Influence of Repeated Aspiration on Viability of Fat Grafts: A Comparative Study

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Abstract

**Background:** Fat grafting has been increasingly widely used in cosmetic and reconstructive surgery. However, the long-term retention of fat grafts is still unpredictable. Many critical variables have been found to significantly affect the viability of fat grafts; still, some of the ordinary impact factors are overlooked.

**Objectives:** We performed this study to find out whether repeated aspiration had an impact on fat grafts through an *in vitro* analysis and a nude mouse model.

**Methods:** A 15 cm by 10 cm rectangle was marked at the lower abdomen. The cannula was gently advanced and retracted through the same incision in a fan fashion within the superficial layer to collect fat samples. Based on the sequence of harvesting, the collected adipose tissue was divided into five groups and labeled as syringes 1, 2, 3, 4, and 5. Part of the sample was dissociated and analyzed using cell staining, Cell Counting Kit-8 assay, and flow cytometry. The other part was injected *in vivo* and analyzed for weight and histology at varying time intervals.

**Results:** Fat grafts from the former syringes were presented with a greater number of viable adipocytes and a higher level of cellular function compared to the latter syringes. Additionally, fat grafts from former syringes had higher graft retention, better vascularity, and less cystic necrosis. Neither the viability of stromal vascular fractions (SVFs) nor the ratio of CD34+CD45− cells within the SVFs were different among the five groups.

**Conclusions:** Repeated aspiration had a negative impact on the adipocytes, but not on the SVFs. With an increasing time of aspiration, the viability of the adipocytes and long-term retention of fat grafts decreased gradually. Harvested fat grafts from the first few syringes may be more suitable for fat grafting.

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variable that generates a certain amount of mechanical damage may negatively affect the fat grafts. Likewise, the back and forth movements of repeated suction against the adipose tissue might have a negative influence on the fat grafts.

To our knowledge, there is no data available with regard to the impact of repeated aspiration on fat grafts, either with a handheld syringe or vacuum machine. The purpose of this study was designed to investigate the mechanical forces generated by repeated aspiration on the viability and long-term survival of fat grafts.

**METHODS**

**Patients**

Six consecutive female patients undergoing abdominal liposuction from May 2014 to December 2014 were enrolled in this study. Informed consent was obtained from each patient, and the study protocol was approved by the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology (IRB no. FWA 00007304). Patients who had systemic metabolic diseases or a history of prior abdominal surgeries were not included in the study. All the procedures were performed by the same surgeon (J.Y.).

**Fat Harvest**

Preoperatively, a rectangle with a base of 15 cm and a height of 10 cm was marked at the lower abdomen of each patient. All the adipose tissue was harvested from the marked area, into which an appropriate amount of tumescent solution (0.1% lidocaine with 1:200 000 epinephrine in normal saline) was infiltrated. A 3 mm, two-sidehole (2.5 × 5 mm), blunt-tipped cannula was attached to a 20 mL syringe for fat harvesting. A light negative pressure was provided by slowly withdrawing the plunger, not exceeding 2 cc of intra-luminal volume of the syringe. The cannula was gently advanced and retracted through the same incision in a fan fashion within the superficial layer to collect all the samples. The collected adipose tissue was divided into five groups, which were marked as syringes 1, 2, 3, 4, and 5 based on the sequence of harvesting. The volume of fat tissue from each group was about 15 mL. Liposapirate from each group was filtered through a 30-mesh stainless steel strainer and then washed three times with sterilized phosphate-buffered saline (PBS) to remove local anesthetics, epinephrine, oil, blood cells, and debris. Purified fat was collected for the study.

**Adipocytes Staining Analysis**

Purified adipose tissue from each group was mixed with isometric 0.1% type I collagenase (Sigma Aldrich, St. Louis, MO) in phosphate-buffered saline, and the mixture was incubated at 37°C in a 5% carbon dioxide incubator for 45 minutes (Thermo Fisher Scientific Inc., Waltham, MA). The digestion was terminated with complete medium [Dulbecco’s Modified Eagle Medium (DMEM)/F12 containing 10% fetal bovine serum; Sigma Aldrich, St. Louis, MO] when the particle morphology of the fat tissue disappeared. After straining through a 200-µm nylon sieve, the digested samples were centrifuged at 1000 revolutions per minute (RPM) for 10 minutes and stratified into four layers: fatty acid, mature adipocytes, culture medium, and stromel pellet. Mature adipocytes were washed four times with DMEM and centrifuged at 1000 RPM for 5 minutes. Last, mature adipocytes were stained with Calcein-AM (2 µmol/L) and Hoechst 33342 (10 µg/L; Dojindo, Kumanoto, Japan). The proportion of viable adipocytes was counted under a Zeiss fluorescence microscope (Carl Zeiss, Oberkochen, Germany).

