
RANDOMIZED CONTROLLED TRIAL

STUDY PROTOCOL

**The study of survival rate of fat in facial autologous fat
transplantation by different purification methods**

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Protocol

1.Summary:

Autologous fat grafting has revolutionized the field of facial soft-tissue augmentation, despite a lack of universally accepted standardization^[1,2]. Contraversy has long been arisen regarding the optimization of different fat-processing techniques. Currently, the three-dimensional (3D) technology has gained an increasing importance in volumetric measurement of fat grafting^[3,4].

The aim of the project is to compare different fat-processing techniques with 3D technology. Patients with facial asymmetry at the Plastic Surgery Hospital will be randomized to one of three fat-processing groups (sedimentation, centrifugation, and cotton pad filtration) for initial fat grafting. These cases will be scanned preoperatively and postoperatively at following time-points (1month: post-1m, 3month: post-3m, 6month: post-6m and 12month: post-12m) with 3D volumetric analysis. A comparative 3D study will be conducted among three groups and then to objectively explore the optimal volume retention of fat-processing technique.

2.Introduction:

Autologous fat grafting has long been considered as a well-established and frequently applied option for facial volume augmentation in plastic surgery^[5,6]. Despite its growing popularity, the volume of transplanted fat has been limited by a variable and unpredictable rate of fat absorption (20%-90%)^[1,2,7]. To minimize this drawback, a wealth of literature has been proposed to optimize each step of the fat grafting, including the techniques of fat-harvesting, fat-processing, and fat-injecting. Unfortunately, no standardized protocol has been adopted^[8-11]. The optimal fat-processing technique remains one of the most controversial issues^[12-13]. To our best knowledge, several common fat-processing techniques have been widely applied in clinic, primarily based on sedimentation^[14], centrifugation^[15], and filtration principles^[16,17]. Many studies published over past decades just have demonstrated clinical results with a certain fat-processing technique; however, it is hard to reach a consensus on which fat-processing technique would reveal greater volume retention unless there is objective and comparative evidence.

Previous evaluation of fat grafting mainly relied on the subjective assessment of two-dimensional (2D) photographs or questionnaires for patients^[18]. Even some

measurements could provide objective-related values such as anthropometric method, water displacement, thermoplastic cast, computed tomography (CT), magnetic resonance imaging (MRI), and ultrasonography scan, however, which has been criticized to their inaccurate, cumbersome, and time-consuming nature^[19-23]. Currently, 3D technology has gained an increasing importance in plastic surgery. Moreover, the term “percentage volume maintenance” has emerged as a promising indicator in evaluating the effect of fat grafting with 3D technology^[4]. However, previous 3D papers just have emphasized the importance of a certain fat-processing technique in clinic. No randomized controlled trial has been performed in 3D study to objectively evaluate the volumetric retention of different fat-processing techniques.

3. Overview of RCTs:

Patients with facial asymmetry at the Plastic Surgery Hospital will be randomized to one of three fat-processing groups (sedimentation, centrifugation, and cotton pad filtration) for initial fat grafting. These cases will be scanned preoperatively and postoperatively at following time-points (1month: post-1m, 3month: post-3m, 6month: post-6m and 12month: post-12m) with 3D volumetric analysis.

4. The research question:

Different fat-processing techniques will be compared head to head with three-dimensional (3D) technology to explore the optimal fat-processing technique for improving the volume retention of grafted fat.

5. RCT population:

(1). Trial site(s) and population(s):

Patients with facial asymmetry from all over of this country seeking for initial facial fat grafting will be enrolled in the Plastic Surgery Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College. These cases will be randomly allocated to either fat-processing group: sedimentation, centrifugation, or cotton pad filtering group. Patients will be underwent the 3D facial scanning preoperatively and postoperatively at post-1m, post-3m, post-6m and post-12m with Artec Spider 3D Scanner (Artec 3D, Luxemburg).

(2). Inclusion and exclusion criteria:

(a). Inclusion criteria: Consecutive patients with facial asymmetry seeking for initial facial fat grafting; After informed consent, patients who would voluntarily

participate in preoperative and postoperative 3D scanning at post-1m, post-3m, post-6m and post-12m on time.

