Cryopreservation of Umbilical Cord Tissue for Stem Cell Harvesting

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Executive Summary

Stem cell transplantation has become an important process used to treat patients with bone marrow diseases. When implanted into patients, stem cells from the umbilical cord have been found to successfully proliferate as new neurons and glia, thereby improving the patients' health. Neurons and glia are imperative for the health and normal function of our nervous system. Neurons are electrically active cells that can produce action potentials to transmit signals based on electrochemical impulses. Glia, which comprise a large part of our nervous systems (90% of the brain alone), were once dismissed as mere padding in the nervous system. However, it is now known that they are actually an integral component of the system, serving to facilitate and ensure the proper transmission of signals between neurons. Damage to or loss of neural cells, whether due to physical injury, removal (as in the case of cancer) or diseases such as Motor Neuron Disease (MND) and Parkinson's disease is severely detrimental to one's health. Using current tissue engineering technology, stem cells harvested from the matrix of the umbilical cord (known as Wharton's Jelly), may be differentiated into neurons or glia, effectively replacing those that were lost or damaged. To ensure biocompatibility, umbilical cord matrix cells from direct relatives are used. Therefore, cryopreservation of these cells is imperative to the stem cell treatment to be used in the future. Our goal is to use FIDAP and GAMBIT software solutions and mesh to compare the effectiveness of glycerol, propylene glycol, and DMSO, three commonly used cryopreservatives, in order to determine the cryopreservation agent that will maximize viability of umbilical cord stem cells.

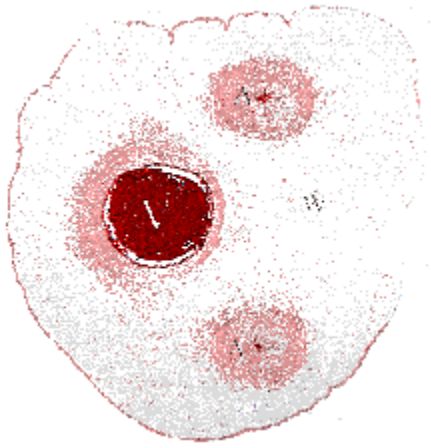
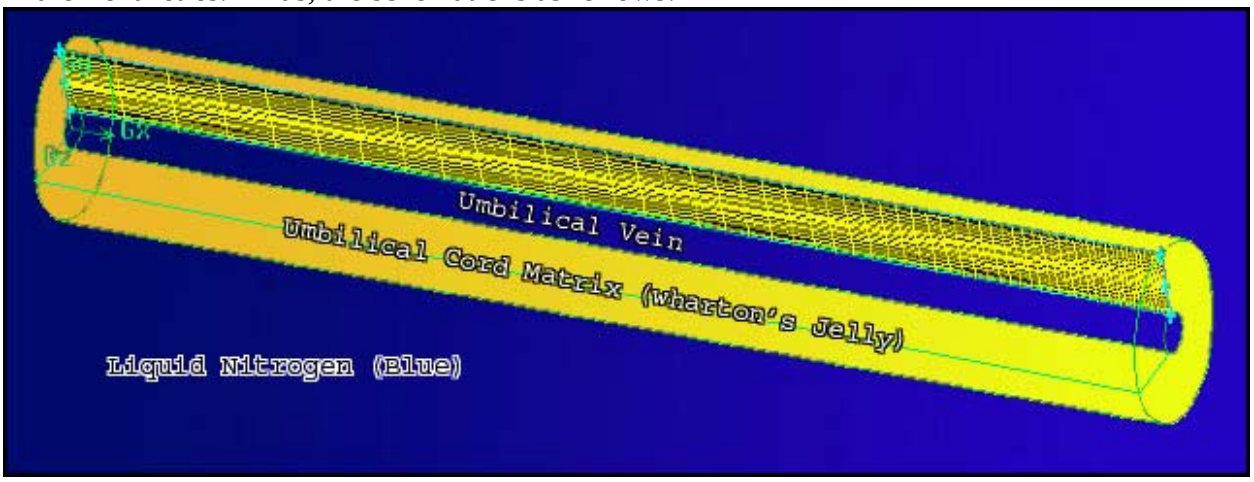


Figure 1. Cross section of normal umbilical cord. Embedded within a spongy, proteoglycan rich matrix know as Wharton's jelly (W) are normally two arteries (A) and one vein (V).
Source:
http://info.med.yale.edu/obgyn/kliman/Placenta/Articles/EOR_UC/Umbilical_Cord.html

Design Objectives and Introduction

The purpose of our project is to compare the percent viability of umbilical cord tissue upon vitrification using three different cryopreservation agents (CPAs). Percent viability refers to the mass of tissue that survives the cryopreservation process per mass of total tissue used. We will simulate cryogenic cooling with liquid nitrogen using maximum allowed concentrations of the CPAs dimethyl sulfoxide (DMSO), propylene glycol, and glycerol. These maximum concentrations of each CPA come from prior research and are used with the assumption that they will yield lower cooling rates necessary to vitrify and thus are more beneficial to cell viability.

The axial-symmetric geometry is used to model umbilical cord tissue because the cord is assumed to be symmetric on both the x and y-axis. This cylindrical cord tissue is 3.6 cm in diameter and has a concentric hollow tube concentric with a smaller 8 mm diameter to account for the single vein within the umbilical cord. According to our model, the hollow cylindrical cord tissue is in contact with liquid nitrogen at –196C both on its inner (vein) and outer surfaces in their entireties. Thus, the schematic is as follows:



In order to develop our umbilical cord tissue cryopreservation model, a number of assumptions were made. They were as follows:

1. Cryoprotectants are evenly distributed in the umbilical cord tissue.
2. Muscle tissue properties approximate stem cell properties.
3. No convective cooling.
4. Constant material properties.
5. No heat generation.
6. Effects of cooling in umbilical arteries can be neglected.
7. The umbilical cord is symmetric.
8. Cooling rates to achieve vitrification are approximated by highest survivability data obtained from the literature.
9. The umbilical cord tissue is submerged in a large reservoir of liquid nitrogen with no fluid flow.
10. The umbilical cord tissue is initially at room temperature.

Once the cooling rates for ethylene glycol, DMSO, and glycerol are calculated, they can be compared with the survivability of umbilical cord tissue data obtained from the literature. By our definition, cells are viable if they achieve 50% survivability. This percentage is adequate because a culture can be grown to replenish the lost cells. In consequence, the optimal cryopreservation agent can be determined with respect to maximization of umbilical cord stem cell preservation time.

The cooling rate profiles were found by using a function from FIPOST to create a FIOUT file. This FIOUT file was a temperature-time history profile for each of our 20 nodes at the center of our mesh. We found the cooling rates at each node by finding the slope of the time vs. temperature plot (between time 0 to time 105.1 sec). From the time history plots (Appendix C), we can see that the cooling rates computed from these two times will be the initial (linear portion) cooling rate. Furthermore, it is standard practice to submerge the umbilical cord in liquid nitrogen for slightly more than 60 seconds for cryopreservation. These values were plotted against the node number using Microsoft Excel. The critical cooling rates for each cryopreservant were obtained from the literature (see Appendix D). These critical cooling rates were compared with our calculated cooling rate profiles to determine the portions of the umbilical cord, based on our mesh that will survive.

Glycerol

For 50% survivability, the cooling rate needs to be above 70 C/min (see Appendix D for data from literature). Therefore, the nodes between 207 and 216 are not viable. By taking the ratio of the volume of viable nodes (which is proportional to the square of the radius of the portion as obtained from the mesh) to the total volume of the model, we found that 12.76% of the sample achieves 50% survivability.

DMSO

For 50% survivability, the cooling rate must be between 0.5C/min and 50C/min. Therefore, the nodes between 1064 and 1058 are viable. Using the same formula as for glycerol, we found that 18.37% of the sample achieves 50% survivability.

Propylene Glycol

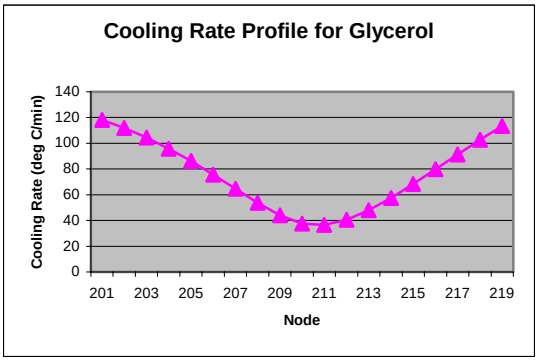
For 50% survivability, the cooling rate must be below 100C/min. Therefore, the nodes between 65 and 72 are viable. As a result, 73.9% of the sample achieves 50% survivability.

