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**Original Article**

**Cryopreserved fat: our clinical experience and applications**

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**Abstract**

**Aim:** Cryopreservation of fat is an effective method for repeat fat grafting, but there are few reports about the clinical use of cryopreserved fat. The aim of this study was to determine the effectiveness and safety of cryopreserved fat for clinical use.

**Methods:** Between Aug 2015 and Dec 2018, we investigated 590 patients who underwent fat harvesting at our clinic. The harvested fat was cryopreserved at a temperature of -196 °C at a cell processing center and injections were performed in our clinic.

**Results:** Of the 590 patients studied, 216 (312 cases) have undergone fat injections so far. Volume augmentations using harvested fat, such as facial and breast augmentations, were performed on 180 patients. For 84 patients, harvested fat was utilized only for revitalization/fertilization purposes, such as to improve skin condition. There were no severe complications in any patients. However, volume maintenance was rarely observed. Skin rejuvenation effects were comparable to that in cases using fresh fat.

**Conclusion:** The clinical use of cryopreserved fat is thought to be safe and effective.

**Keywords:** Cryopreserved fat, frozen fat, fat grafting, skin rejuvenation

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INTRODUCTION

Fat grafting is used worldwide for volume augmentation. Recently, it has become clear that fat grafting has a regenerative effect (revitalization/fertilization) which leads to rejuvenation\(^1\)\(^-\)\(^4\).

However, one of the biggest problems of fat grafting is the unpredictable retention rate for volume augmentation. Additionally, skin rejuvenation effects vary\(^7\). Injections must often be repeated to achieve the targeted results\(^7\)\(^-\)\(^10\).

Another problem associated with fat grafting is the painful process of harvesting fat, which is also time-consuming for both patients and doctors. Accordingly, it would be advantageous to both patients and plastic/aesthetic surgeons if fat could be harvested during one procedure and cryopreserved for later use.

The cryopreservation method has been researched for a long time, and many doctors conclude that the use of cryopreserved fat is useful and safe when appropriate methods are used\(^11\)\(^-\)\(^13\). However, most of these studies are experimental, and documented clinical use of such fat is scarce\(^14\)\(^,\)\(^15\).

The aim of this study was to determine the safety and benefits of the clinical use of cryopreserved fat.

METHODS

Patients and methods
Patients: Table 1.

From Aug 2015 to Dec 2018, we harvested fat from 490 patients and sent it to a cell processing center (CPC) (CellSource Co., Ltd., Tokyo, Japan) for cryopreservation at -196 °C.

Flowchart of harvesting, cryopreserving, and repeat injection
At our clinic, we harvest patients’ fat for same-day use. The residual fat is sent to the CPC. The CPC (CellSource Co.) cryopreserves the fat using their own cryoprotective agent at -196 °C. The company will then send back the thawed fat to our clinic for repeat injections [Figure 1].

Anesthesia
Usually, we use a combination of local anesthesia with tumescent technique and intravenous anesthesia.

Harvest in the clinic
When harvesting the patients’ fat, we use a tumescent technique (20 mL of 8.4% sodium hydrogen carbonate, 1 mL of epinephrine, and 50 mL of 1.0% lidocaine per 1000 mL of saline) with suction pressure of less than 1 atm.

Choice of donor site
Fat is typically harvested from the thighs, lower abdomen, and flanks (so-called LFD: localized fat deposit)\(^17\), because these areas bleed the least and are the easiest sites from which to harvest fat.

First injection at the clinic
We usually inject the fat on the same day it is harvested. Fat grafting is used not only for volume augmentation but also for regenerative effects such as skin rejuvenation. When we use the fat for volume augmentation, we apply the so-called “Coleman technique”, and for skin rejuvenation, we use the nanofat or squeezed fat technique\(^5\)\(^,\)\(^6\).
The main injection sites for volume augmentation are the breasts and the face (such as the forehead and cheeks). For skin rejuvenation, the main injection sites are the lower eyelids, the area surrounding the lips, and areas with signs of facial aging, e.g., wrinkles. Sometimes fat grafting is used for scar treatment such as in cases of double eyelids, liposuction revision, or contracture release in percutaneous aponeurotomy (so-called rigottomy).

How to send the fat
After fat is utilized on the day of harvest, the remaining fat is collected in a bag (FB-bag: CellSource Co.) which is then packed in an ADIPORTER box (CellSource Co., Ltd., Tokyo, Japan). After packing, the bag is sent to the CPC at CellSource Co. in a refrigerated state (below 10 °C) [Figure 2].

