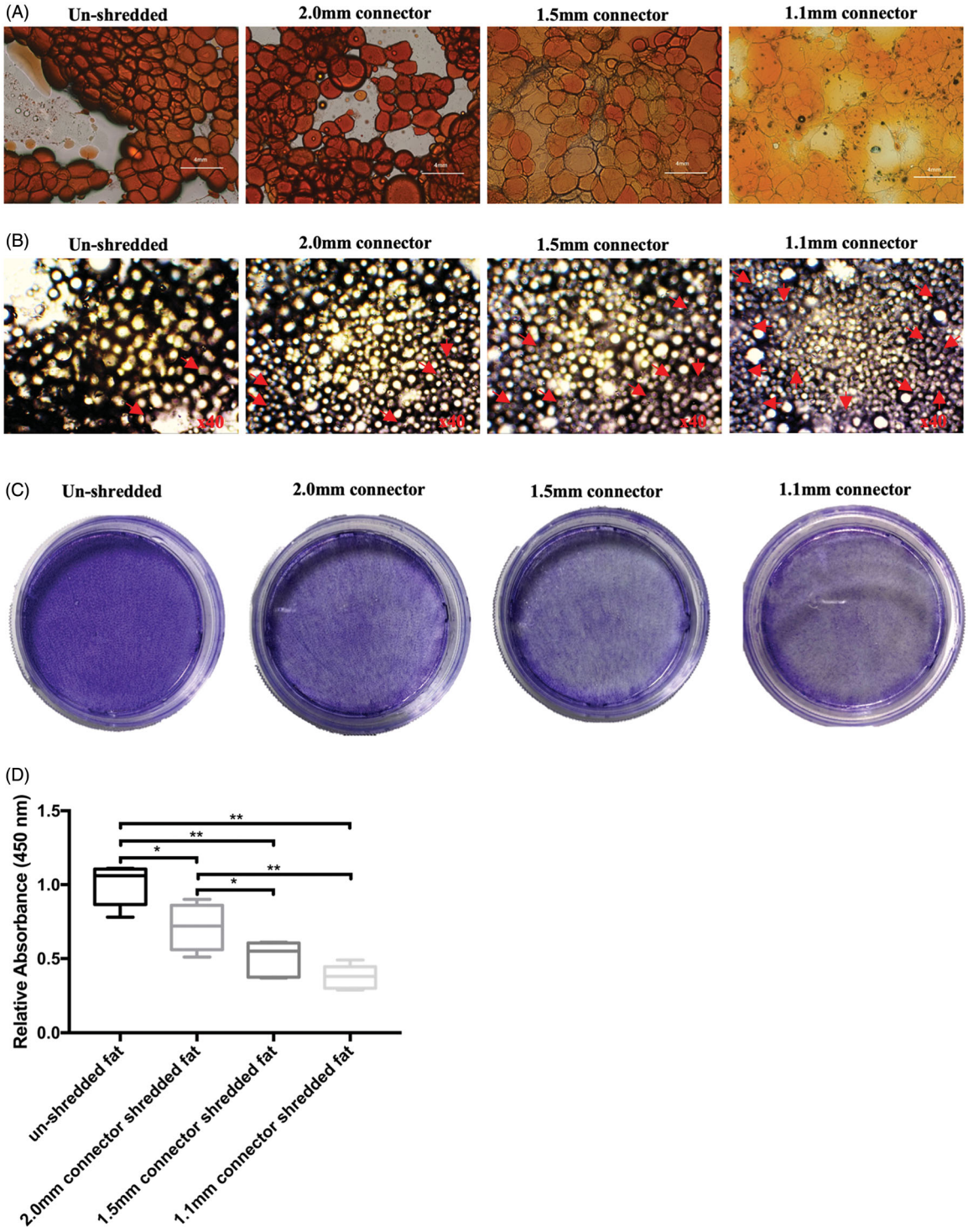

Figure 2. Effects of the Luer-Lok connectors on fat cells and adipose-derived stem cells (ADSCs) from Nanofat. (A) Oil Red O staining of fat tissue obtained from un-shredded fat, 2.0-mm Luer-Lok connector-shredded fat, 1.5-mm Luer-Lok connector-shredded fat and 1.1-mm Luer-Lok connector-shredded fat. Adipocyte clusters are divided into lobes and lobules by connective tissue septa of variable density. (B) Trypan Blue staining assay in un-shredded fat and the 2.0-, 1.5- and 1.1-mm Luer-Lok connector-shredded fat groups. Blue (red arrow): Trypan Blue-positive cells (damaged). (C) Crystal violet staining in ADSCs from un-shredded fat, 2.0-mm Luer-Lok connector-shredded fat, 1.5-mm Luer-Lok connector-shredded fat and 1.1-mm Luer-Lok connector-shredded fat. (D) Relative absorbance of crystal violet staining in the figure of panel C. $^{*}p < .05$; $^{**}p < .01$.