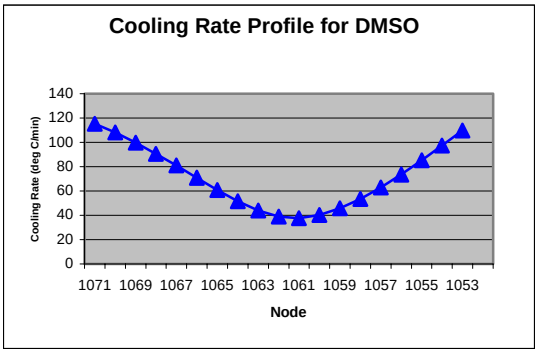
For all three cryoprotectants in solution, we found that when the umbilical cord tissue was first immersed (t=2 seconds) in the –196 C liquid nitrogen, the three surfaces (top, right, inner) immediately cooled to a much lower temperature than the rest of the tissue. However, by the time 105 seconds had elapsed, the temperature drop was better distributed throughout the tissue with only the middle cylindrical shell not being as affected. At the center of the umbilical cord (left side of the axis-symmetric model), we plotted the temperature of nodes 195 (glycerol), 192 (DMSO), and 160 (propylene glycol) as a function of time. From these plots, we saw that in all three cases, the temperature exponentially decays. (see Appendix C)