**Metabolic Activity of Adipocytes by Cell Counting Kit-8 Assay**

Cell Counting Kit-8 (CCK-8; Donjido, Kumanoto, Japan) provides sensitive colorometric assays to measure cell viability in cell proliferation and cytotoxicity assays. Compared to 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) assays, CCK-8 assay provides higher sensitivity and stability, shorter handling times, and less cytotoxicity.

10⁵ of adipocytes from each group were prepared, which were determined by cell staining with Hoechst 33342 (flattened nucleus located on the periphery). From each group, 90 µL adipocytes were inoculated into a pre-incubated 96-well plate. CCK-8 solution (10 µL) was added to each well of the plate, and the mixture was incubated at 37°C for 3 hours. Measurements of the absorbance were made three times at 450 nm using a microplate reader (Thermo, Multiskan Spectrum).

**Stromal Vascular Fractions Viability Analysis, Culture, and Multi-Differentiation**

Stromal pellets at the bottom of each group were collected and processed twice with red blood cell lysis buffer (Fushenbio, Shanghai, China). Then the remnant stromal vascular fractions (SVFs) were centrifuged, re-suspended by 300 µL PBS, and divided into three equal parts. A 10 µL sample of the first part was stained with Trypan Blue and counted on the hemocytometer to evaluate the viability of the SVFs. The procedure was repeated at least three times. The second part was cultured using DMEM/F12 medium supplemented with 10% fetal bovine serum (Sigma Aldrich, St. Louis, MO) in a 25 cm² culture flask (Corning, New York, NY). Cells were passaged when reaching 70-80%
confuence. And cells at subculture 3 were cultured in an adipogenic and osteogenic differentiation medium for 21 days, then stained with Oil Red-O and Alizarin Red S solution (Cyagen Biosciences, Santa Clara, CA).

**Ratio of CD34 + CD45- Cells Within SVFs**

A 100 µL sample of the third part was incubated with 5 µL CD34-PE and CD45-PerCP/Cy5.5 (PE: Phycoerythrin, PerCP/Cy5.5: Peridinin chlorophyll/Cyanin 5.5) (eBioscience, San Diego, CA) at 4°C for 40 minutes. The mixture were centrifuged, re-suspended with 500 µL PBS, and analyzed using a BD LSR II flow cytometer (BD Biosciences, Franklin Lakes, NJ). Data acquisition and analyses were performed by FACSDiva software (BD Biosciences, Franklin Lakes, NJ).

**Animal Model**

According to the random number table (The RAND Corporation, Santa Monica, CA), 11, 40 six-week-old BALB/c-nu/nu female mice (Vital River, Beijing, China) were divided into five groups and their adipose tissue was divided into syringes 1, 2, 3, 4, and 5. All the mice were housed in a specific pathogen free level laboratory and cared for in accordance with institutional guidelines. Following previously published animal models, 0.3 mL aliquots of purified lipoaspirate from each group were injected subcutaneously at 4 para-vertebral points. An 18-gauge Coleman cannula attached to a 1-cc syringe was used (Figure 1A).

**Fat Graft Persistence and Histology**

Fat grafts were harvested at 2 and 10 weeks to determine the short- and long-term fat graft persistence (Figure 1B). Harvested fat grafts were fixed in 4% paraformaldehyde, dehydrated in graded alcohols, cleared in xylene, embedded in paraffin, sectioned at 4-µm in thickness, stained with hematoxylin and eosin, and sealed with neutral balsam. The slides were analyzed according to the following histologic parameters: (1) intact and nucleated fat cells; (2) cysts and vacuoles; (3) inflammation, the infiltration of lymphocytes, and macrophages; and (4) fibrosis and other components of connective tissue. Each of these parameters was graded on a semi-quantitative scale of 0 to 5 where 0 indicated absence; 1, minimal presence; 3, moderate presence; 4, moderate to extensive presence; and 5, extensive presence. All the slides were examined by two independent pathologists in a blind manner.12

**Statistical Analysis**

All data were presented as mean ± standard deviation (mean ± SD) and analyzed using SPSS 17.0 (SPSS, Chicago, IL). A one-way analysis of variance test was used to compare the proportions of viable adipocytes, absorbance, and fat graft persistence between each group. The Mann-Whitney non-parametric test was used to compare the histological parameter scaling results between the groups. Confidence level was set at 95% and a p-value < .05 was considered statistically significant.