compared to the 2.0-mm Luer-Lok connector group (Figure 2(B)). Additionally, during the 7-day growth of cultured ADSCs, crystal violet staining was employed to investigate whether mechanical shear force would affect ADSC yield. All samples were digested and incubated under the same conditions. After incubation for 24 h, we observed a decline in the number of ADSCs in the 2.0-mm Luer-Lok connector group and a robust decrease in the 1.5- and 1.1-mm Luer-Lok connector groups (Figure 2(C,D)). However, the number of cells was visibly greater in the un-shredded fat and 2.0-mm Luer-Lok connector-shredded fat

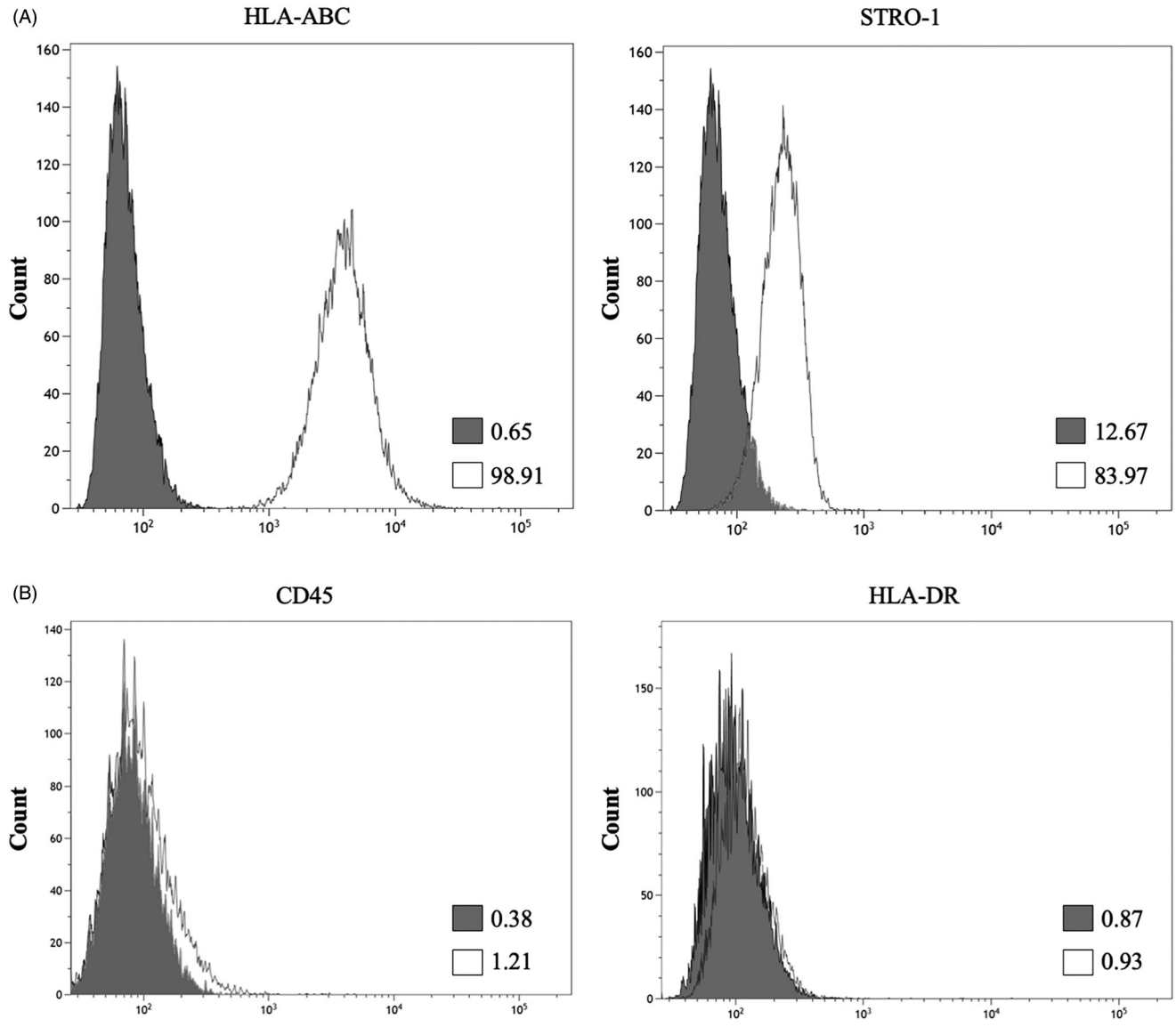

Figure 3. Immunophenotype of ADSCs. (A) Cells were analyzed by flow cytometry using positive markers of ADSCs, including HLA-ABC- and STRO-1. (B) Cells were analyzed by flow cytometry using negative markers of ADSCs, such as CD45 and HLA-DR. The shaded curves represent the isotype controls, and the open curve represents the stem cell sample. The shifting of the open curve indicates positivity of the sample for positive biomarkers ($n = 3$).

compared to the other two groups (Figure 2(C,D)). These findings suggest that a smaller diameter of the Luer-Lok connector yields fewer ADSCs.

ADSCs in different Luer-Lok connector-shredded fat samples have similar biological properties

Since fat cells were damaged during the emulsifying process, we wanted to determine if the biological characteristics of ADSCs changed as well. The immunophenotype of ADSCs was detected by flow cytometry. The results showed that there was no immunotype difference among the ADSCs derived from the four groups. As shown in