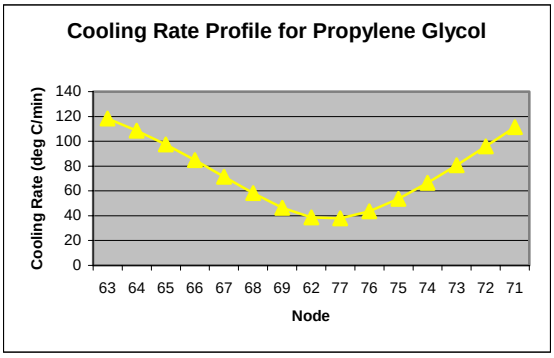
Results and Discussion

Cooling Rate Profiles for CPAs at centers

A. 14.3% Glycerol as cryoprotectant B. 6.06% DMSO as cryoprotectant C. 7.86% Propylene Glycol as cryoprotectant

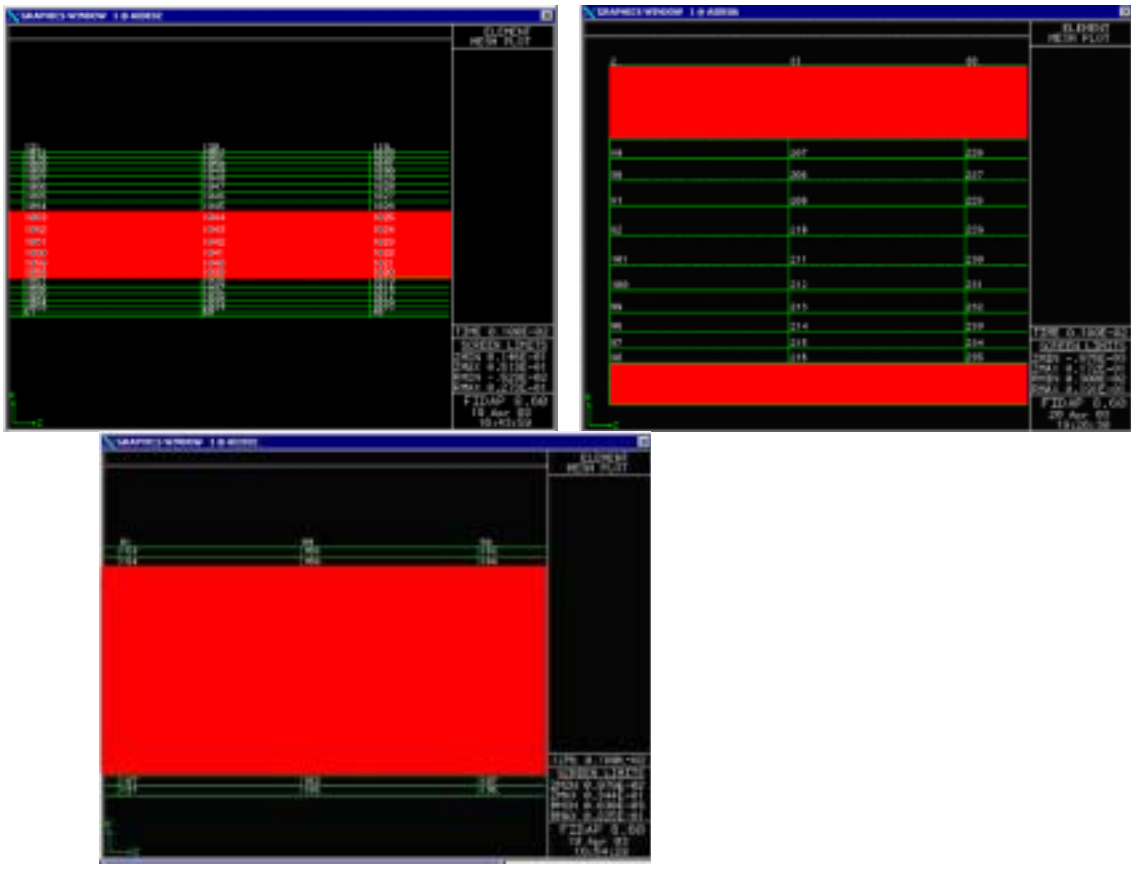






<u>Viability of cells (red = living) due to</u>

A. 14.3% Glycerol as cryoprotectant B. 6.06% DMSO as cryoprotectant C. 7.86% Propylene Glycol as cryoprotectant

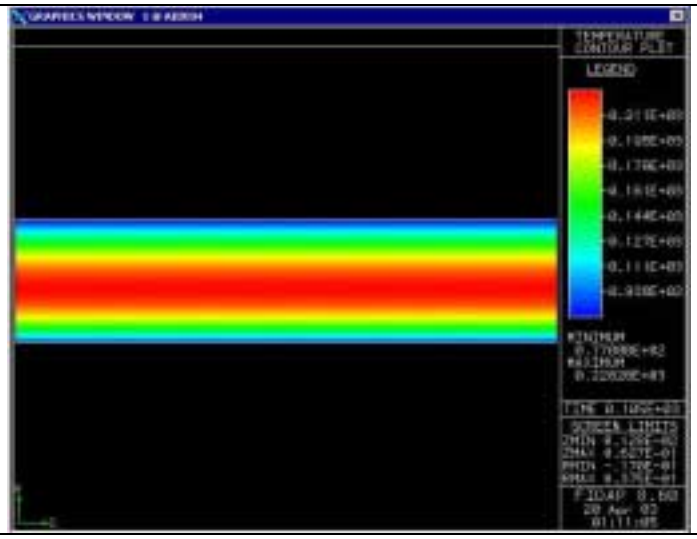
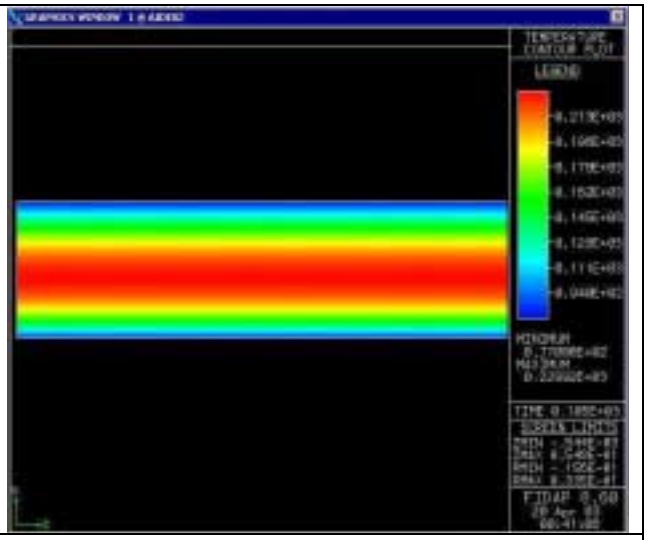
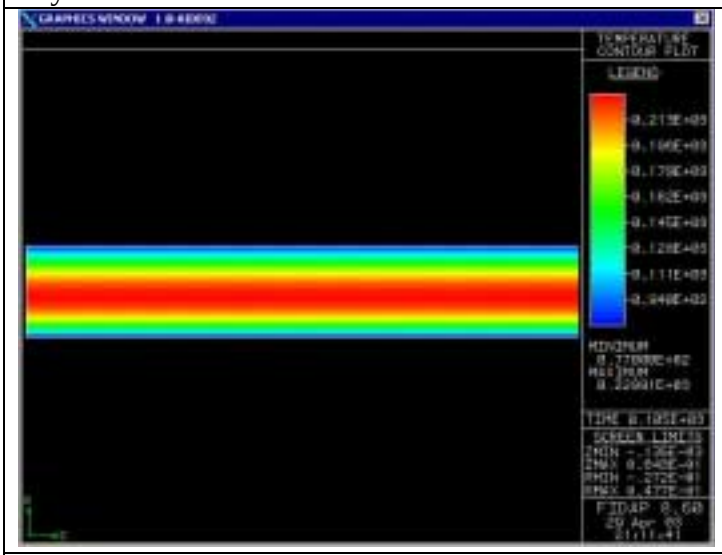
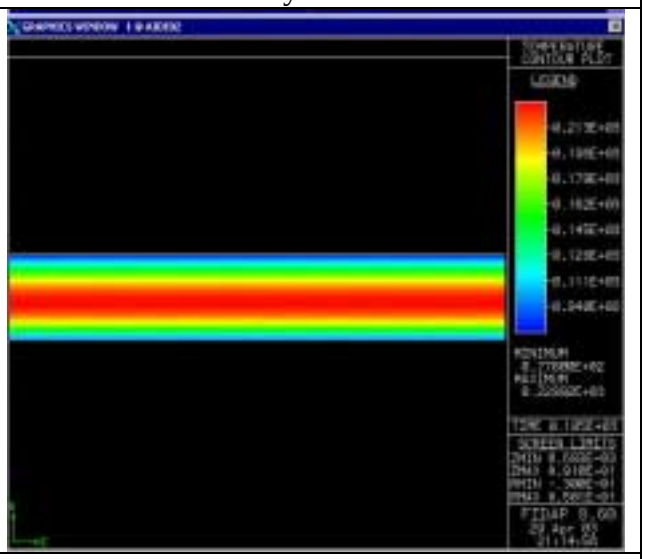


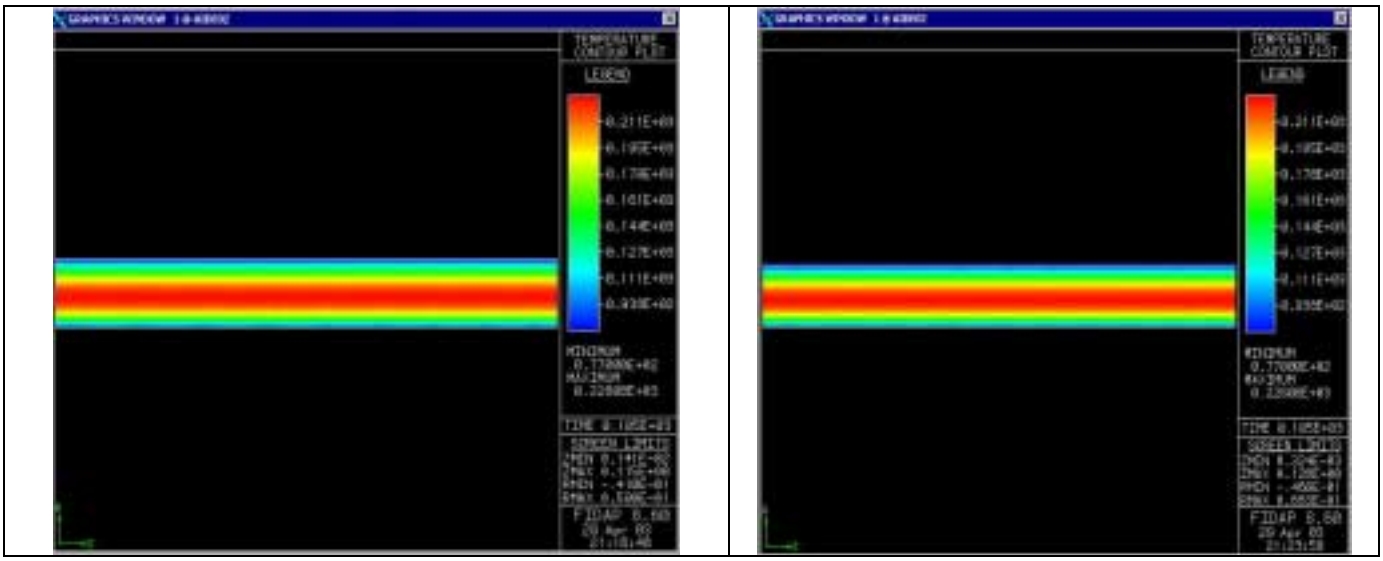
Sensitivity Analysis

The sensitivity analysis showed that individually changing the density, thermal conductivity, and specific heat by 10% did not have a significant effect on the temperature-time profile. Because of toxicity levels, only small concentrations of cryopreservation agents could be used. As a result, the media had properties that were very similar to those of human tissue. Therefore, it can be concluded that the property values we obtained from the literature were accurate enough for our model. Refer to Appendix C for the figures.

Our sensitivity analysis also showed that changing the concentrations of the cryopreservation agents did not have a significant effect on the temperature-time profiles. When the concentrations of propylene glycol, DMSO, and glycerol in the umbilical cord tissue were increased by twenty percent, the temperature contours remained relatively constant. Thus, using cryopreservant concentrations that were equal to the toxicity levels is sufficient for maximum survivability.

Original temperature contour plot at t = 105 for Propylene Glycol	After increasing the concentration of Propylene Glycol by 20%

	
Original temperature contour plot at t = 105 for Glycerol	After increasing the concentration of Glycerol by 20%
	
Original temperature contour plot at t = 105 for DMSO	After increasing the concentration of DMSO by 20%