(b). Exclusion criteria: Patients who did not complete preoperative or postoperative 3D scanning; Patients who did secondary treatment within the postoperative follow-up period; Except to facial fat grafting, patients who did other facial surgeries in the meantime.

(3). Sources or methods of recruitment:

Cases of facial asymmetry undergoing initial facial fat grafting at the Plastic Surgery Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, were enrolled in this study. These patients were randomly allocated to either fat-processing group: sedimentation, centrifugation, or cotton pad filtering group for treatment.

(4). Information for participants:

This protocol has been approved by the Ethical Institutional Committee of the Plastic Surgery Hospital, Chinese Academy of Medical Sciences. It will be emphasised that the enrolment in the study is voluntary, that she or he can withdraw at any time from all or part of the study. The study will be explained verbally, according to the information sheet and consent will be recorded with a signature.

6. Allocation of interventions:

In the study, three fat-processing groups are sedimentation group, centrifugation group, and cotton pad filtering group. Above three groups will be numbered 1, 2, and 3. Eligible patients will be registered in our hospital in order, and the randomisation sequence will be generated according to such numbers. Patients will be randomly allocated to either fat-processing group for fat grafting.

7. The interventions:

Inclusive patients will be randomly allocated to either fat-processing group (sedimentation, centrifugation, or cotton pad filtration) for initial fat grafting. Apart from different fat-processing techniques, the fat-harvesting and fat-injecting techniques applied will be common for patients. Lower abdomen will be chosen to be the donor sites of all cases. After infiltration with tumescent solution, fat tissue aspiration will be manually performed using a blunt-tipped cannula (2.5 mm in diameter) connected to a 20-ml syringe. Next, the harvest fat tissue will be washed with normal saline two times, and then processed with either fat-processing technique. For the sedimentation technique, the syringes filled with lipoaspirates were put upside

down statically for 15 minutes to separate them into distinct layers by gravity. For the centrifugation technique, the syringes were placed into a centrifuge and spun at 1000rpm (161g) for 3minutes. For the cotton pad filtering technique, harvested fat was directly poured from each syringe onto the sterilized cotton pad (30cm×20cm×1.5cm in specification), which was wrapped with multiple pieces of gauze. The aspirated fat was gently rolled and kneaded along the cotton pad using a sterile scalpel handle for 5 minutes, allowing oil and aqueous portions to be absorbed sufficiently by the syphonage of the cotton pad. Finally, the residual fat tissue left on the cotton pad was collected again for implantation.

The facial scanning of these cases will be obtained preoperatively and postoperatively at post-1m, post-3m, post-6m and post-12m through the Artec Spider 3D Scanner, with high resolution (up to 0.01mm) and superior accuracy (up to 0.03mm). The skilled technician will hold the scanner to capture facial images with an optimum object-to-scanner distance of 450mm to 700mm in approximately 10s. After image acquisition, the pre- and postoperative 3D data will be converted into reverse-engineering software Geomagic Qualify 12.0 (Raindrop Geomagic, USA) for image registration. The volume difference can be precisely calculated with Boolean Operation by software 3-matic 7.0 (Materialise, France), with result measured in cubic millimeters. Finally, the percentage volume maintenance of grafted fat could be obtained using the following equation: the percentage volume maintenance (%) = (3D volume difference / injected fat amount) ×100.

8.Outcome assessment:

(1) Outcome assessment:

Currently, the 3D technology has gained an increasing importance in plastic surgery. Its accuracy and validation in evaluation of facial fat grafting has been proved by our group²². Moreover, the term “percentage volume maintenance” has emerged as a promising indicator in evaluating the effect of fat grafting with 3D technology²³. In this study, the percentage volume maintenance of grafted fat could be obtained using the following equation: the percentage volume maintenance (%) = (3D volume difference / injected fat amount) ×100.

The percentage volume maintenance of different fat-processing techniques will be measured with 3D softwares and will be analysed with variance analysis. SPSS

17.0 will be performed in the study. The percentage volume maintenance of different fat-processing groups will be expressed as mean \pm SD. A value of $P<0.05$ will be considered as statistically significant. The effect of patient gender, age, different fat-processing groups, and body mass index (BMI) in every follow-up period will be recorded and analyzed with covariance analysis to estimate the reliability of the study.

