Viability of Fat Cells Over Time After Syringe Suction Lipectomy

The Effects of Cryopreservation

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Abstract: The purpose of this study was to determine the late decline in viability of fat cells over time for fat tissue stored at −15°C and −70°C after harvest from abdominal liposuction. A total of 16 females were recruited for this study. The viability of fat cell specimens was measured after freezing for 1, 3, 7, 14, 28, and 56 days. A number of viable mature adipocytes were evaluated by fluorescence microscopy after staining with fluorescein diacetate and propidium iodide. The glycerol-3-phosphate dehydrogenase activity was measured in lipoaspirates before digestion and the XTT reduction assay was performed. In addition, the XTT reduction assay was also performed on isolated lipocytes and preadipocytes.

The viability of mature adipocytes was very low for both the −15°C and −70°C samples after 1 day of freezing (13.3% ± 7.4% and 12.6% ± 6.3%, respectively). There was no statistically significant difference between the samples stored at 2 temperatures. The GPDH activity of the lipoaspirates frozen, for 1 day, at −15°C and −70°C was 25.1% ± 10% and 28.7% ± 11%, respectively. For the XTT test, the fractional enzyme activity of the lipoaspirates frozen, for 1 day, at −15°C and −70°C was 30.0% ± 10.9% and 36.1% ± 12.3%, respectively. In addition, the adipocytes had low activity from day one: 15.4% ± 7.2% at −15°C and 11.5% ± 5.6% at −70°C. Furthermore, the preadipocytes had a low activity of 8.0% ± 6.0% at −15°C and 8.6% ± 3.8% at −70°C. At 8 weeks, there were few viable mature adipocytes and the activity of the cells was very low by XTT and GPDH testing.

The results of this study showed that the viability of adipocytes declined rapidly after frozen storage for 1 day at both −15°C and −70°C, and decreased gradually in storage after 8 weeks; at which time only approximately 5% of the fat cells were alive. These findings suggest that the present fat preservation storage techniques using a −15°C freezer or a −70°C deep freezer are both inadequate to maintain the viability of fat cells.

Key Words: fat viability, cryopreservation, fat graft

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Autogenous fat grafts are used as soft tissue augmentation in the field of aesthetic and reconstructive plastic surgery; its clinical application is continuously expanding. However, the viability of grafted fat has been reported to be very variable, in the range of 30% to 80% depending on the investigation, and is the subject of continuous debate.1–4 The reasons for this variability include different donor site preparation techniques, harvesting methods, instruments used, fat grafting and injection techniques, grafting interval, and methods of analysis. All of these variations have made it difficult to compare studies.5–7

Most recently, significant amounts of adult stem cells have been found in aspirated fat tissues.8 Since these stem cells are now known to differentiate into fat, cartilage, bone, muscle, and nerve, adipose-derived stem cells have become a focus of attention in regenerative medicine.8–10 Harvested fat tissues can potentially be used, not just as graft materials for soft tissue augmentation, but for disease specific treatment in the near future. However, before these tissues can be used in the clinical setting, many unsolved problems need to be addressed. Safe cryopreservation of the aspirated fat tissues is one of these problems.

With the current procedures used, the fat absorption rate is frequently inadequate. Although there have been significant advancements made in this field, additional progress is needed because currently repeated grafting is often required to augment the tissue loss from fat absorption. Ideally, fresh fat should be harvested from patients for each grafting procedure. However, to minimize the inconvenience for patients, surgeons typically harvest enough fat initially and preserve tissues in a commercial refrigerator for later use. The typical storage temperature is about −20°C in the freezer. Reportedly, fat that has been stored for 3 to 12 months has been successfully used for regrafting. However, there is limited information on the clinical outcome of regrafting using stored cryopreserved fat tissues.

Prior studies on the time-dependant viability of cryopreserved fat tissues have shown a wide range of results. Schuller-Petrovic11 reported that slow freezing of the tissue to −20°C, shortly after harvesting, had no harmful effect on the adipocytes. Sommer and Sattler12 reported that live adipocytes were found after cryopreservation at −20°C for 3 years. However, Wolter et al13 reported that the adipocytes were destroyed after 48 hours of freezing at −20°C and that reuse of adipose tissues cryopreserved at −20°C provides an injection of mostly dead cells. These contradicting results have led to confusion about the effect of cryopreservation on adipose tissues.