Because of the different properties of each CPA and their specific cooling rates that determine the survivability of tissue, we were unable to predict which cryoprotectant agent would yield the highest survivability before running our model. However, because current studies seem to use DMSO more often than the others, it was though likely to be the optimal CPA for vitrification. Our results showed that glycerol yielded the lowest percent survival (12.76%), then DMSO (18.37%), and propylene glycol yielded the highest percent survival (73.9%). These results are shown graphically in the "Results and Conclusions" section. We also constructed a model for the CPA ethylene glycol, but we were unable to find the relationship between the cooling rate and percent survivability of cells in any published literature. Further study would be needed to make any conclusions.

Conclusion and Design Recommendations

Based on our analysis, propylene glycol gives the largest portion of umbilical cord tissue with 50% survivability out of the three cryopreservation agents. Our model indicates that 73.9% of the cord tissue will be viable upon vitrification.

Although our results were suitable for this project, there are some things that can be taken into consideration in the future if our project were to be refined. The properties that were used in our governing equation were based on muscle tissue properties from published literature because those for umbilical cord tissue were unavailable. Seeing that fetal tissue has some physiological differences to adult muscle tissue, using exact values for tissue properties could give a more precise result.

We would also recommend that our design be improved by including the effects of cooling in the umbilical arteries. The two arteries found in the umbilical cord tissue are small in diameter compared to the one large vein, but they could cause asymmetrical cooling in the tissue if liquid nitrogen were allowed to fill them. In addition, the structure of the umbilical cord in

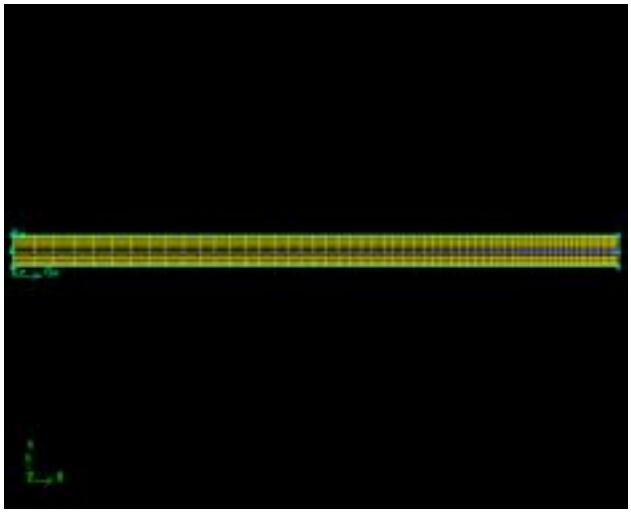
real life would not be totally symmetric. Therefore, to get more accurate results, the umbilical cord should be modeled in 3-D.

