Adipose Tissue Preservation: Comparison of four reagents with storage at low temperature

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In clinical plastic surgery practice, frequently autologous fat transfer is performed. This procedure is performed for the purpose of treating soft tissue deformities or for cosmetic surgery, where abdominal fat—after suction—might be transferred to the breast or glabellar area.

In procedures like abdominoplasty and liposuction, there is abundant fat available, and when fat grafting is performed concurrently with each procedure, donor site morbidity is not clinically relevant for the purpose of the fat grafting procedure.

Thus, a second fat grafting treatment is often needed, and unless the original fat cells collected from the donor site are adequately preserved, cells must be collected once again.

For the group undergoing cryopreservation, four 15 mL falcon tubes were labeled “A”, “B”, “C”, and “D” as below.

Instead, these four formulas were used because the literature has used these chemicals (DMSO, HSA, and/or Trehalose) independently.

In this study, through visual analysis, we compared the efficacy of 4 different cryopreservation agent formulas labeled A, B, C and D as above in Table 1 in preserving adipocyte structure and fatty tissue histology after cryopreservation with each of the formulas and then staining by the three techniques of H and E, Trichrome and Perilipin.

In this experiment we combined the chemicals to get combined benefits. Adipocytes were then divided into two groups: one group that would undergo cryopreservation and another group that would not but would be used for controls as fresh fat.

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All the slides show the typical viable fatty tissue. Many adipocytes in their characteristic "honeycomb-like" architecture. There are also some blood vessels with red cells in them. Adipocytes are rounded in shape, with their nucleuses pushed towards the periphery. Their appearance is more consistent with white than brown adipose tissue (cells have a single large vacuole of triglyceride rather than the multiple vacuoles characteristic of brown adipose tissue).

In conclusion, no difference in the microscopic appearance of fatty tissue architecture or individual fat cell or even stromal structure was observed between the fresh fat tissue samples and the cryopreserved tissue after fourteen days. Thus, these results indicate that each of the four cryopreservation formulas lead to excellent preservation of both fat tissue structure integrity and even fat cell viability, with no apparent difference between the four formulas. Further investigation can proceed with any of these cryopreservatives.

Cryopreservant Solution Preparation

Following a literature review, 4 cryopreservation solutions were prepared with the following compositions:

Table 1:

| Cryopreservant A: 10% DMSO + 2% HSA |
| Cryopreservant B: 10% DMSO + 2% HSA + 0.5 M Trehalose |
| Cryopreservant C: 20% DMSO + 2% HSA |
| Cryopreservant D: 20% DMSO + 2% HSA + 0.5 M Trehalose |

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For the group undergoing cryopreservation, four 15 mL falcon tubes were labeled “A”, “B”, “C”, and “D” as below.

These four tubes were then left in 4 degree Celcius freezer and then after 14 days, cryopreserved adipocytes were fixed, sectioned, stained by H&E, Masson’s Trichrome, and Immunohistochemistry using Perilipin antibody, and imaged via electron microscopy (images are presented in the Results section below).

For each of the three stains (H and E, Trichrome, and Perilipin), three sections of each treatment (no treatment/control, cryopreservant A, cryopreservant B, cryopreservant C, cryopreservant D) were generated. For each of the three stains (H and E, Trichrome, and Perilipin), three sections of each treatment (no treatment/control, cryopreservant A, cryopreservant B, cryopreservant C, cryopreservant D) were generated.