Figure 3, ADSCs isolated using a 2-mm Luer-Lok connector were labeled with positive markers, such as HLA-ABC and STRO-1 (Figure 3(A)), but did not express negative markers, such as CD45 and HLA-DR [8,9] (Figure 3(B)). In addition, both Nanofat and un-shredded fat presented the same cluster of differentiation markers and shared similar positive cell percentages. These results suggest that ADSCs from different Nanofat may possess the same regenerative capacity.

Higher mechanical shear force yields lower ADSC viability

To explore whether the Luer-Lok connector could damage ADSCs, we determined the growth ability of ADSCs in the four groups by the CCK-8 assay. As shown in Figure 4(A), the number of active ADSCs obtained from shredded fat using 1.5-mm and 1.1-mm Luer-Lok connectors was notably lower than that of the 2-mm Luer-Lok connector (Figure 4(A)). In contrast to the cells from un-shredded fat, there was no significant change in cell viability in the 2-mm Luer-Lok connector group (Figure 4(A)). Even though the growth curve was similar in all four groups, cells from the 2-mm Luer-Lok connector group showed higher viability than those of the 1.5-mm and 1.1-mm Luer-Lok connector groups (Figure 4(A)). We then detected the activity of β-galactosidase in different groups, which is often used to assess cell aging [10]. The results showed that cells from un-shredded fat had a greater aging cell population than the other three groups (Figure 4(B)). Although un-shredded fat had a slightly higher number of β-galactosidase blue staining-positive cells than the other three Nanofat groups ($p < .01$), the average SAβ-gal % for both Nanofat and un-