For cost considerations, propylene glycol or glycerol would be the more inexpensive choices of the three cryoprotectants that we used. They were listed as costing approximately $25 and $29 respectively for 500 mL, compared to $41 for 500 mL of DMSO. Because our model found that propylene glycol generated the highest percentage of cell viability, combined with its low price it is the best choice of cryopreservation agent for umbilical cord preservation.

Another option in stem cell harvesting would be to cryopreserve the umbilical cord blood from the two arteries and the vein rather than the tissue itself. Because it is more cost effective to preserve vials of a liquid instead of an entire sample of human tissue, it would be financially advantageous for researchers to work with umbilical cord blood rather than umbilical cord tissue.

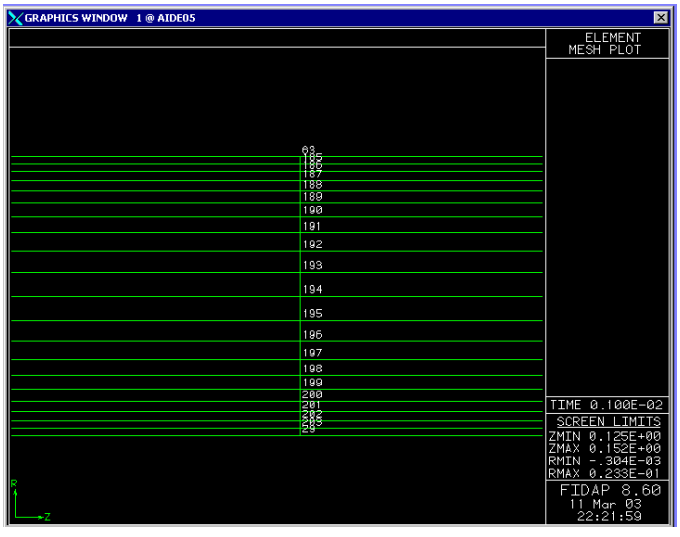
Appendix A:

1. Geometry:



2. Mesh:

The axi-symmetric mesh to the right is a model of the cylindrical tissue of the umbilical cord. The mesh was created using Gambit software.



3. Governing equation:

To model the cooling of our umbilical cord, the energy equation was used. Because convection was not included in our study and heat was not generated, these terms were excluded from our governing equation.

$$\rho Cp\,\frac{\partial T}{\partial t} = k\left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\,\frac{\partial T}{\partial r}\right) + \frac{1}{r^2}\frac{\partial^2 T}{\partial \theta^2} + \frac{\partial^2 T}{\partial z^2}\right]$$

4. Boundary Conditions:

The umbilical cord is assumed to be submerged in liquid nitrogen after the CPAs have been used in vitrification. Liquid nitrogen is assumed to be at 77 K and this is the temperature at the boundaries:

$$T\big|_{top} = T\big|_{bottom} = T\big|_{right} = 77\ K$$

5. Initial Conditions:

Assuming that the umbilical cord tissue is at room temperature, the initial condition is $T = 294$ K at $t = 0$.

6. Properties

To obtain our properties, we took a weighted average of the properties of each cryoprotectant and the properties of muscle tissue based on the percentage of cryoprotectant used.

	Glycerol	DMSO	Propylene glycol	Ethylene glycol
Mass Percentage used (toxic level (%))	14.28	6.06	9.30	7.86
Specific heat (J/kg-K)	3459.1908	3532.6506	3275.3730	3560.3214
density (kg/m3)	1097.418	1072.7574	1067.2000	1071.5502
conductivity (W/m-K)	0.4494	0.4593	0.4674	0.4591
Dimensions (m)				
diameter after birth	0.036			
radius after birth	0.018			

radius of vein	0.004
length	0.3

Appendix B

PROBLEM command:
Geometry Type – Axisymmetric (we used symmetry to model half of an umbilical cord (lengthwise) as a rectangle above the x-axis (to account for the hollow vein at the center) rotated about the x-axis)

Flow Regime – Incompressible (there is no fluid flow in this problem)

Simulation Type – Transient (we are modeling temperature changes with time)

Convective Term – Linear (because there is no fluid flow, there is no nonlinear portion of the convective term)

Momentum Equation – No Momentum (no fluid flow in this problem)

Temperature Dependence – Energy Equation (we are modeling changes in temperature and therefore, the energy equation is used to model our system)

SOLUTION command:
S.S. – 10
ACCF – 0

We used the default values for a transient solution in our solution command.

TIMEINTEGRATION command:

Time Integration – Backward

Number of Time Steps (Nsteps) – 1000 (Number of timesteps used to Obtain a solution)

Starting Time (Tstart) – 0 (seconds)

Ending Time (Tend) – 5400 (seconds)

Time Increment (DT) – 0.001 (initial timestep increment)

Time Stepping Algorithm (Variable) – 0.001 (allows program to adjust timestep size to fit the solution)

Increment Max (INCM) – 10 (limits the size of timestep increase to 10 seconds)

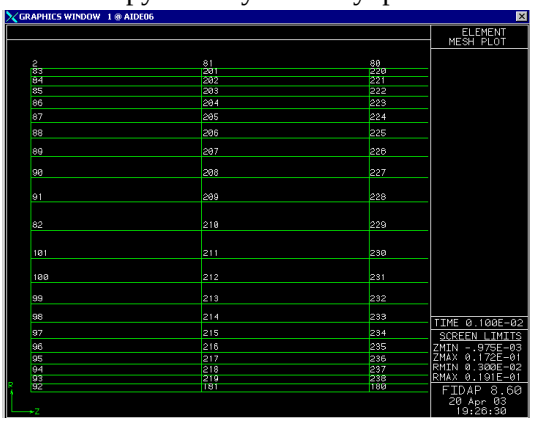
Mesh Convergence

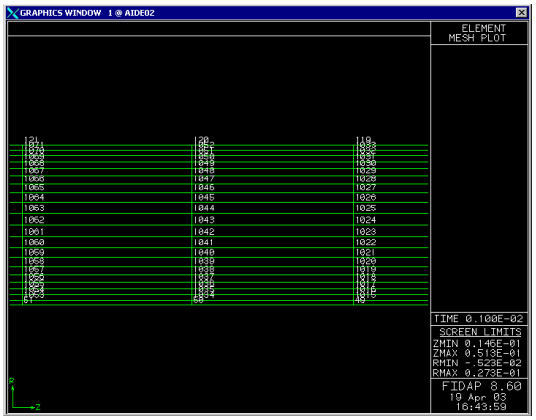
There was no change in the temperature contour at the time of our interest (t = 1500) with an increased number of elements and increased grading of the mesh. Furthermore, the contour lines are smooth and not jagged. We conclude that our mesh has converged. (Refer to Appendix C for mesh / mesh convergence diagrams)

