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

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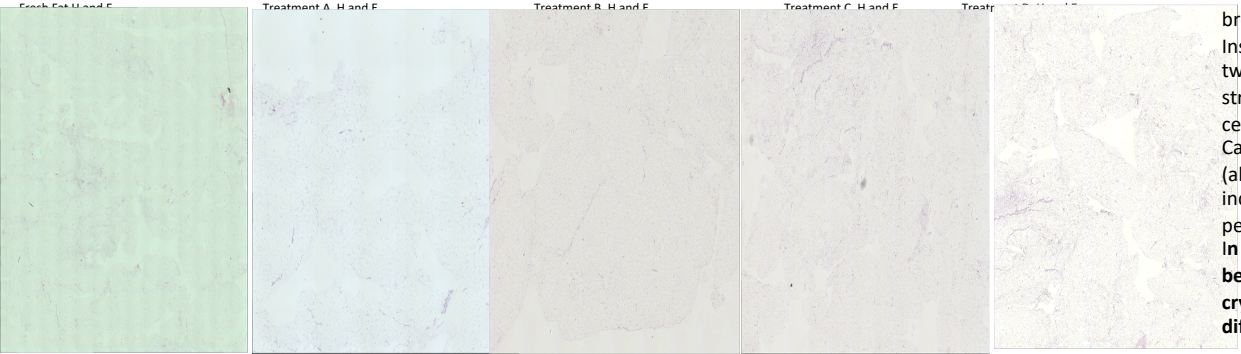
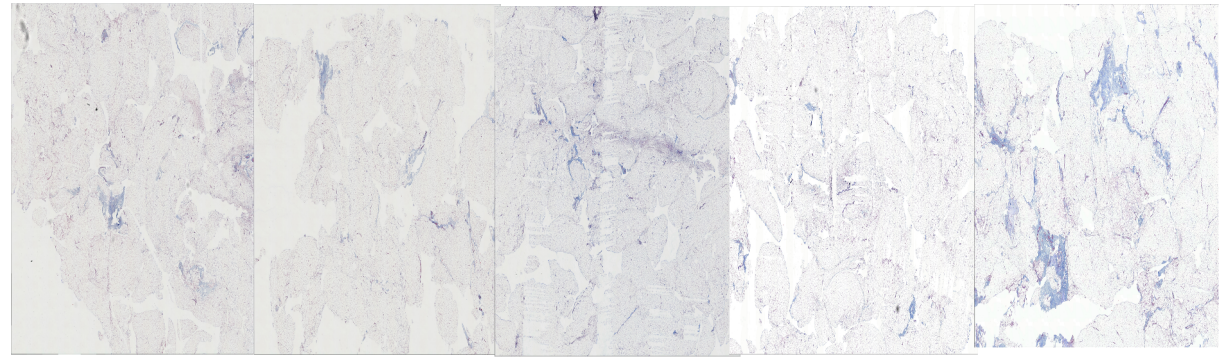
- In clinical plastic surgery practices, frequently autologous fat transfer is performed. This procedure is performed for the purpose of treating soft tissue deformities or for cosmetic surgery, where abdominal fat—for instance—might be transferred to the breast or gluteal area.
- In procedures like abdominoplasty and liposuction, there is abundant fat available, and when fat grafting is performed concomitantly with such procedures, donor site morbidity is not clinically relevant for the purpose of the fat grafting procedure.
- However, fat grafting is not an efficient procedure, as 37% of the total grafted fat cell volume does not heal well and does not persist long-term and hence is lost from the recipient site.
- Thus, a second fat grafting treatment is often needed, and unless the original fat cells collected from the donor site are adequately preserved, fat cells must be collected once again. This raises the issue of donor site morbidity because the original donor site fat may no longer be available.
- Given these clinical issues, a good method for cryopreserving adipose tissue for the purpose of repeat tissue grafting procedures is required.
- The overall propose of this study is to assess efficacy of four different cryopreservants in the long-term preservation of adipose tissue, with the aim of eventually using cryopreserved adipocytes in tissue grafts.
- To achieve this purpose, adipocytes were cryopreserved for 14 days, and then their structure was analyzed by electron microscopy to assess 1.) viability of fatty tissue and 2.) integrity of the adipocyte structure.
- Cryopreservant Solution Preparation
- Following a literature review, 4 cryopreservant solutions were prepared with the following compositions:

Table 1:

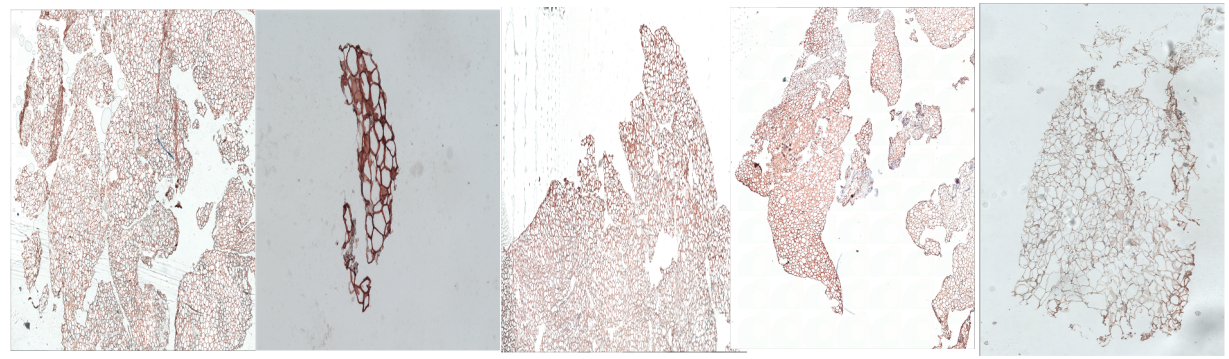
- Cryopreservant A: 10% DMSO + 2% HSA
- Cryopreservant B: 10% DMSO + 2% HSA + 0.2 M Trehalose
- Cryopreservant C: 20% DMSO + 2% HSA
- Cryopreservant D: 20% DMSO + 2% HSA + 0.2 M Trehalose
- These four formulations were used because the literature has used these chemicals (DMSO, HSA, and/or Trehalose) independently.
- 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.
- For the group undergoing cryopreservation, four 15 mL falcon tubes were labeled “A”, “B”, “C”, and “D” corresponding to the same alphabet crryopreservant as in Table 1. Then, into each tube, 7 mL of the corresponding cryopreservant and 7 mL adipocytes were added.
- These four tubes were then left in 4 degrees Celsius 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 as below).
- The other group of adipocytes were not cryopreserved. Instead, these adipocytes were fixed, sectioned, stained by H&E, Masson’s Trichrome, and Immunohistochemistry using Perilipin antibody, and imaged via electron microscopy and analyzed as fresh fat and used as control against the frozen fat samples as 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.



Fresh Fat, Trichrome | Treatment A, Trichrome | Treatment B, Trichrome | Treatment C, Trichrome | r | Treatment D, Trichrome



Fresh Fat, Perilipin | Treatment A, Perilipin | Treatment B, Perilipin | Treatment C, Perilipin | Treatment D, Perilipin



In this study, through visual analysis, we compared the efficacy of 4 different cryopreservation agent formulas labeled A, B, C and D as above inTable 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.

The pictures labeled as one of the three formulas that is as Treatment A, B, C or D (Table 1) are after 14 days of cryopreservation using each of the formulas and treated with each of the three types of stains. Only the “fresh fat” slides are from day 0 and with no treatment and serve as controls to compare the histological appearance for assessment of viability.

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).

Inspecting the stromal vascular viability using stromal vascular fraction across all slides showed maintained stromal integrity and structure following two days of cryopreservation. The stromal vascular fraction contains smooth muscle cells, endothelial cells, and mature adipocytes. The fact that stromal integrity was maintained after 14 days makes it more likely that the overall integrity of the fatty tissue was also maintained. Viable stromal cells suggest that a fat grafting procedure will be successful.

Careful visual examination of the slides revealed normal fat cells predominating in almost the whole slide for all the figures presented in Results (although it is worth nothing that Treatment A, Perilipin stain, contained few adipocytes, probably because the section for the sample had incidentally few adipocytes on it). Each individual fat cells appears with a typical signet ring shaped plasma membrane and features a nucleus at the periphery, a visible fat droplet, and a thin layer of cytoplasm.

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 cryopreservants.