Liu et al14 reported that cells maintain some degree of metabolic activity because they are only partially frozen at −20°C, the temperature provided by commercial freezers. The glass-transition temperature of water that completely stops a cell’s metabolic activity is −130°C. Therefore, many laboratories use −70°C as the appropriate temperature for their “deep freezer” to store cells and tissues.

The purpose of this study was to determine the late decline of viability in fat cells, over time, for fat tissue stored at −15°C in a common commercial refrigerator (R-B59AM, LG, Korea) that is used by many aesthetic surgeons and at −70°C in a deep freezer (Ultra low deep freezer, Sanyo, Japan) that is used in many laboratories. The goal of the study was to clarify some of the issues.

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The XTT assay were performed without any other procedure after the samples were checked in the same manner. The completed samples were checked at 1 minute intervals. After cooling, the completed samples were directly into the refrigerator and deep freezer. The temperature was measured by a sensor (Center 308 type k thermometer, Gilwoo, Seoul) and placed into a Sanyo, Japan) with a thermometer. The lipoaspirate was aliquoted into 1 mL E-Tube (microtubes, Axygen Scientific, California) with a thermo probe. The adipocytes were harvested from the fat tissues, and the adipocytes were collected after gravity separation for 5 minutes to minimize cell damage; then the adipocytes were separated from the pellets by centrifugation at 1000 rpm for 10 minutes.

Debated with regard to cryopreservation of fat tissues by objective analysis.

**MATERIALS AND METHODS**

**Fat Harvest and Cryopreservation**

Healthy patient volunteers (mean age, 37.5 years; standard deviation, 9.7; 16 women) undergoing elective liposuction body-contouring procedures provided consent to participate after study approval was obtained from Keimyung University, Dongsan Medical Center. Fat tissue specimens were harvested from the abdomen of the donors. The harvested areas were first injected with tumescent solution (Hartmann 1000 mL, 2% lidocaine 20 mL, 1:1000 epinephrine 1 mL). After 5 minutes, the fat tissues were harvested using a blunt-tipped cannula measuring 3 or 3.7 mm in diameter connected to a 60-mL Tulip syringe (Tulip BioMed, San Diego, CA). Liposuction aspirates were obtained by one surgeon. The lipopapillaroplasty (approximately 300–500 mL) was collected in a bottle at the time of liposuction and transferred immediately to the laboratory. The specimens were then spun at 3000 rpm for 2 minutes to separate the fat tissues; the lipopapillaroplasty were then separated into fat tissues, blood, cell debris, and supernatant. The blood, cells, debris, and supernatant were removed using a transfer pipette. The remaining layer was used for testing.

Assessment of the cell integrity was carried out by the viability staining test. Measurement of the cell stability was determined by the glycerol-3-phosphate dehydrogenase (GPDH) assay. The measurement of the metabolic activity was evaluated by the XTT assay (Viability staining test, 2 g; GPDH assay, 1 g; XTT assay: fat tissues, 0.5 g; adipocytes; 200 μL; preadipocytes, 2 g).

**The Freezing and Thawing Rate**

Five lipopapillaroplasty specimens from 5 donors were used to evaluate the actual freezing and thawing rate with a commercial refrigerator (R-B59AM, LG, Korea) and deep freezer (Ultra low deep freezer, Sanyo, Japan). The lipopapillaroplasty was aliquoted into 1 g samples in a 1.5 mL E-Tube (microtubes, Axygen Scientific, California) with a thermo sensor (Center 308 type k thermometer, Gilwoo, Seoul) and placed directly into the refrigerating and deep freezer. The temperature was checked at 1 minute intervals. After cooling, the completed samples were retrieved and then thawed at room temperature; the thawing rates were checked in the same manner.