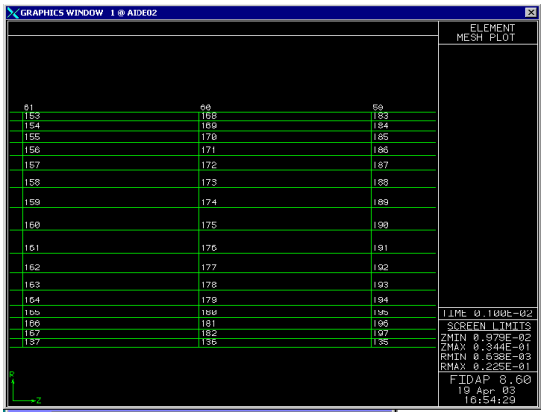
Appendix C

Meshes used for CPAs

A. 14.3% Glycerol as cryoprotectant B. 6.06% DMSO as cryoprotectant C. 7.86% Propylene Glycol as cryoprotectant

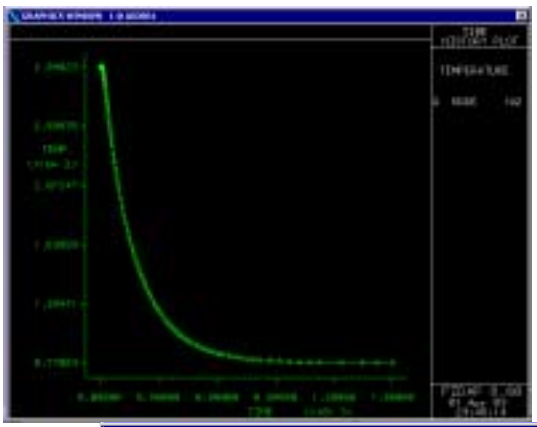


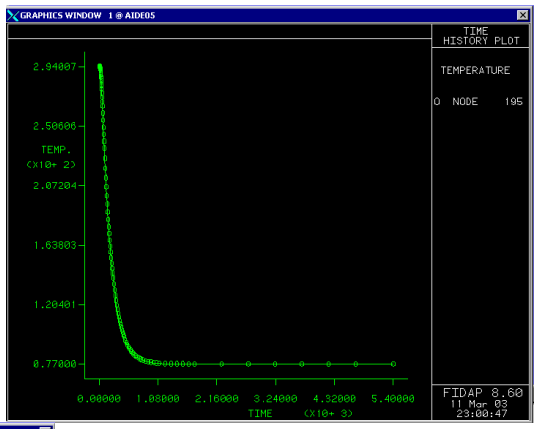


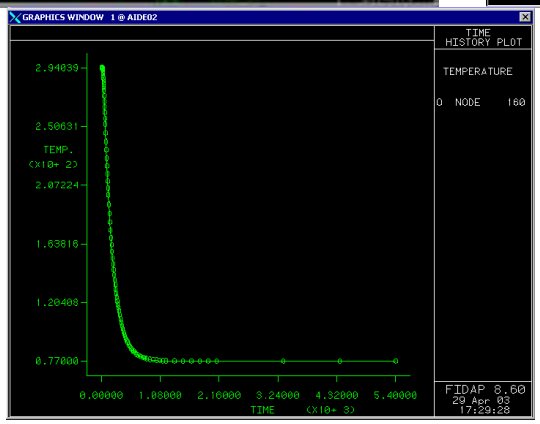


Time History Plot at the center for CPAs

A. 14.3% Glycerol as cryoprotectant B. 6.06% DMSO as cryoprotectant
C. 7.86% Propylene Glycol as cryoprotectant





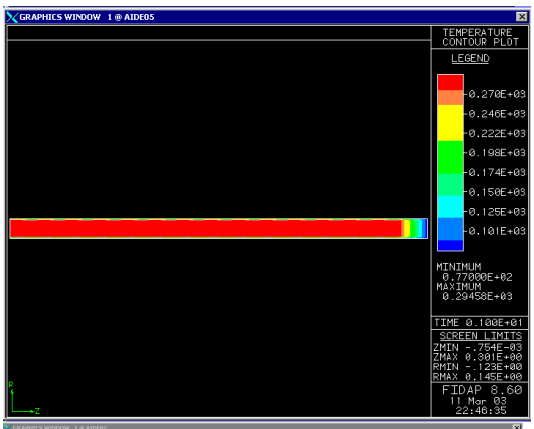


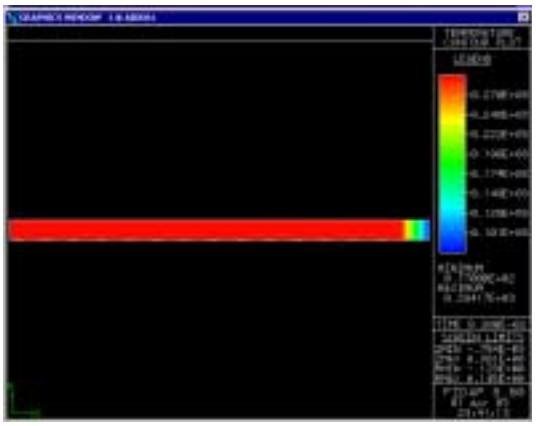
Temperature Contours at t = 2 seconds for CPAs

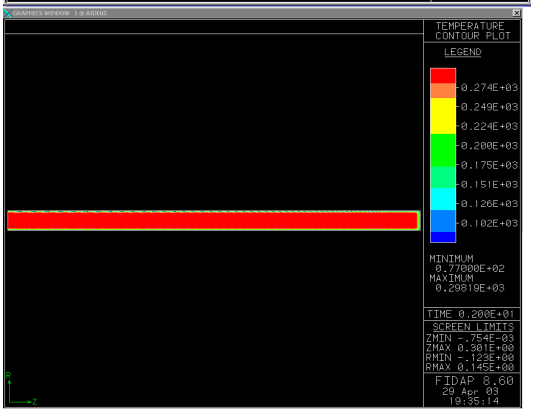
A. 14.3% Glycerol as cryoprotectant

B. 6.06% DMSO as cryoprotectant

C. 7.86% Propylene Glycol as cryoprotectant







Temperature Contours at t = 105 seconds for CPAs

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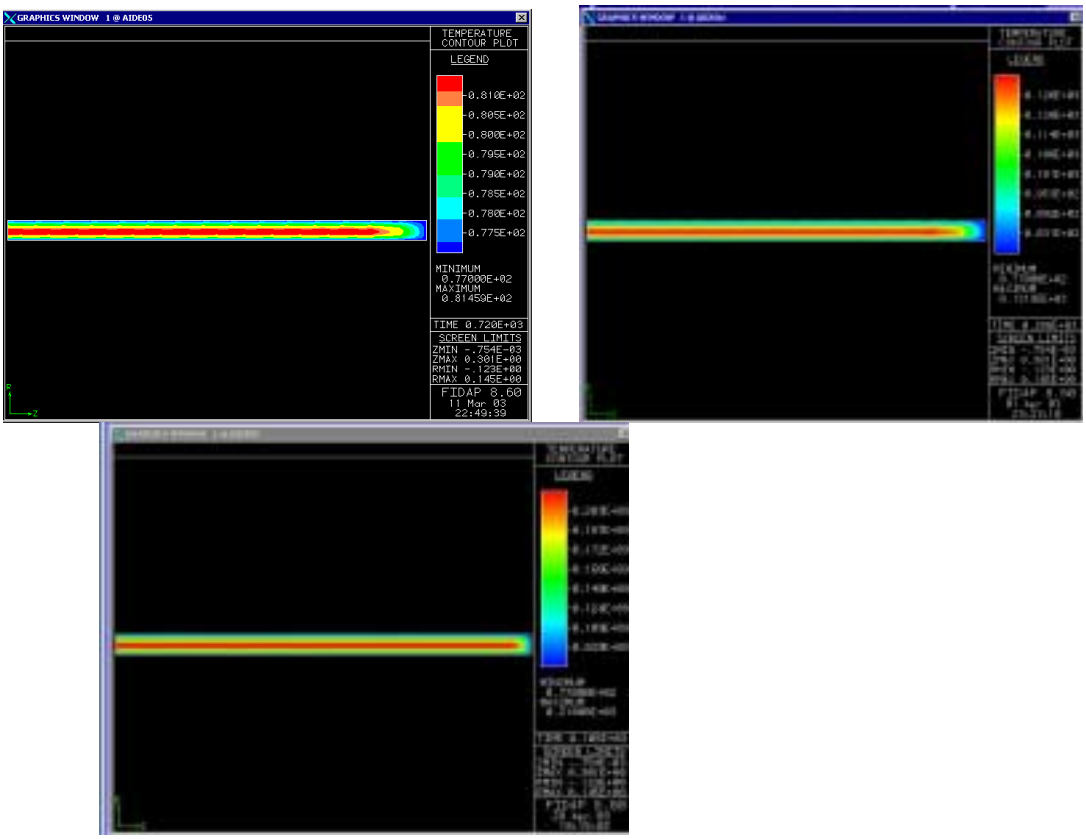
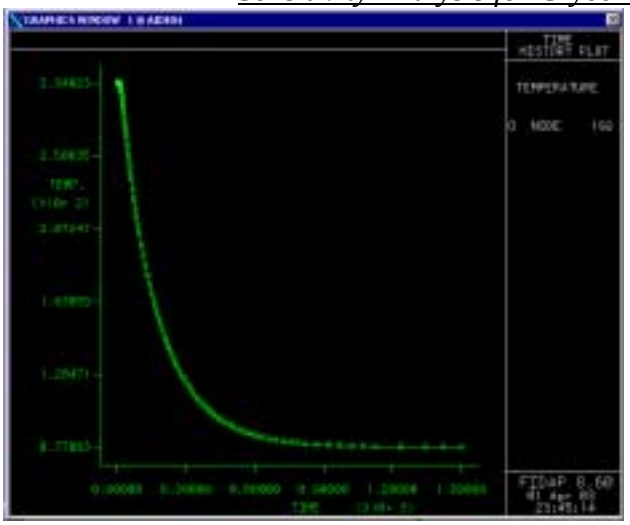
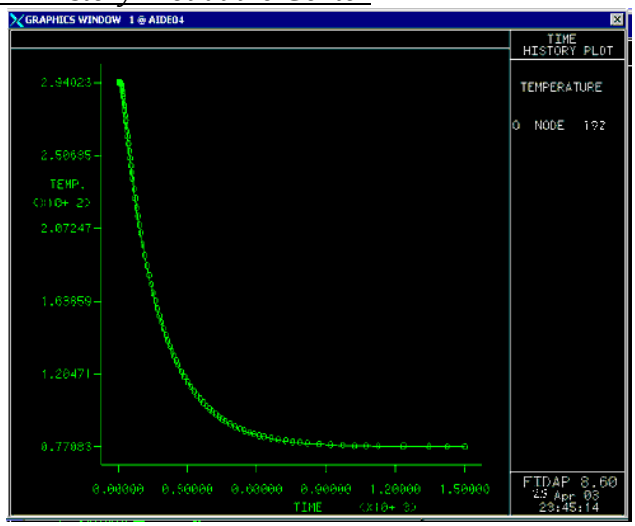
C. 7.86% Propylene Glycol as cryoprotectant