**RESULTS**

The patients’ average age was 32.5 years (range, 24-47 years). The mean body mass index was 23.4 (range, 21.3-26.9).

**Adipocytes Staining Analysis**

The proportion of viable adipocytes was 94.1% in syringe 1, 93.7% in syringe 2, 86.8% in syringe 3, 81.5% in syringe 4, and 80.4% in syringe 5, respectively. In general, the proportion of viable adipocytes decreases with an increasing sequence of harvesting. There were no significant differences between groups 1 and 2 (p = 0.72) or groups 4 and 5 (p = 0.06). A statistically significant difference could be detected between groups 2 and 3 (p < 0.001 and groups 3 and 4 (p < 0.05; Figures 2A-D and 3A).

**Metabolic Activity of Adipocytes**

The absorbance of fat cells plus CCK-8 solution is 1.989 ± 0.261 in group 1, 1.997 ± 0.215 in group 2, 1.915 ± 0.152 in group 3, 1.858 ± 0.172 in group 4, and 1.815 ± 0.240 in group 5. With an increasing sequence of harvesting, the absorbance of each group decreases gradually. The variation trend is in accordance with the results of a cell staining analysis. Statistically significant differences were found among all the groups except for groups 1 and 2 (p = 0.93), and groups 4 and 5 (p = 0.32; Figure 3B).

**SVFs Viability, Multi-Differentiation, and Surface Marker Analysis**

Viability of SVFs from each group were: 92.07 ± 2.66% in group 1, 91.09 ± 3.03% in group 2, 91.52 ± 1.59% in group 3, 92.40 ± 2.84% in group 4, and 92.58 ± 1.90% in group 5 (Figure 4). There is no statistically significant difference among the five groups. Additionally, SVFs isolated from any of the five groups possessed a high capability of multidirectional differentiation (Figure 5A-C). Further, the ratio of CD34 + CD45- cells in SVFs were 49.72 ± 4.45% in group 1, 50.58 ± 4.45% in group 2, 49.25 ± 5.89% in group 3, 50.32 ± 3.45% in group 4, and 50.73 ± 3.49% in group 5. No statistically significant difference could be found among these five groups (Figure 6A and B).
Graft Persistence

The average graft weight at 2 weeks of syringes 1, 2, 3, 4, and 5 were 194.75 ± 8.18 mg, 190.58 ± 7.60 mg, 179.67 ± 10.86 mg, 173.25 ± 15.04 mg, and 171.92 ± 12.20 mg, respectively. The average graft weight at 10 weeks of each group was 150.17 ± 9.45 mg, 148.58 ± 10.23 mg, 139.42 ± 8.89 mg, 130.75 ± 10.40 mg, and 128.08 ± 11.32 mg, respectively. At both 2 and 10 weeks, a greater percentage of fat grafts survived for syringes 1, 2, and 3 compared to syringes 4 and 5 ($p < 0.001$). A statistically significant difference could be found between syringes 3 and 4 and between 3 and 5 ($p < 0.05$; Figure 7).

Histological Analysis

Generally, the histological appearance of fat samples from syringes 1, 2, and 3 present healthier adipocytes with less vacuoles, inflammation, and fibrosis, and better vascularity compared to syringes 4 and 5 at 10 weeks. No significant difference could be found between syringes 4 and 5, regardless of cyst formation, inflammation scale, the level of vascular density, or development of fibrosis. There is a statistically significant difference between syringes 1 and 3 for the level of inflammation ($p < 0.05$); other than that, no statistically significant difference could be found among syringes 1, 2, or 3 in terms of the established evaluation criteria (Figures 8A-T and 9A-E).