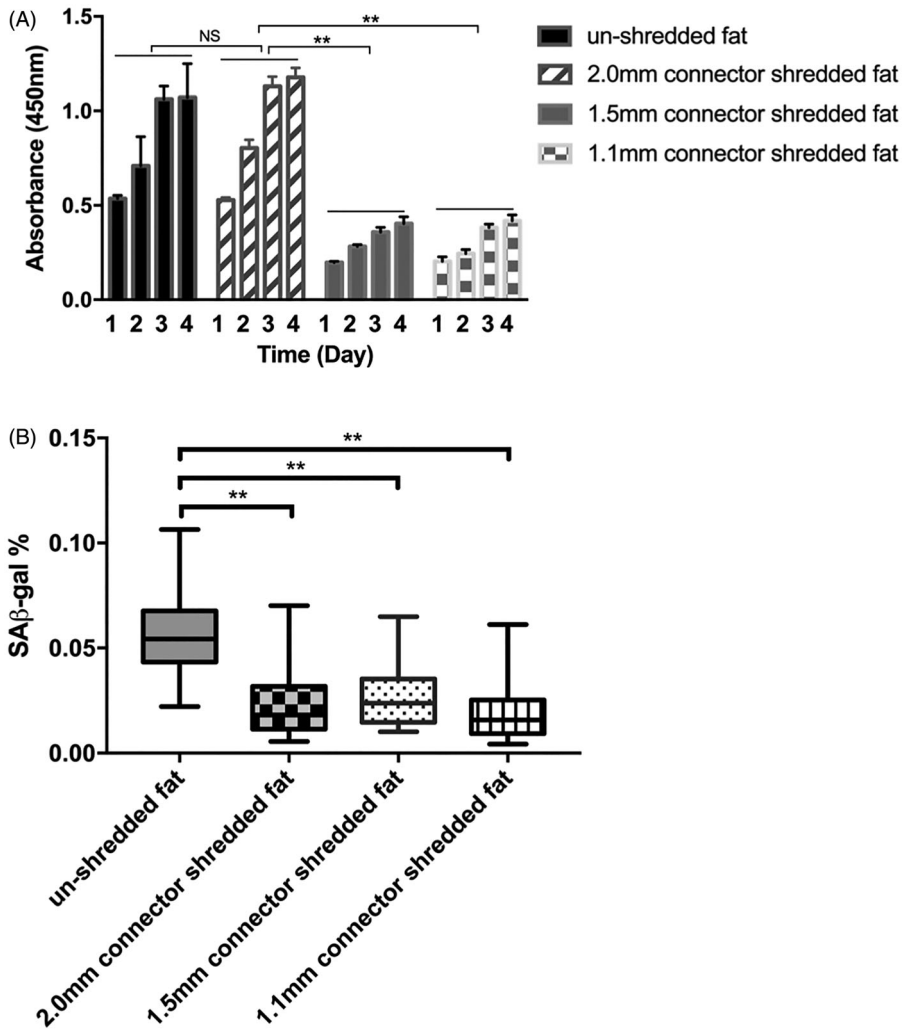

Figure 4. Viability of ADSCs extracted using different Luer-Lok connectors. (A) Cell proliferation was measured using the Cell Counting Kit-8 (CCK-8) assay. ADSCs from all four groups were grown and maintained for five days. (B) Senescence assay using senescence-associated β-galactosidase (SAβ-gal) staining in the four cell groups. Positively stained cells were manually scored under a microscope. SAβ-gal %=number of blue stained cells/total number of cells counted)×100%. Results were obtained from three independent experiments. $^{**}p < .01$.

shredded fat was less than 0.1%, which indicated that a small difference in the percentage of aging cells between these groups probably had no impact on their growth and viability (Figure 4(B)). These results suggest that mechanical shear force not only damages fat cells but also ADSC viability. Thus, the suitable size of Luer-Lok connector diameters for preparing Nanofat might be above 1.5 mm.

Fat emulsions prepared with less mechanical shear force have a better rejuvenation effect

Twenty-seven patients ranging from 20 to 55 years were treated with Nanofat prepared using 2.0-mm, 1.5-mm and 1.1-mm Luer-Lok connectors (Table 1). After injection with Nanofat from different Luer-Lok connectors, all patients were monitored for 6 months. During the follow-up period, no major complications, such as cyst formation, uneven appearance and infection, were observed. However, mild swelling and redness were noted during the first 10–14 days after treatment. Of note, once treated with Nanofat from the 2-mm Luer-Lok connector for 6 months, the fine lines in glabella were completely filled and the skin quality was significantly improved (Figure 5(A), the image below vs. the image above). Another case study revealed that the injection of Nanofat from the 2-mm connector notably reduced lower eyelid pigmentation and corrected fine lines after 6 months of treatment (Figure 5(B), images below vs. above). Following the use of Nanofat derived from the 1.5-mm Luer-Lok connector, the lower eyelid skin was moderately improved (Figure 5(C), the image below vs. the image above). However, there was no significant therapeutic effect from treatment with Nanofat from the 1.1-mm Luer-Lok connector (Figure 5(D), the image below vs. the image above). We were also able to determine the differences in therapeutic effect between these groups using outcome evaluations from doctors and all patients. As shown in Table 2, the average doctor and patient self-evaluation scores were 4.083 ± 0.2289 and 4.167 ± 0.2072 in the 2-mm Luer-Lok connector group, 2.429 ± 0.202 and 2.143 ± 0.2608 in the 1.5-mm group and 1.00 ± 0.189 and 0.75 ± 0.25 in the 1.1-mm group. The average evaluation scores were significantly lower in the 1.5- and 1.1-mm connector groups than in the 2.0-mm connector group. Thus, the LA obtained using a slightly larger mechanical shear force (2-mm connector) might have a better rejuvenation effect than those obtained with less mechanical shear forces (1.5- and 1.1-mm connectors).