Sensitivity Analysis for Glycerol – History Plot at the Center

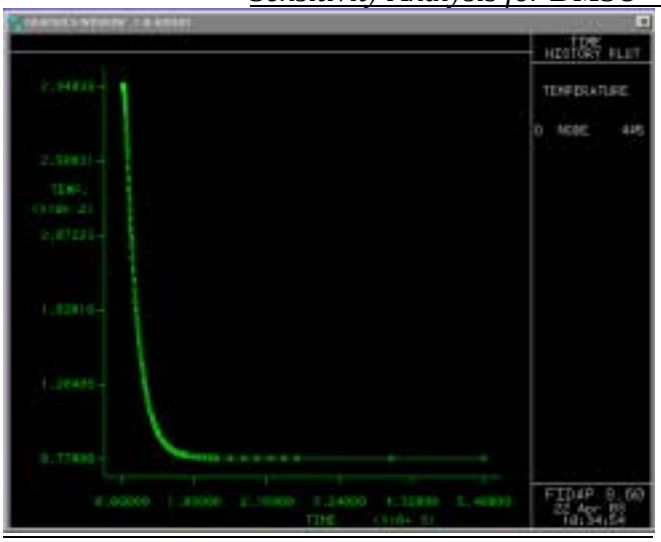


Original density, thermal conductivity, and specific heat values

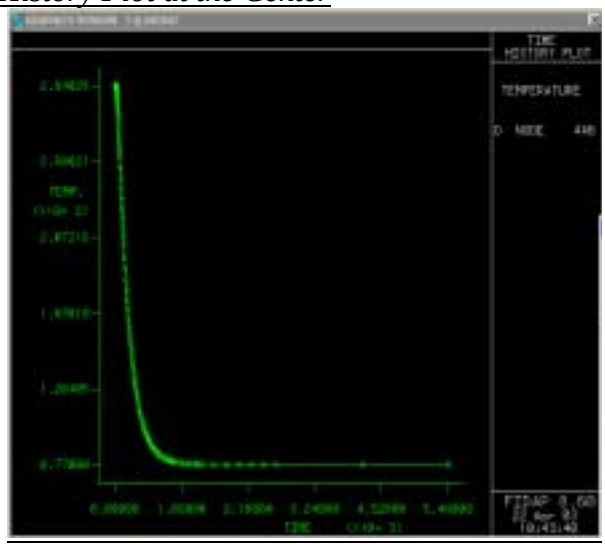


Density, thermal conductivity, and specific heat values increased by 10%

Sensitivity Analysis for DMSO – History Plot at the Center

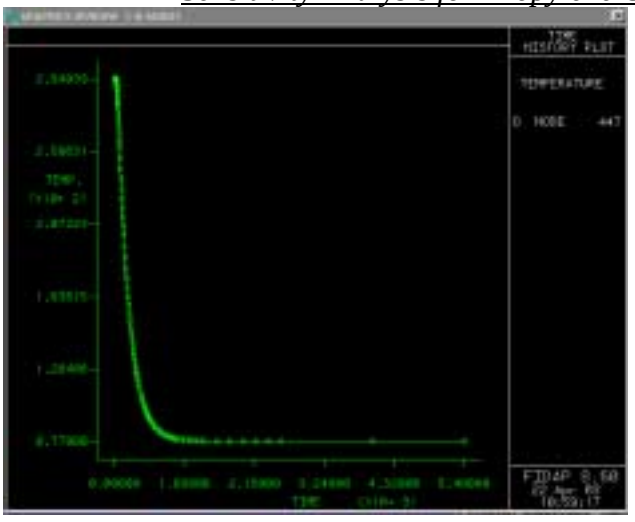


Original density, thermal conductivity, and specific heat values

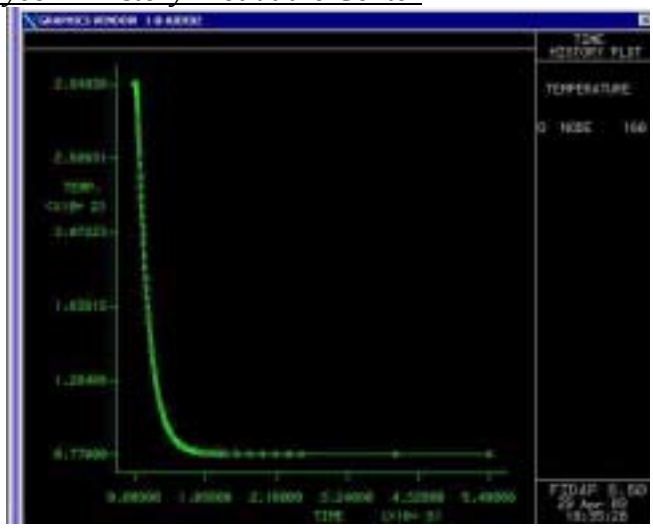


Density, thermal conductivity, and specific heat values increased by 10%

Sensitivity Analysis for Propylene Glycol – History Plot at the Center

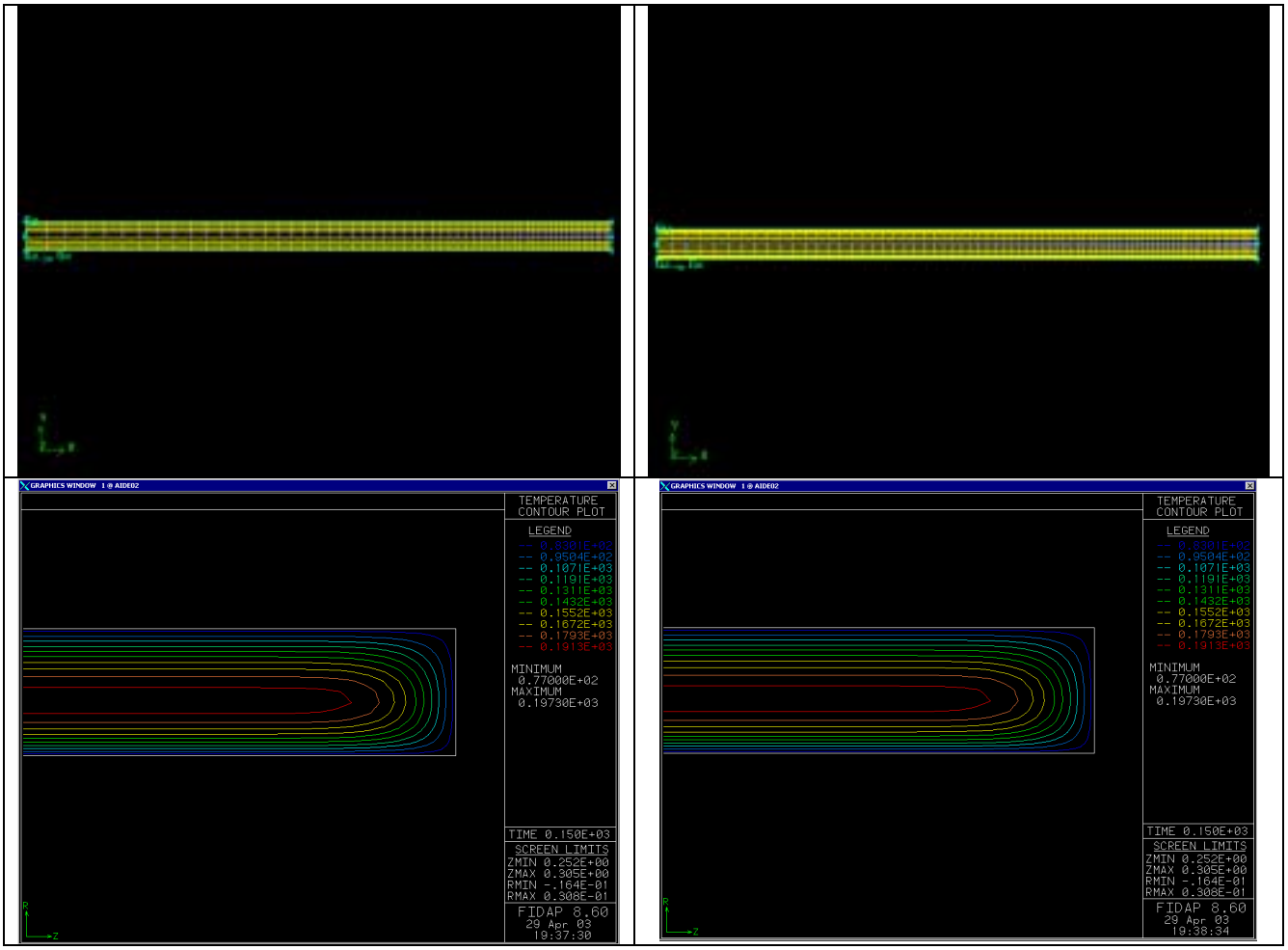


Original density, thermal conductivity, and specific heat values

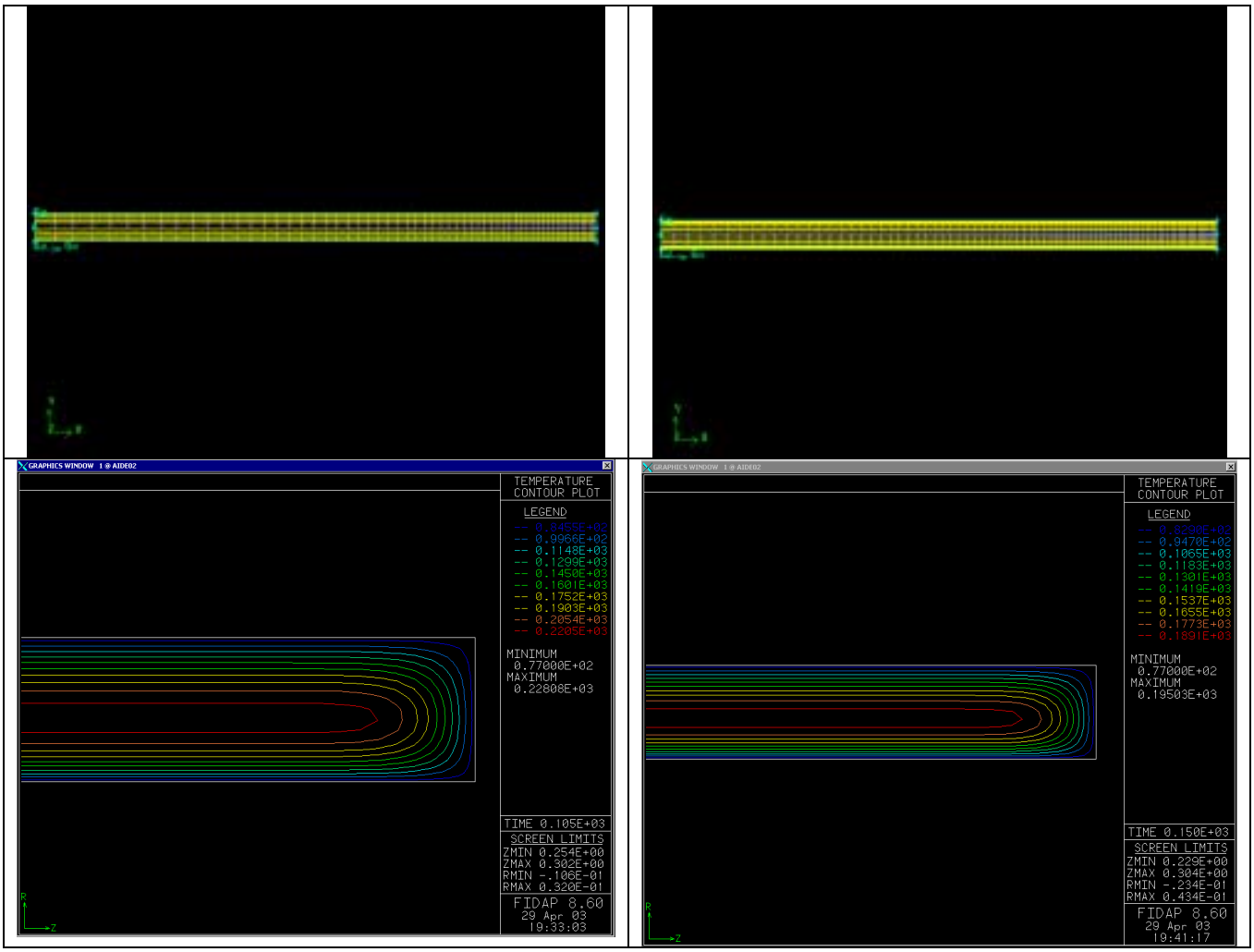


Density, thermal conductivity, and specific heat values increased by 10%

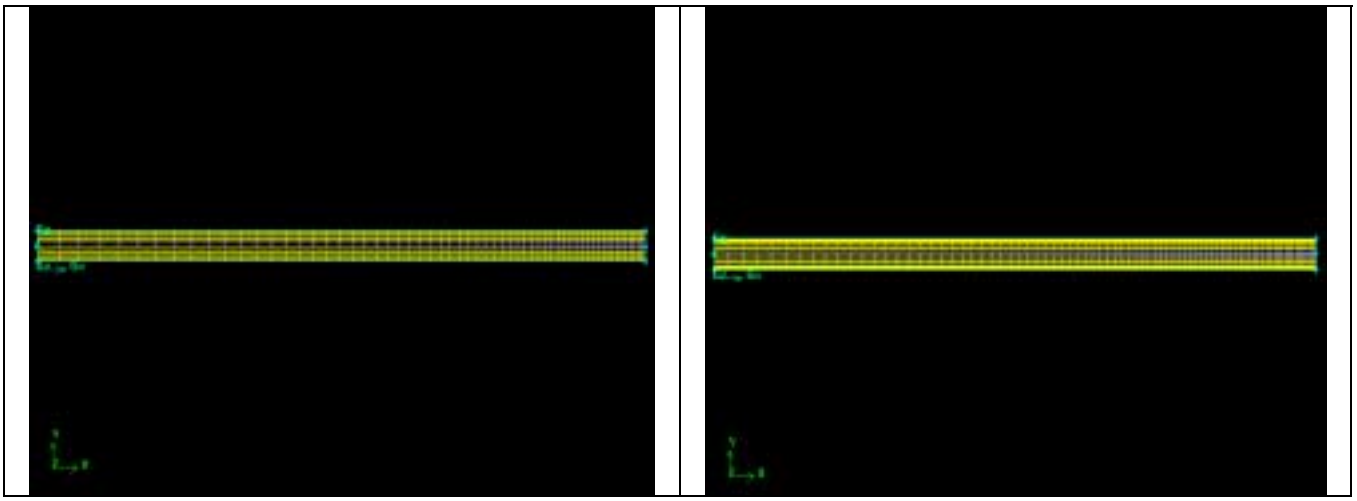
Mesh Convergence For Glycerol

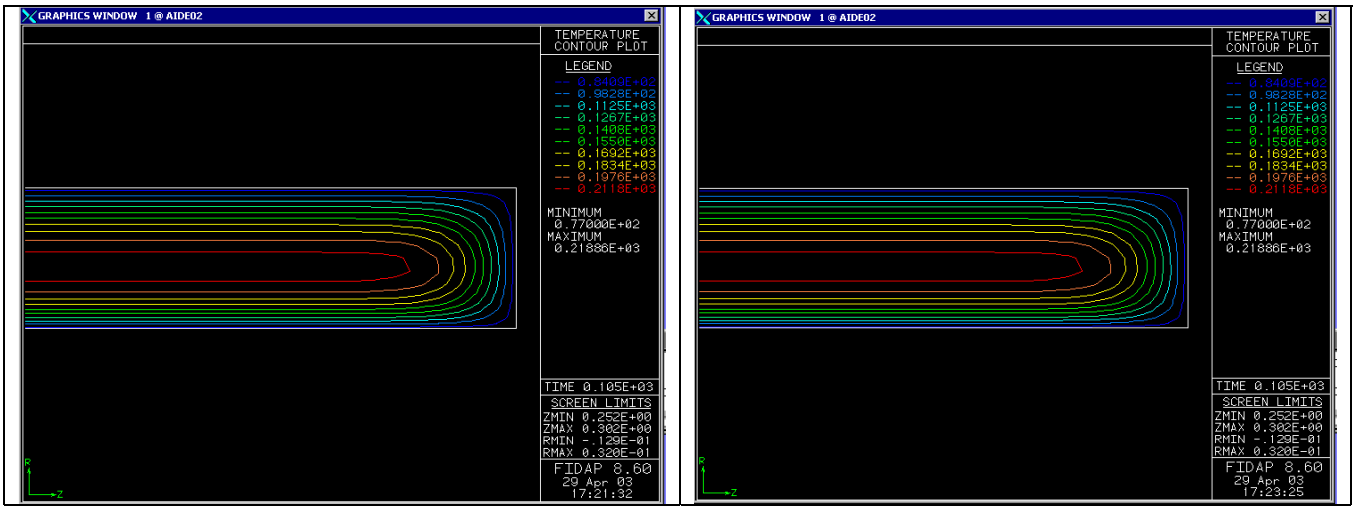


Mesh Convergence For DMSO



Mesh Convergence For Propylene Glycol




GRAPHICS WINDOW 1 @ AIDE02
TEMPERATURE
CONTOUR PLOT
LEGEND
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-- 0.9828E+02
-- 0.1125E+03
-- 0.1267E+03
-- 0.1408E+03
-- 0.1550E+03
-- 0.1692E+03
-- 0.1834E+03
-- 0.1976E+03
-- 0.2118E+03
MINIMUM
0.77000E+02
MAXIMUM
0.21886E+03
TIME 0.105E+03
SCREEN LIMITS
ZMIN 0.252E+00
ZMAX 0.302E+00
RMIN -.129E-01
RMAX 0.320E-01
FIDAP 8.60
29 Apr 03
17:21:32
R
Z
GRAPHICS WINDOW 1 @ AIDE02
TEMPERATURE
CONTOUR PLOT
LEGEND
-- 0.8409E+02
-- 0.9828E+02
-- 0.1125E+03
-- 0.1267E+03
-- 0.1408E+03
-- 0.1550E+03
-- 0.1692E+03
-- 0.1834E+03
-- 0.1976E+03
-- 0.2118E+03
MINIMUM
0.77000E+02
MAXIMUM
0.21886E+03
TIME 0.105E+03
SCREEN LIMITS
ZMIN 0.252E+00
ZMAX 0.302E+00
RMIN -.129E-01
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FIDAP 8.60
29 Apr 03
17:23:25
R
Z

Appendix D:

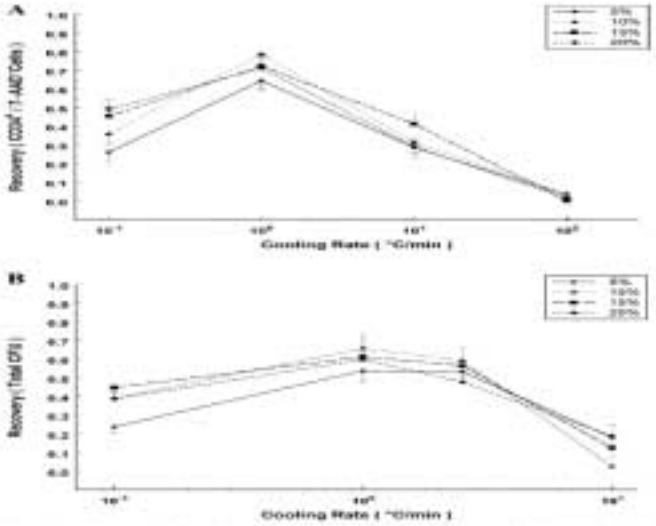