(2). Timing of outcome assessment:

According to the follow-up period of this study, the outcome will be collected in four periods. The percentage volume maintenance of inclusive patients will be obtained at post-1m, post-3m, post-6m and post-12m with 3D volumetric analysis.

9.Post-recruitment retention strategies:

Provide study materials and a 24-hour service. Give collaborators regular information about the progress of the study. Help ensure complete data collection at discharge and at post-1m, post-3m, post-6m and post-12m. Respond to any questions about the trial.

10.Safety monitoring and adverse events:

Graduate students in the department will be appointed to deal with any ethical issues that may arise while the trial is in progress.

This is a clinical research, and it would have some unexpected adverse events in future. Firstly, fat absorption in postoperative period is inevitable. After post-6m, maybe some patients will too impatient to wait until post-12m and seek for a secondary fat grafting to obtain facial symmetry again. Then, these cases will be excluded. Secondly, the poor compliance of patients is another main reason. The follow-up time points of this subject are respectively at post-1m, post-3m, post-6m, and post-12m. If some patients live very far away from Beijing, and the cost of several follow-up visits will be high and troublesome. The data of these cases will be also lost.

11.Data collection and management:

Data collection and management is considered to be the crucial stage of the project, which consist three steps. Firstly, whichever fat-processing technique the patient will be performed in clinic operating rooms, the amount of injected fat would be meticulously recorded in milliliters. Secondly, the 3D facial scanning of patients should be operated by the same skilled technician. During image acquisition, patients will be instructed to close their eyes and sit upright, with the Frankfurt horizontal

plane parallel to the ground. The skilled technician will hold the scanner to capture facial images with an optimum object-to-scanner distance of 450mm to 700mm. If the patient moved during the operation, the procedure should be asked to repeat again. Thirdly, the 3D volumetric analysis should be measured by the same technician. The volume difference could be precisely calculated by software Geomagic Qualify 12.0 (Raindrop Geomagic, USA) and 3-matic 7.0 (Materialise, France), with the result measured in cubic millimeters. In sum, the percentage volume maintenance of grafted fat could be obtained using the following equation: the percentage volume maintenance (%) = (3D volume difference / injected fat amount) × 100. Then, the percentage volume maintenance of different fat-processing groups at post-1m, post-3m, post-6m and post-12m could be carefully recorded.

After each data collection periods, the data management unit will produce a summary report including: (1) number of patients that were included in the study in that period; (2) missing data rates; and (3) inconsistent data rates.

12. Sample size:

In the study, three fat-processing groups will be analysed (cotton pad filtration, centrifugation, sedimentation). In sample size design, it is necessary to calculate each of the comparison groups separately. The maximum value of the calculation results should be chosen as the final sample size of this study. Then, the sample size can meet the requirements of all corresponding hypotheses.

(1). Hypothesis1:

Autologous fat processed by cotton pad filtration will have a significant higher volume retention than did by sedimentation.

$$H_0 : x_T - x_c \leq 0$$

$$H_1 : x_T - x_c > 0$$

x_T : the percentage volume maintenance of cotton pad filtration

x_c : the percentage volume maintenance of sedimentation

Hypothesis2:

Autologous fat processed by cotton pad filtration will have a significant higher volume retention than did by centrifugation

$$H_0 : x_T - x_c \leq 0$$

$$H_1 : x_T - x_c > 0$$

x_T : the percentage volume maintenance of cotton pad filtration

x_C : the percentage volume maintenance of centrifugation

(2). Sample size justification (Hypothesis1):

The significance level of the test was both bilateral 5% and the confidence level was 80%, and the random grouping ratio between the experimental group and the control group was 1:1. According to our pre-experiment and the statistical principle, 44 subjects could be obtained. Considering the 20% drop rate during the study, 55 subjects could be required for each group.