**Fat Cell Isolation**

The cryopreserved fat tissues were thawed slowly at room temperature over 20 minutes. After thawing, the fat tissues were digested with 5 mL of 0.1% collagenase type I solution (Worthington Biochemical Corporation, Lakewood, NJ) in phosphate buffered saline at 37°C for 40 minutes, and the collagen degradation reaction was stopped with DMEM (Gibco BRL, Grand Island, NY) containing 10% FBS (HyClone, Logan, UT) with an equal volume following incubation. The treated specimens were stained with a 200 μm strainer and the adipocytes were collected after gravity separation for 5 minutes to minimize cell damage; then the adipocytes were separated from the pellets by centrifugation at 1000 rpm for 10 minutes.

**Analysis of the Isolated Fat**

**Mature Adipocyte Counts**

After the specimens were digested with collagenase and strained with a 200 μm strainer, the specimens were divided into an oil layer, a layer with mature adipocytes, blood, and layer with preadipocytes by gravity separation. The adipocytes with 400 μL were taken from the middle of the mature adipocyte layer. Twelve micromolar of fluorescein diacetate and 3750 nanomolar of propidium iodide were prepared in phosphate-buffered saline, and added to adipocytes in equal volume, and mixed by inverting 3 times. After 5 minutes, 10 μL of the adipocytes were placed on a disposable Hemocytometer (Digital Bio, Seoul, Korea) and examined under ultraviolet illumination with a fluorescence microscope, the Axivert 200 Microscope (Carl Zeiss, Gottingen, Oberkochen, Germany) at ×50. Viable adipocytes fluoresced bright green (particular around the edge), whereas dead cells appeared black; the nuclei of the dead cells fluoresced red owing to uptake of the propidium iodide. Three fields of view were evaluated for the analysis, and the mean number of stained cells, before and after freezing, were compared. The adipocyte size was limited to a maximum of 150 μm. Cells in excess of this size are illustrated by cysted oil (Fig. 2).

**GPDH Assay**

Measurement of G3PDH, dissolved in the intra extract solution (undamaged adipocytes; intracellular GPDH) and the extra washing solution (damaged adipocytes; extracellular GPDH) from fresh and freeze-thawed fat tissues, was carried out using the procedure reported by Ramsay et al. Measurements of G3PDH, dissolved in the intra extract solution (undamaged adipocytes; intracellular GPDH) and the extra washing solution (damaged adipocytes; extracellular GPDH) from fresh and freeze-thawed fat tissues, was carried out using the procedure reported by Ramsay et al. Measurements of G3PDH, dissolved in the intra extract solution (undamaged adipocytes; intracellular GPDH) and the extra washing solution (damaged adipocytes; extracellular GPDH) from fresh and freeze-thawed fat tissues, was carried out using the procedure reported by Ramsay et al.

Fat tissues were washed with phosphate-buffered saline and centrifuged at 2000 rpm for 3 minutes, then the washing solution was transferred to another tube. This process was performed again and the total washing solution (2 mL) was centrifuged at 15,000 rpm for 10 minutes at 4°C, and the extracellular GPDH of the damaged adipocytes was harvested. The fat tissues were transferred in a tube with 2 mL of phosphate buffered saline and homogenized with a tissue homogenizer (IKA-Werke GmbH & Co. KG, Staufen, Germany). The homogenate was then centrifuged at 15,000 rpm for 10 minutes at 4°C, and the intracellular GPDH of undamaged adipocytes was harvested.

The fat tissues were transferred in a tube with 2 mL of phosphate buffered saline and homogenized with a tissue homogenizer (IKA-Werke GmbH & Co. KG, Staufen, Germany). The homogenate was then centrifuged at 15,000 rpm for 10 minutes at 4°C, and the extracellular GPDH of the damaged adipocytes was harvested. The fat tissues were transferred in a tube with 2 mL of phosphate buffered saline and homogenized with a tissue homogenizer (IKA-Werke GmbH & Co. KG, Staufen, Germany). The homogenate was then centrifuged at 15,000 rpm for 10 minutes at 4°C, and the intracellular GPDH of undamaged adipocytes was harvested.