Fig. 5. Effect of cooling rate on the recovery of $CD34^+$ cells equilibrated in 5–20% w/w Me_2SO. (A) Flow cytometry with 7-AAD. Means ± SEM ($n = 6$). (B) Clonogenic assay with results expressed as total CFU. Means ± SEM ($n = 4$). Recovery expressed relative to pre-freeze untreated controls.

Source: Hunt et. al

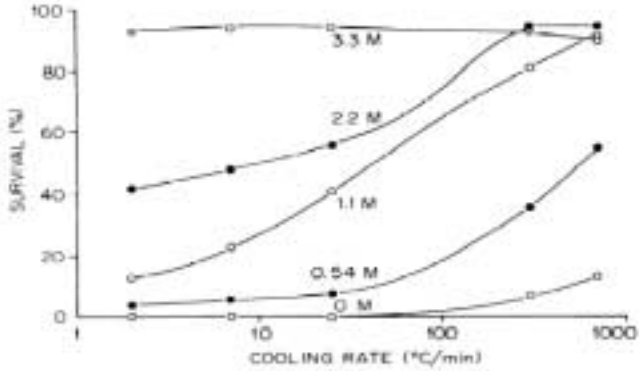

FIG. 17. Effect of various concentrations of glycerol on relationship between survival of human red blood cells and cooling rate. Reprinted from Ref. 67 by courtesy of Marcel Dekker, Inc. based on data of Morris and Farrant (76).

Source: Mazur

TABLE 3

Effect of Cryoprotectant Concentration and Permeation Temperature on TR Activity Following Freezing and Thawing, Using Slow, Medium, and Fast Cooling

Cooling rate (°C/min)	Permeation temperature (°C)	PG concentration			
		10%	20%	40%	Medium 199
		TR activity			
−1	4	76 ± 11.7%*	73.1 ± 19%*	52.4 ± 7.7%	50.2 ± 8.4%
	22	48.5 ± 10.4%	48.6 ± 11.7%	54.1 ± 16.4%	52.2 ± 7.6%
−60	4	55 ± 16.2%	43.5 ± 7.4%	42.6 ± 7.9%	40.5 ± 10.3%
	22	50.2 ± 11.7%	43.5 ± 7%	45.4 ± 10.9%	42.2 ± 8.7%
>100	4	40.6 ± 8.5%	35.6 ± 13.5%	36.7 ± 8.4%	32.5 ± 7.7%
	22	48.1 ± 12.1%	27.8 ± 9.1%	18.4 ± 8.6%	30.4 ± 8.5%

Source: VILLALBA et. al

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National Toxicology Program
- toxicity levels
ntp-server.niehs.nih.gov

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www.solvaypolyglycerol.com/product/properties/0,5531,-EN-1000048,00.html

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Umbilical Cord Matrix, a Rich New Stem Cell Source,
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RAFAEL VILLALBA, JOAN BENITEZ,† ENRIQUE DE NO-LOWIS,
LUIS F. RIOJA, AND J. LUIS GO´ MEZ-VILLAGRA´ N

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Cryopreservation of umbilical cord blood: 2. Tolerance of CD34þ cells to multimolar dimethyl sulphoxide and the efect of cooling rate on recovery after freezing and thawing
Charles J. Hunt,a,* Susan E. Armitage,b and David E. Peggc

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- prices of CPA's
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