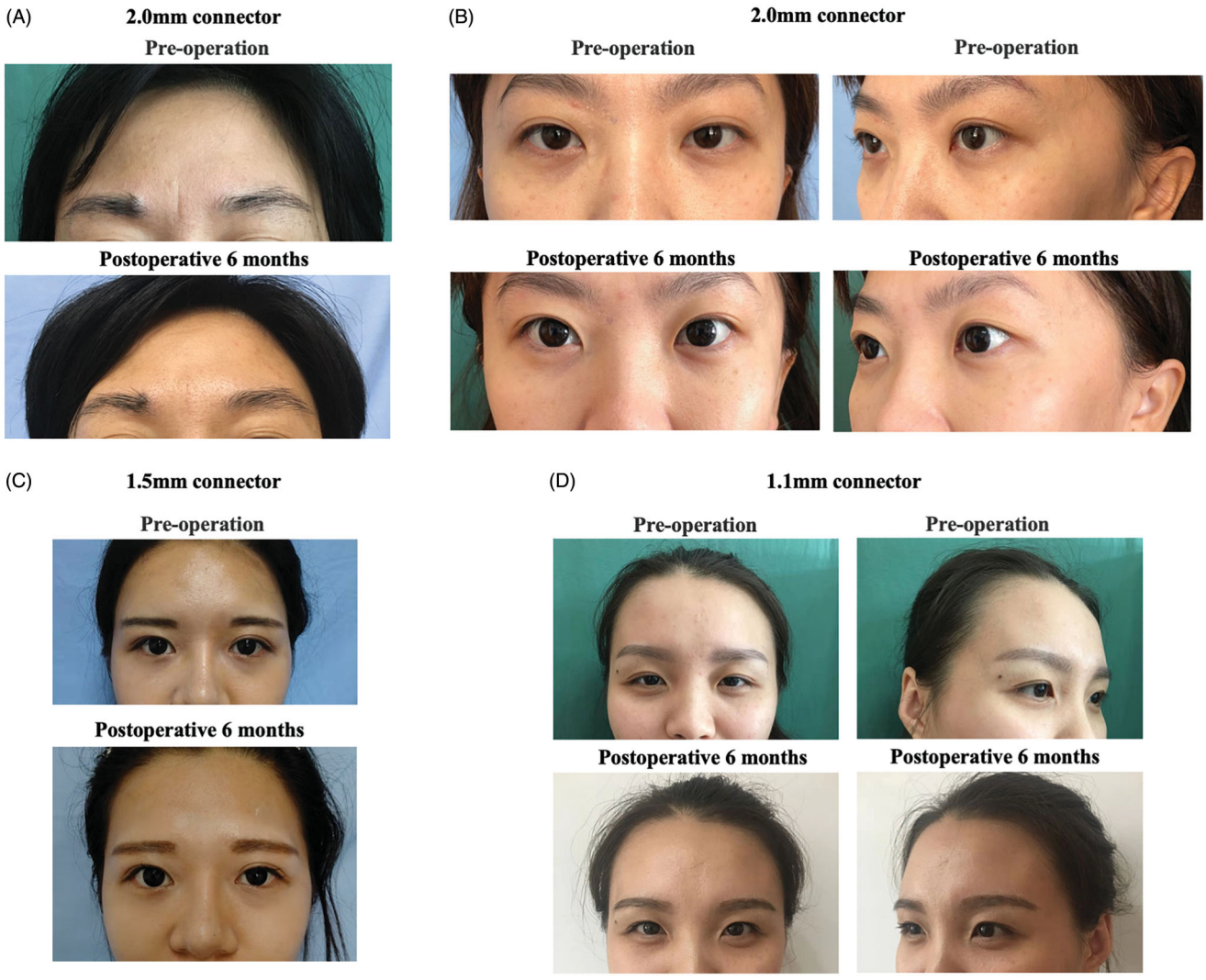

Figure 5. Assessment of the therapeutic efficacy of different Nanofat obtained from various Luer-Lok connectors. (A) Case 1: a 24-year-old woman presented for rejuvenation in the glabella. Two milliliters of Nanofat prepared using the 2.0-mm Luer-Lok connector was delivered through a 30 G needle. The image above was taken before surgery, and the images below were taken 6 months postoperatively. (B) Case 2: a 27-year-old woman presented with dark coloration (left images) and fine lines (right images) of the lower eyelid skin. Four milliliters of Nanofat prepared using the 2.0-mm Luer-Lok connector was delivered through a 30 G needle. The image above was taken before surgery (patient wore double eyelid tapes), and the image below was taken 6 months postoperatively (patient wore colored contact lenses). (C) Case 3: a 28-year-old woman presented with dark coloration of the lower eyelid skin. Four milliliters of Nanofat prepared using the 1.5-mm Luer-Lok connector was delivered through a 30 G needle. The image above was taken before surgery, and the images below were taken 6 months postoperatively. (D) Case 4: a 24-year-old woman presented with dark coloration (left images) and fine lines (right images) of the lower eyelid skin. Four milliliters of Nanofat prepared using the 1.1-mm Luer-Lok connector was delivered through a 30 G needle. The image above was taken before surgery, and the images below were taken 6 months postoperatively.

Table 2. Comparative analysis of clinical features.

	2.0 mm	1.5 mm	1.1 mm
Gender			
Female	9	7	6
male	3	0	2
Age (years)	30.83 ± 2.437	36.57 ± 3.316	29 ± 1.547
Donor site			
Front-lateral thigh	5	3	4
Lower abdomen	7	4	4
Area involved			
Lower eyelid	4	3	3
Glabella	3	1	1
Forearm scar tissue	1	0	0
Evaluation			
Doctor evaluation	4.083 ± 0.2289	2.429 ± 0.202**	1.00 ± 0.189**
Patient evaluation	4.167 ± 0.2072	2.143 ± 0.2608**	0.75 ± 0.25**

**$p < 0.01$.

Discussion

Fat grafting and stem cells have been commonly used in plastic surgery for soft tissue volume restoration and skin rejuvenation [11]. ADSCs are able to secrete vascular endothelial growth factor (VEGF), hepatocyte growth factor and transforming growth factor-β, which can potentially increase angiogenesis [12–14]. However, the traditional method of isolating SVF from the LA is time-consuming, complicated and expensive. Therefore, Nanofat grafting is the simplest method to obtain ADSCs in clinical procedures [15].