Cryopreservation and storage of fat
The process of cryopreservation and storage at -196 °C was performed in the CPC at CellSource Co.

The details of this process are confidential matters for the company. Roughly speaking, delivered fat is washed using Ringer’s lactate solution. The fat is mixed with a cryoprotectant and divided into 4- to 5-mL aliquots in syringes. These syringes are gradually frozen at a controlled rate until the temperature reaches...
They are then transferred to liquid nitrogen and stored at -196 °C. Using this process, we can store many syringes of patients’ fat after single harvesting sessions.

**Return of fat**

When we want to use cryopreserved fat, we use an Internet web-ordering service from the CellSource CPC to place an order. After the CPC receives the order, the patient’s cryopreserved fat is thawed rapidly (37 °C) and the cryoprotectant liquid is washed out. The CPC sends back that fat to our clinic in a refrigerated state (below 10 °C) [Figure 3].

**Injection of thawed cryopreserved fat**

The thawed fat must be injected within 48 hours of receiving it. We follow the “Coleman technique” for volume augmentation, and use the “nanofat (emulsified fat) technique” for skin rejuvenation. The fat is usually used at multiple injection sites. For example, the first injection of cryopreserved fat may be used for breast augmentation and the second injection for forehead volume augmentation and/or scar treatment. We may use the cryopreserved fat many times if the supply lasts. Therefore, we can inject each patients many times easily.
Follow-up of patients
We followed-up each patient after at one month and 3-6 months after the first injection using fresh fat, and also at one month and 3-6 months after repeat injections using thawed cryopreserved fat.

Comparison of stromal vascular fraction
We compared the amount of stromal vascular fraction (SVF) in fresh fat before sending it to the CPC with the SVF of thawed cryopreserved fat. SVF was digested by collagenase (Wako Pure Chemical, Osaka, Japan). Cell count and viability of SVF were performed by KUNA-STEAM Automated Fluorescence Cell Counter (Logos Biosystems, South Korea).

RESULTS
Of the 490 patients who underwent fat harvesting in our clinic, 216 patients (312 cases) received fat grafting with cryopreserved fat. The characteristics of those patients are shown in Table 2. The number of patients who received a varying number of injections from one harvesting session is shown in Table 3. The ways of using cryopreserved fat are shown in Table 4. The injected volume of cryopreserved fat was from 0.2 to 24.0 mL for the face and from 4.0 to 100.0 mL for the body.

There were no severe complications in any patients. Mild complications occurred in 5 patients (2.3%), all of which were temporary pigmentation [Table 5].
A comparison of sent and returned fat volumes showed that fat volume decreased 34.4% if it was not centrifuged before being sent, and decreased 51.3% if centrifuged before sending [Table 6].

SVF in fresh fat was $7.1 \times 10^5$/mL (before sending) and $14.8 \times 10^5$/mL in thawed cryopreserved fat (returned fat) ($n = 5$). These amounts reflect the amounts of SVF/mL that were concentrated from cryopreserved fat as compared to fresh fat.

We show some cases below

**Case 1: facial rejuvenation using cryopreserved fat [Figure 4]**
A 46 y.o. patient disliked her bony face and wanted to look younger. She did not want to undergo painful harvesting many times. Therefore, we planned serial injections after one harvesting session followed by the cryopreservation of her fat. She received facial rejuvenation surgery (first operation) involving fat grafting to her forehead (20.0 mL), cheeks (8.0 mL each), and lips (1.5 mL each) with thread lift (Silhouette Soft; Sinclair Pharma, London, UK) using the bidirectional floating method for her sagging cheeks, and her residual fat was sent for cryopreservation. After her first operation, she received two more fat grafting procedures using her cryopreserved fat within one year (three and six months after her first operation). She looked younger and healthy after these operations.

**Case 2: facial rejuvenation using cryopreserved fat [Figure 5]**
A 47 y.o. patient hated her bony forehead and complained of looking older than her real age. Her first operation involved facial fat grafting to her forehead (22.0 mL), malar area (3.0 mL each), cheeks (5 mL each), upper and lower eyelids (1.0 mL each, and 1.5 mL each, respectively) and chin (2.3 mL) with lower orbital fat removal. After her first operation, she received fat grafting using her cryopreserved fat two more times within two years (6 and 18 months after first operation). Postoperative photographs [Figure 5] are one year after her last fat grafting procedure using cryopreserved fat (3.5 years after her first injection), where she appears more youthful than she did prior to her first operation.