**Effect of Cryopreservation on Viability of Fat Cells**

**FIGURE 1. Timetable of cryopreservation and viability analyses.**

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preadipocytes, 2 g) directly after harvesting and after 1, 3, 7, 14, 28, and 56 days of cooling to −15°C and −70°C. The cell Proliferation Assay Kit (Wel Gene, Daegu, Korea) was used to measure cell viability. Briefly, the XTT reagent solution was mixed with Dulbecco Modified Eagle Medium (Gibco BRL, Grand Island, NY) of equal volume. The working solution was transferred into samples (fat tissues, 0.5 mL; adipocytes, 200 µL; preadipocytes, 100 µL). After 3 hours at 37°C, 100 µL of solution from each of the samples was transferred to a 96-well plate and the absorbance was measured at 450 nm and 690 nm (Microplate reader, Bio Rad, California). Heat-treated fat tissues, adipocytes, and preadipocytes (100°C, 10 minutes), served as a negative control. All values were expressed as relative values compared with the fresh samples. Measurement results of the fresh samples were calculated as a percent and the metabolic activity of the viable cells was assessed. Each sample had duplicated measurements performed.

Statistical Analysis
All data in this study were expressed as mean ± standard deviation. Statistical analysis was performed using the Mann-Whitney U test. A P value <0.05 was considered statistically significant.

RESULTS
The Freezing and Thawing Rate
The mean time to reach −15°C and −70°C was 21 minutes and 13 minutes, respectively (Fig. 3). The lipoaspirates that were placed in the deep freezer were frozen very rapidly compared with the commercial refrigerator. Within 1 minute, the specimens cooled to below zero in the deep freezer, while the specimens in the refrigerator took 4 minutes to reach 0, and then cooled slowly. The average thawing time at room temperature was 20 minutes in the commercial refrigerator and 27 minutes 30 seconds in the deep freezer.

Viability of Mature Fat Cells
After the assessment of cell integrity by viability staining tests, we found that the adipocyte viability of the fresh fat tissues was 79.7% ± 8.4%. After freezing at −15°C and −70°C for 1 day, we found that the adipocyte viability was 13.3% ± 7.4% and 12.6% ± 6.3%. There was a statistically significant decrease noted between the fresh samples and those that were frozen for one day (P < 0.05). A representative view of the fluorescence stained adipocytes is shown in Figure 2, A), a sample tested directly after harvesting, B), a sample frozen at −15°C for 1 day, C), a sample frozen at −70°C for 1 day, and D), a sample in cysted oil. Empty white arrows indicate viable adipocytes; white arrows indicate propidium iodide-stained nuclei; blue arrows indicate damaged cells; yellow arrows indicate oil (magnification ×50). Scale bar = 100 µm.
The stability of fresh fat cells was 82.4%.

GPDH Assay

Fluoresced more brightly green than did the viable cells at arrows indicate cysted oil staining. The viable cells at stained nuclei, blue arrows indicate damaged cells, and yellow arrows indicate viable adipocytes, white arrows indicate propidium iodide staining. Values are mean ± SEM; *P < 0.05.

TABLE 1. Assessment of Fresh and Cryopreserved Fat Tissue: Adipocyte and Preadipocyte Viability