Over the years, many methods of preparing this liquid fat have been developed. Mashiko et al. compared two different Nanofat products obtained through two mechanical processing techniques, squeeze and emulsification. The author believes that sharp cutting (squeeze) of the fat tissue may be less traumatic to ADSCs than blunt cutting (emulsification), implying that sharp cutting is

a better way to prepare Nanofat [16]. Gentile et al. also compared three different modified Nanofat grafting procedures (supercharged-, evo- and centrifuged-modified Nanofat). Among the three methods, supercharged-modified Nanofat offers the highest concentration of cells and the best clinical evaluation scores [17]. In our study, using the common Luer-Lok connector, large chunks of fat tissue could be shredded into small sizes. We compared three different diameters of Luer-Lok connectors as well as un-shredded fat. Surprisingly, the smaller diameter of the Luer-Lok connector, which resulted in a higher mechanical shear force, yielded fewer SVF cells. In terms of clinical outcomes, compared to the 1.5-mm and 1.1-mm Luer-Lok connectors, the 2.0-mm connector-shredded fat showed promising results in treating fine lines, decreasing pigmented lower eyelid areas and softening scar texture. However, a second session of Nanofat grafting may be considered 4–6 months after the first procedure for patients who request additional improvements. Additionally, stress induced by the different handling processes in fat grafting and/or withdrawal can greatly modify the SAβ-gal activity, such as H_2O_2 exposure [18]. In the present study, relative to treatment with different diameters of Luer-Lok connectors, more aging cells were observed in the un-shredded fat group. The reason for this finding is likely that the larger fat mass in the un-shredded group slightly affected the growth of a small number of cells, thereby motivating them into the aging cycle during the seven-day culture of ADSCs. However, the differences were so small that they may not have affected ADSC viability. Therefore, the different cell viabilities of ADSCs in the four groups were not associated with the number of aging cells.

In conclusion, this study presents the development of a simple procedure for preparing Nanofat based on a previous technique. Less traumatic procedures can provide higher concentrations of ADSCs; however, more studies are needed to further confirm the clinical impacts of Nanofat obtained via various techniques.

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Disclosure statement

All of the authors declared no potential conflicts of interest with respect to the research, authorship and publication of this article.

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