**Case 3: repeat rigotomy (needling) using cryopreserved fat [Figure 6]**
A 41 y.o. patient had undergone breast implant removal and simultaneous fat grafting (SIEF)\(^{[18,20]}\). However, three months after the operation, her right breast became deformed due to capsule contracture. Therefore, we performed a rigotomy (percutaneous aponeurotomy) with fat grafting using fresh fat followed by serial injections using cryopreserved residual fat. After receiving five total rigottomies with fat grafting (two with fresh fat, three with cryopreserved fat), her breasts developed a nearly natural appearance.
Figure 4. Case 1: facial rejuvenation using her cryopreserved fat. A: front view B: diagonal view. (left) Preoperative. (right) 6 months after second cryopreserved fat grafting. This figure is used with permission from Ohashi et al. published in Plast Reconstr Surg Glob.

Figure 5. Case 2: facial rejuvenation using her cryopreserved fat. A: front view B: diagonal view. (left) Preoperative. (right) 3.5 years after her first injection. This figure is used with permission from Ohashi published in Clin Plast Surg.
Case 4: hand rejuvenation using residual fat [Figure 7]
A 65 y.o. woman received fat grafting for breast augmentation and requested her cryopreserved residual fat be used as well. Four months after the first operation, she received fat grafting in her hands (16.0 mL for each hand) for hand rejuvenation without any additional harvesting needed.

DISCUSSION
Fat grafting is a major procedure for volume augmentation of areas such as the breasts, buttocks, and face. It has become clear that there are other merits of fat grafting including but not limited to skin rejuvenation, improve of fibrous scars, burn relief, alleviation of scleroderma symptoms, and healing of radiation damage. This is due to the revitalization/fertilization and regenerative effects of fat.[4,7,21,22]

However, one of the biggest demerits of fat grafting is the unpredictable nature of results involving varying maintenance rates for volume augmentation and the unpredictable degree of revitalization/fertilization and regeneration effects.

Due to varying effects, we often administer repeat injections to achieve satisfactory results. But repeat harvesting of fresh fat has detrimental impacts on patients with regard to pain and high costs. Therefore, it is in the interest to both aesthetic/plastic surgeons and patients to be able to preserve and use cryopreserved fat.

There are concerns regarding the viability of cryopreserved fat.[23,24] Many authors have recently suggested that slow cooling and fast thawing with cryoprotective agents may improve the viability of stored fat to a degree comparable to that of fresh fat.[25-27]
Improper cryopreservation techniques may compromise fat viability; however, with adequate cryopreservation techniques such as slow cooling and fast thawing, preservation at -196 °C (below -85 °C) and the addition of cryoprotective agents foster high fat viability [27-29].

We trust the CPC (CellSource Co. in Tokyo) to use proper cryopreservation techniques. Our responsibilities, therefore, only include packaging and sending residual fat. To use the cryopreserved fat, we then recall the fat [refer to Figure 1]. This ordering system allows small clinics to use cryopreserved fat without needing high-cost cell processing facilities on site. This makes it easier for clinics to start using cryopreserved fat safely while ensuring the stable quality of the fat. Having specialists conduct cell processing also benefits patients by preserving the quality of their fat for repeat injections.

Regarding volume and SVF count of cryopreserved fat, our results showed that the volume of fat decreased after cryopreservation; however, the amount of SVF per mL increased. This may be attributed to the fact that SVF (and adipose-derived stromal cells) is stronger than normal adipocytes in response to stress from factors such as ischemia, mechanical damage (during transportation), and cryopreservation [6]. According to our results, cryopreserved fat is very useful for rejuvenation and fertilization/revitalization.

Lastly, our cases indicated that cryopreserved fat has comparable viability as fresh fat; however, further studies are needed to compare the retention rate and regeneration effects of fresh and cryopreserved fat in a pathological study.

In summary, we did not experience any severe complications in any of our 216 patients over three and half years. Our results indicated that fat grafting with cryopreserved fat closely mimics that with fresh fat, making cryopreserved fat a safe and useful source for repeat fat grafting injections.

DECLARATIONS

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Authors’ contributions
The author contributed solely to the article.

Availability of data and materials
Not applicable.

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Conflicts of interest
The author declared that there are no conflicts of interest.

Ethical approval and consent to participate
In Japan, the Regenerative Medicine Safety Act came into effect as of November 25, 2014 under an institutional framework for promoting the implementation of regenerative medicine. This act, which covers clinical research and private practice, stipulates three risk-dependent standards and the procedures for notification of plans for regenerative medicine as well as the standards of cell culture and processing facilities and the licensing procedures to ensure the safety of regenerative medicine.
Consent for publication
The author obtained consent for publication from all patients we show in this article.

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