<table>
<thead>
<tr>
<th>Rate (%)</th>
<th>No. of Viable Adipocytes (%)</th>
<th>GPDH Assay of Fat Tissues (%)</th>
<th>XTT Assay of Fat Tissues (%)</th>
<th>XTT Assay of Adipocytes (%)</th>
<th>XTT Assay of Preadipocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>79.7 ± 8.4</td>
<td>82.4 ± 4.93</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1 d</td>
<td>13.3 ± 7.4</td>
<td>25.1 ± 10</td>
<td>30 ± 10.9</td>
<td>15.4 ± 7.2</td>
<td>8.0 ± 6.0</td>
</tr>
<tr>
<td>-15°C</td>
<td>12.6 ± 6.3</td>
<td>28.7 ± 11.2</td>
<td>36.1 ± 12.3</td>
<td>11.5 ± 5.6</td>
<td>8.6 ± 3.8</td>
</tr>
<tr>
<td>-70°C</td>
<td>11.0 ± 2.9</td>
<td>14.0 ± 2.68</td>
<td>20.1 ± 4.59</td>
<td>13.4 ± 5.8</td>
<td>5.3 ± 3.2</td>
</tr>
<tr>
<td>3 d</td>
<td>12.3 ± 5.2</td>
<td>30.9 ± 5.88</td>
<td>29.4 ± 11.7</td>
<td>16.3 ± 9.5</td>
<td>8.9 ± 5.9</td>
</tr>
<tr>
<td>-15°C</td>
<td>9.8 ± 4.9</td>
<td>18.1 ± 9.09</td>
<td>16.0 ± 7.8</td>
<td>9.8 ± 4.8</td>
<td>3.3 ± 1.0</td>
</tr>
<tr>
<td>-70°C</td>
<td>9.3 ± 2.7</td>
<td>28.6 ± 8.34</td>
<td>24.6 ± 6.9</td>
<td>10.3 ± 6.1</td>
<td>4.0 ± 4.0</td>
</tr>
<tr>
<td>1 wk</td>
<td>8.8 ± 1.9</td>
<td>14 ± 2.85</td>
<td>15.1 ± 8.0</td>
<td>4.8 ± 3.4</td>
<td>1.1 ± 1.7</td>
</tr>
<tr>
<td>-15°C</td>
<td>9.3 ± 2.1</td>
<td>21.5 ± 7.87</td>
<td>22.6 ± 11.21</td>
<td>5.6 ± 3.8</td>
<td>3.3 ± 4.5</td>
</tr>
<tr>
<td>-70°C</td>
<td>9.3 ± 6.1</td>
<td>13.9 ± 6.03</td>
<td>10.0 ± 8.5</td>
<td>4.4 ± 4.5</td>
<td>0</td>
</tr>
<tr>
<td>2 wk</td>
<td>5.4 ± 4.2</td>
<td>18.9 ± 4.27</td>
<td>14.4 ± 7.7</td>
<td>4.7 ± 4.5</td>
<td>1.3 ± 1.2</td>
</tr>
<tr>
<td>-15°C</td>
<td>5.8 ± 6.8</td>
<td>16.1 ± 4.05</td>
<td>4.8 ± 3.8</td>
<td>1.9 ± 3.4</td>
<td>2.1 ± 4.2</td>
</tr>
<tr>
<td>-70°C</td>
<td>4.5 ± 4.0</td>
<td>18.8 ± 3.14</td>
<td>6.6 ± 2.4</td>
<td>5.1 ± 6.8</td>
<td>2.3 ± 4.5</td>
</tr>
</tbody>
</table>

GPDH indicates glycerol-3-phosphate dehydrogenase.

After freezing at -15°C and -70°C for 3, 7, 14, 28, and 56 days, we found that the cell stability was 14.0% ± 2.68% and 30.9% ± 5.88% (at 3 days), 18.1% ± 9.09% and 28.6% ± 8.34% (at 7 days), 14% ± 2.85% and 21.5% ± 7.87% (at 14 days), 13.9% ± 6.03% and 18.9% ± 4.27% (at 28 days), and 16.1% ± 4.05% and 18.8% ± 3.14% (at 56 days). The samples frozen at -70°C had higher activity than did the samples frozen at -15°C for all times evaluated. There was a statistically significant difference at 3 and 7 days between the 2 temperature levels (P < 0.05) (Fig. 5, Table 1); these values correlated with the decreased cellular integrity.

XTT Assay

Lipoaspirates

The measurement of the fat tissue metabolic activity by the XTT assay showed that the fresh samples had 100% activity. After freezing at -15°C and -70°C for 1 day, we found that the metabolic activity was 30% ± 10.9% and 36.1% ± 12.3%. There was a statistically significant decrease between the fresh samples.
and the one day frozen samples \((P < 0.05)\). After freezing at \(-15°C\) and \(-70°C\) for 3, 7, 14, 28, and 56 days, we found that the cell stability was 20.1% ± 4.59% and 29.4% ± 11.7% (at 3 days), 16.0% ± 7.8% and 24.6% ± 6.9% (at 7 days), 15.1% ± 8.0% and 22.6% ± 11.21% (at 14 days), 10.0% ± 8.5% and 14.4% ± 7.7% (at 28 days), and 4.8% ± 3.8% and 6.6% ± 2.4% (at 56 days). Samples frozen at \(-70°C\) had higher activity than did the samples frozen at \(-15°C\) for all times evaluated. There was a statistically significant difference at 7 days between the temperature levels \((P < 0.05)\) (Table 1); this value correlated with decreased cellular metabolism.