DISCUSSION

Autologous fat grafting has been widely used for soft-tissue defect reconstruction for increasing indications in both cosmetic and reconstructive breast surgery; lipoatrophy; lip, nose, or tempora augmentation; correction of unsightly scars; face or hand rejuvenation; and more. However, the survival rate and long-term retention of the fat grafts varies greatly. To date, there is still no consensus on the optimal technique for fat grafting.$^{13-21}$

The approaches by which the fat tissue is harvested, processed, and re-injected all have an influence on the fat graft viability and long-term survival. According to an evidence-based review on fat grafting by Gir et al,$^6$ techniques that
use low-pressure suction by means of larger bore cannulas appear to increase adipocyte viability. Lee et al pointed out that adipocytes are highly resistant to forces of positive and negative pressure alone, whereas they are remarkably susceptible to the effects of shear stress. Thus, different mechanical forces may generate varying degrees of cell injuries. Likewise, might the repetitive motions of fat harvesting also have a negative influence on the fat grafts?

Various studies had been conducted to evaluate the impact of different fat harvest or preparation techniques on fat grafts. Pu et al conducted a comparative study between the Coleman technique and the conventional machine liposuction. It turned out that the former technique was superior to the latter one, presented with more viable adipocytes and optimal cellular function. Ferguson et al also demonstrated that the LipiVage System (syringe aspiration at low pressure; Lewisville, TX) was better than the conventional liposuction technique, with higher viable adipocytes count. Agostini et al compared the histomorphology and adipocyte viability of lipoaspirated samples harvested using wet and dry techniques. Though the fat collected with the wet technique was more preserved than that harvested with the dry technique, no substantial differences could be found. Besides, Erdim et al and Ozsoy et al both pointed out that larger cannulas tend to increase fat cell viability compared to smaller ones. In a controlled animal model of fat grafting, Kirkham et al found that the use of a 5-mm aspiration cannula led to improved fat graft retention and quality compared to a 3-mm cannula. The 5-mm group presented both less infiltrate and fibrosis. Further, several published research studies all pointed out that centrifugation forces greater than 3 000 RPM caused more cellular damage. All the above-mentioned approaches, different as they seemed, had one thing in common: they brought

**Figure 2.** Calcein-AM/Hoechst 33342 staining to assess the viability of the isolated adipocytes. (A) Bright field. (B) Viable adipocytes were stained green by Calcein-AM. Lipid droplets were unstained. (C) Both viable and dead adipocytes were dyed blue by Hoechst 33342. (D) Merged figure to calculate the proportion of viable adipocytes.
about more aggressive mechanical injuries against the fat grafts, which was consistent with our initial hypothesis.

In the present study, fat grafts were collected at the same frequency, under the same pressure, and from the same site. The only variable is the sequence of harvesting. We found that fat grafts from the former 1, 2, and 3 syringes were presented with a greater number of viable adipocytes, higher level of cellular function, better graft retention, and more integral architecture compared to syringes 4 and 5. With continuous aspiration in the same limited area, the viability and metabolic function of the adipocytes worsened gradually. However, neither the viability of stromal vascular fractions or the ratio of CD34 + CD45- cells within the SVFs were different among the five groups.

In addition, two types of fluorescent dyes (Calcein-AM and Hoechst 33342) were applied to stain and calculate the proportion of viable adipocytes. Calcein-AM, a cell-permanent dye, can be used to determine cell viability in most eukaryotic cells. In live cells, the non-fluorescent Calcein-AM is converted to a green-fluorescent calcein after acetoxymethyl ester hydrolysis by intracellular esterases.30 Hoechst 33342, a nucleic acid stain, is a popular cell-permeant nuclear counterstain that emits blue fluorescence when bound to double-stranded DNA. Unlike trypan blue, which is a vital stain used to selectively color dead tissues or cells blue, fluorescent dyes can easily distinguish viable cells from cell debris and oil drops.31 Further, it is unnecessary to determine the absolute number of viable adipocytes through direct cell counting. Actually, it is slightly inaccurate to compare group difference through direct adipocytes counting. First, the original weight of fat tissues to be examined cannot be guaranteed to be exactly the same, and second, 1 gram of fat tissue can generally yield a half million adipocytes, so even the very little changed weight of fat tissue may greatly influence the ultimate absolute number of adipocytes.25,32,33 Thus, in the present study, the proportion of viable adipocytes was calculated to reflect the whole viability of fat graft.