Sample size justification (Hypothesis2):

Likely, 41 subjects could be obtained. Considering the 20% drop rate during the study, 52 subjects could be required for each group.

$$\text{Equation of Sample size calculation: } n = \frac{2(\mu_{1-\alpha} + \mu_{1-\beta})^2 \sigma^2}{(x_T - x_C)^2}$$

In sum, the sample size of each fat-processing group should be 55 subjects.

13. Analysis strategies:

SPSS 17.0 was performed in the study. The percentage volume maintenance of different fat-processing groups was calculated with 3D technology and was analysed with variance analysis, which was expressed as mean \pm SD. A value of $P < 0.05$ was considered as statistically significant. The effect of patient gender, age, different fat-processing groups, and body mass index (BMI) in every follow-up period have been associated with the percentage volume maintenance. Above variables were recorded and analysed with covariance analysis to estimate the reliability of the study.

14. Ethical aspects of RCTs:

The patient will be recruited in our hospital. It will be emphasised that the enrolment in the study is voluntary, that she or he can withdraw at any time from all or part of the study, and that any decision she or he takes in this respect will have no bearing on the medical care she and her family receive. The study will be explained verbally, according to the information sheet and consent will be recorded with a signature.

15. RCT management:

(1) Registering the trial:

This study has been registered in Chinese Clinical Trial Registry on 30 November

2014. The Registration identification number is ChiCTR-IOR-14005599.

(2) Trial management:

The overall progress of the study will be monitored by a scientific and administrative steering committee. The membership of this committee are Rongwei Wu, Xiaonan Yang, Xiaolei Jin, Haibin Lu, Zhenhua Jia, Binghang Li, Haiyue Jiang and Zuoliang Qi. Rongwei Wu will be responsible for the case recruitment. Xiaolei Jin will be appointed as clinical observer and coordinator for patients. Haibin Lu and Zhenhua Jia will be responsible for data collection and data entry. Xiaonan Yang will be responsible for statistical analysis of the study. Binghang Li, as the skilled technician, who will be responsible for 3D facial scanning and 3D volumetric analysis of patients. Zuoliang Qi will be the supervisor of this trial, who will have the responsibility of supervising the data collection made by the data collectors on a weekly basis. The follow-up time points of this study are respectively at post-1m, post-3m, post-6m, and post-12m. Thus, the multiple visits maybe face many difficulties in clinical. The committee will meet monthly, or more frequently as needed.

(3). Local coordination:

Patients will be returned to the hospital at post-1m, post-3m, post-6m, and post-12m. Our committee will be responsible for the coordination for them, and no local coordination will be created.

(4). Research governance and good clinical practice:

Research governance is a mechanism for ensuring that all research on human subjects complies with relevant legal and professional standards. This study has been approved by the Ethical Institutional Committee of the Plastic Surgery Hospital, Chinese Academy of Medical Sciences. The research has been well designed. After informed consent, patients understand the purpose of the research. The research will also protect patient confidentiality.

16.Reporting, dissemination and notification of results:

(1). Publication policy:

The review process and authorship for publications and presentations will follow the guidelines set forth in the Policy and Procedures Manual of the Global Network for Health Research. Any submission for publication or for presentation at professional conferences must adhere to these principles.

Prior to the submission or application for presentation, all manuscripts, posters or

oral presentations, or other reports of the outcomes of this research effort must be approved by a majority of the members of the above Committee. This committee's membership includes (1) Rongwei Wu, (2) Xiaonan Yang, (3) Xiaolei Jin, (4) Haibin Lu, (5) Zhenhua Jia, (6) Binghang Li, (7) Haiyue Jiang ; and (8) Zuoliang Qi.

Each author should have participated sufficiently in the work to take public responsibility for the content. Authorship credit should be based only on substantial contributions to (a) conception and design, or analysis and interpretation of data; and to (b) drafting the article or revising it critically for important intellectual content; and on (c) final approval of the version to be published. Conditions (a), (b), and (c) must all be met.

(2). Dissemination the results:

Outcomes will be spread as much as possible through the following:

- a) Publication in a widely spread first-class journal (e.g. *JAMA Facial Plastic Surgery*)
- b) Presentation in congresses and local, national and international meetings
- c) Presentation and discussion of outcomes at the participating hospitals
- d) Recording at Cochrane Library

17.References

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