Adipocytes

The measurement of the adipocyte metabolic activity by the XTT assay showed that the fresh samples had 100% activity. After freezing at \(-15°C\) and \(-70°C\) for 1 day, the metabolic activity was 8.0% ± 6.0% and 8.6% ± 3.8%. There was a statistically significant decrease between the fresh and 1 day frozen samples \((P < 0.05)\). After freezing at \(-15°C\) and \(-70°C\) for 3, 7, 14, 28, and 56 days, we found that the cell stability was 5.3% ± 3.2% and 8.9% ± 5.9% (at 3 days), 3.3% ± 1.0% and 4.0% ± 4.0% (at 7 days), 1.1% ± 1.7% and 3.3% ± 4.5% (at 14 days), 0% and 1.3% ± 1.2% (at 28 days), and 2.1% ± 4.2% and 2.3% ± 4.5% (at 56 days). There was no statistical difference between the temperature levels (Fig. 6, Table 1); these values correlated with decreased cellular metabolism.

DISCUSSION

Viability of fresh fat and cryopreserved fat at \(-15°C\) and \(-70°C\) was analyzed chronologically by fluorescein diacetate/prodium iodide (FDA/PI) fluorescence staining, intracellular GPDH activity, and the XTT assay. We observed that the viability of the cryopreserved fat rapidly decreased to below 80% on the first day of freezing independent of the temperature. These findings suggest that \(-15°C\) is not an ideal temperature for lipoaspirate fat preservation. In addition, our results showed that even at \(-70°C\), which is thought to be the ideal preservation temperature for cells and tissues, there was no difference in the cell viability.

The accurate analysis of fat cell viability is challenging because mature human adipocytes do not proliferate or adhere well to culture conditions; cell integrity and viability can not be tested with proliferation tests used for fibroblasts and preadipocytes. Therefore, adipocytes are studied under the electron microscope.
with trypan blue staining to determine cell viability.12,19 Even this method is technically limited because advanced skills and experience are required for accurate analysis. Further, dead cells without nuclei or mitochondrial activity may be interpreted as live cells when the shapes of the cells appear normal under the microscope. This technical bias may be one of the major contributing factors for the wide range of results reported.

The methods used for cell viability analysis are categorized mainly into 2 groups: cell membrane integrity assays and functional assays. The integrity assay includes trypan blue, FDA/PI stains, and the GPDH assay. The functional assays include MITT, XTT, and acid phosphatase. These techniques are currently used for adipocyte viability analysis.13,20

The FDA/PI fluorescence stain can be a useful tool for distinguishing living cells from nonliving cells. Under the fluorescence microscope, the living cells are seen with a green stain when the intracellularly absorbed fluorescein diacetate is converted to fluorescein by nonspecific esterase. By comparison, the dead cells are seen with a red stain when propidium iodide penetrates the cells through a damaged membrane and incorporates into the nuclei (Fig. 2). To date, there are no studies on FDA/PI staining used to directly count the number of stained cells among mature adipocytes for viability measurements. However, in this study such measurements were performed and the mature adipocytes from the fresh fat tissues showed about an 80% viability; however, after 1 day of cryopreservation at −15°C and −70°C, the viability was reduced to 13.3% and 12.6%, respectively. Thereafter, the viability of the adipocytes gradually diminished for 8 weeks until the statistical end point was reached, and no significant difference in the cell viability was observed between the 2 temperature groups. By contrast, although a significant portion of the adipocytes was lost on the first day of cryopreservation, those cells that survived were viable for about 2 months under the conditions of cryopreservation.

The GPDH assay detects cell membrane integrity by marking the escaped intracellular elements that pass through the damaged membrane. All of the chronological analyses of adipocytes showed that the extracellular GPDH levels were higher at −15°C compared with −70°C and this difference was statistically significant for day 3 and 7 of cryopreservation. This indicates that the cryopreservation of the adipocytes was superior at −70°C than at −15°C. According to Wolter et al.,13 the XTT results suggested more viable cells, and the GPDH level was significantly higher at −80°C when compared with −15°C. They suggested that more cells were destroyed, but that the surviving intact cells were more viable at a storage temperature of −80°C. This contradicts our study results.