CCK-8 assay was used to determine the metabolic activity of adipocytes. CCK-8 assay, like the MTT and XTT assays,
utilized a highly-soluble tetrazolium salt (WST-9) to produce a water-soluble formazan dye (generated by dehydrogenases in viable cells), which was proportional to the number of living cells. Apart from being nonradioactive and more convenient, CCK-8 assay was more sensitive than assays using other tetrazolium salts, such as MTT, XTT, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, or water soluble tetrazolium salt. Xiong et al compared CCK-8 with MTT in measuring the viability of human promyelocytic leukemia cells. He pointed out the linear correlation of optical density (OD) value and cell viability detected by CCK-8 was better than that of MTT. In our study, OD value measured by CCK-8 correlated well with the proportion of viable adipocytes from each group (data not shown).

Besides the adipocytes, the other regenerative cells also play an important role in determining the long-term maintenance of fat grafts. A large number of studies have stated that fat grafts enriched with SVFs present better grafting results than mere fat grafts. SVFs consist of at least four subgroups: blood-derived cells (CD45+), adipose-derived stem cells (ASCs) (CD31-CD34+CD45−), endothelial (progenitor) cells (CD31+CD34+CD45−), and pericytes (CD31-CD34-CD45−). In particular, the concentration of CD34+ progenitor cells within the SVFs may predict retention of the fat grafts. A study conducted by Yin et al investigated the effect of water-jet force on SVFs compared to the classic Coleman technique, and found a significantly higher ratio of CD34+CD45− cells within the water-jet group than the Coleman technique group; meanwhile, the water-jet group presented better fat graft survival. In our study, neither the viability of SVFs nor the ratio of CD34+CD45− cells within the SVFs were significant different among the five groups. In this sense, SVFs may be more resistant to

**Figure 5.** Stromal vascular fractions (SVFs) isolated from any group are all highly viable and possess great viability of multi-directional differentiation. Typical figures are presented. (A) Adipose-derived stem cells from passage three (100 ×). (B) Oil Red O staining of adipose-derived stem cells (ASCs) under adipogenic differentiation (100 ×). (C) Alizarin Red S staining of ASCs under osteogenic differentiation (100 ×).
mechanical forces than the adipocytes, just like as they are more resistant to ischemia and hypoxia.\textsuperscript{40}

Further, the classic nude mouse model was used to compare the long-term retention of fat grafts of different syringes, and the outcomes were also consistent with the \textit{in vitro} experiments. The first few syringes presented with better architecture, increased vascularity, decreased fibrosis, and higher long-term survival. Therefore, fat tissue from the first few syringes may be more suitable for fat grafting; in the clinical practice, harvesting a certain amount of adipose tissue from multiple areas may be preferred to obtain a higher survival of grafted fat. In the next step, we are preparing to conduct a randomized controlled clinical trial to further confirm the efficacy of our investigation. If proven true, we are more than thrilled to have it applied immediately in the clinical practice, since the approach is easily accessible to both new and skilled practitioners.

Actually, whether the approach is absolutely applicable depends on the gap between fat grafts supplies and demands. Theoretically, for those large-volume fat transplantation cases, like breast and buttock augmentation, it is rather difficult to harvest a sufficient volume of fat grafts while completely avoiding repetitive aspiration. But for those small-volume fat transfer cases, the approach is entirely feasible. So certain necessary conditions are required to apply this approach: (1) patients with relatively sufficient subcutaneous fat storage; (2) small volume fat transplantation, usually less than 100 mL, like facial fat grafting, hand/genital rejuvenation, touch-up procedures for breast augmentation or reconstruction with implants, etc.; and (3) patients’ willingness to bear the possible discomfort following an increased amount of tumescent solution injection. Besides, large-volume fat grafting cases should usually be avoided.

Additionally, as for the sample size of this investigation, it was taken into account at the time of designing. In a study by Smith et al\textsuperscript{41} to explore the effect of harvesting and preparation techniques on adipocyte graft survival, liposaptrates from three patients were analyzed \textit{in vitro}, and five freshly harvested tissue specimens were injected into the severe combined immune deficiency mice to study the \textit{in vivo} viability. The authors also pointed out that the minimum number of specimens analyzed \textit{in vivo} should be no less than five. Further, in an investigation by Li et al\textsuperscript{42} six female patients were enrolled in a study to determine the best donor site for tissue harvesting and isolation of SVF cells for fat graft survival. Similarly, nine female