The XTT assay is a functional assay that uses a microplate reader to detect water soluble orange formazan XTT that is converted from yellow tetrazolium salt XTT in metabolically active cells. Compared with the XTT levels in lipoaspirated fresh fat, fat XTT levels after 1 day of cryopreservation at −15°C and −70°C were 30% and 36.1% of the fresh fat, respectively; the XTT levels were generally higher for all other cryopreservation time points at −70°C compared with −15°C. This finding suggests that the metabolic activity of the cells preserved at −70°C was higher indicating more viable cells were preserved at the lower temperature. From the results of the XTT and the GPDH analysis, we observed that the viability and metabolic activity of the preserved cells at −70°C was superior to those at −15°C, contradicting the findings of Wolter et al.13 The XTT levels from the filtered adipocytes, from the lipoaspirated fats that underwent collagenase treatment, were 15.4% and 11.5% with cryopreservation at −15°C and −70°C compared with the fresh fat. The XTT levels of the further segregated processed preadipocytes were 8.0% and 8.6% at −15°C and −70°C. As demonstrated, the metabolic activity of the processed adipocytes was lower than the lipoaspirates, and the processed preadipocytes had lower metabolic activity than the adipocytes. These findings show that cell damage can occur due to other factors in addition to cryopreservation such as collagenase reactions and the cell separation process. This suggests that cell viability may be maximized by minimizing manipulations of the lipoaspirated fat cells.

Cell damage caused by cryopreservation has been explained by following 2 theories: intracellular ice formation and osmotic stress.21 Because cells are partially frozen at −15°C, the metabolic activity of the cells is still present. A temperature of −130°C is required to stop metabolic activity completely; however, from a practical point of view, temperatures this low may cause additional cell damage.13 Therefore, many have chosen −80°C for cryopreservation. Recently, Matsumoto et al22 demonstrated that the stem cell harvest rate, from cryopreserved fat cells that were frozen 1°C every 15 minutes to −80°C, for 1 month, were noticeably reduced compared with fresh fat. The controlled-rate slow freezing, which reduces intracellular ice formation, and rapid thawing in a 37°C water bath, which reduces recrystallization has been considered the method to use to reduce cell damage. The results of the actual freezing and thawing rates, following the current protocol of the aesthetic surgeon, revealed that the rapid freezing and slow thawing had the opposite effects of what was expected for safe preservation. The centrifuged fat that was aliquoted into a sterile syringe and placed into a −20°C freezer, is as the current standard clinical practice, was confirmed to be inadequate for cryopreservation.

Consistent with other studies, our study results demonstrated that cryopreservation of lipoaspirated fats at −15°C and −70°C results in a limited number of viable cells. By contrast, fat grafting from a harvest of 5% fat cells that were still viable after 8 weeks of cryopreservation might be effective, and is supported by anecdotal clinical evidence of partially successful fat grafting with cryopreserved fat.

It is not yet clear how quickly cryopreserved fat cells deteriorate. MacRae et al23 and Wolter et al13 showed that cell viability is significantly reduced within the first 2 days of cryopreservation at −20°C; however, they did not evaluate the viability during the first 24 hours of cryopreservation. Our results showed that most fat cells are destroyed within the first 24 hours of cryopreservation at −15°C and −70°C and that the surviving cells were intact for 8 weeks. Matsumoto et al22 reported that fat cells preserved at room temperature were markedly damaged during the first 24 hours, and that the stem cell harvest rates for fats preserved at 4°C, compared with fresh fat, were markedly reduced. Therefore, most prior studies, in addition to our findings, have shown that most of the cells from lipoaspirates, even those with minor injury, die during the first 1 to 2 days of cryopreservation. Therefore, for effectively maintaining viability of lipoaspirates, an appropriate cryoprotective agent should be used in addition to cryopreservation.13,24,25 Further studies are needed to develop safe and clinically practical preservation methods.

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