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\caption{Ratio of CD34 + CD45- cells within stromal vascular fractions (SVFs). (A) Typical flow cytometry scatter plot. (B) Ratio of CD34 + CD45- cells in SVFs were 49.72 ± 4.45% in group 1, 50.58 ± 4.45% in group 2, 49.25 ± 5.89% in group 3, 50.32 ± 3.45% in group 4, and 50.73 ± 3.49% in group 5. No statistically significant difference could be found among these five groups.}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure7.png}
\caption{Average weight of fat grafts harvested at 2 and 10 weeks. At both 2 and 10 weeks, a greater percentage of fat grafts survived for syringes 1, 2, and 3 compared to syringes 4 and 5 (\textit{p} < 0.001). A statistically significant difference could be found between syringes 3 and 4 and syringes 3 and 5 (\textit{*p} < 0.05).}
\end{figure}
Figure 8. Typical example photographs of Hematoxylin and eosin-stained adipose tissue harvested at 2 and 10 weeks from each group. Samples from syringe 1 at (A) 2 weeks (40×); (B) 2 weeks (100×); (C) 10 weeks (40×); and (D) 10 weeks (100×). Samples from syringe 2 at (E) 2 weeks (40×); (F) 2 weeks (100×); (G) 10 weeks (40×); and (H) 10 weeks (100×). Samples from syringe 3 at (I) 2 weeks (40×); (J) 2 weeks (100×); (K) 10 weeks (40×); and (L) 10 weeks (100×). Samples from syringe 4 at (M) 2 weeks (40×); (N) 2 weeks (100×); (O) 10 weeks (40×); and (P) 10 weeks (100×). Samples from syringe 5 at (Q) 2 weeks (40×); (R) 2 weeks (100×); (S) 10 weeks (40×); and (T) 10 weeks (100×). Low-magnification images were further magnified in the right column. There was better integral architecture, less collagen bands, decreased areas of fibrosis, and less infiltration of macrophages in syringes 1 and 2 compared to syringes 4 and 5. There is a statistically significant difference between syringes 1 and 3 in the level of inflammation at 2 weeks; other than that, no statistically significant difference could be found among syringes 1, 2, or 3 in terms of the established evaluation criteria at either time. No significant difference could be found between syringes 4 and 5 regardless of cyst formation, inflammation scale, the level of vascularity, or development of fibrosis at either 2 or 10 weeks.
patients were enrolled in another in vitro and in vivo study to compare different processing methods on fat grafting. After full consideration, we decided to set the number of enrolled patients at six. Besides, all the subjects were females, as male patients were lacking. Originally, a certain number of male patients were intended to be included in the investigation, however, the number of male patients who came for liposculpture procedures were rather limited. We were unable to get sufficient male patients to participate in the investigation. Interestingly, among all the references we looked up, the gender information of the enrolled subjects was either unmentioned or entirely female. Maybe these investigators suffered the same situation as we did. Admittedly, whether gender difference has an effect on fat grafting is worthy of further study.

Moreover, a single sized cannula was applied to harvest the fat grafts. Though not all surgeons harvest fat grafts with the 3-mm sized cannula, as far as we know few studies, among all those investigations on the influence of various variables on fat grafts viability, have introduced a second variable—harvesting cannula size on the basis of their original research (processing, reinjection, cryopreservation, etc.), other than those in search of more advantageous harvesting cannulas. In the present study, we mainly focus on the influence of the repetitive motions of harvesting cannula on the viability of fat grafts; though the whole investigation may not be optimal, we assume it is sufficient to confirm our original hypothesis.

Our study has its own limitations. First, gender composition. Patients enrolled in our study were entirely female, and male patients were lacking. Hormone status due to gender differences may also influence the viability of fat grafts. Second, only cell components were analyzed; however, the local vasculogenic and anti-inflammatory cytokines were neglected. Finally, the entire procedures were conducted using the wet technique: a control group of patients undergoing fat harvesting with the dry technique would enhance the strength of the design of the study.
Figure 9. Histopathology parameters of (A) integrity, (B) cysts or vacuoles, (C) inflammation, (D) vascularity, and (E) fibrosis (*$P < 0.05$, **$P < 0.001$).
CONCLUSIONS

In summary, the present study has provided cell viability assessment, metabolic activity analysis of the adipocytes, viability of SVFs, ratio of CD34+CD45− cells within SVFs in vitro, and graft retention in vivo to demonstrate that there are substantial differences in adipose tissue harvested from different syringes. With continuous aspiration, the cellular function of adipocytes and the viability and long-term retention of fat grafts decreased gradually. Fat tissue from the first few syringes may be more suitable for fat grafting. Harvesting a certain amount of adipose tissue from multiple areas may be preferred to obtain higher survival of fat grafts.

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