Production Assistance for Cellular Therapies

Welcome to the PACT Educational Web Seminar

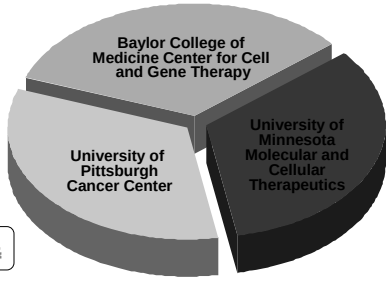
February 21, 2008

12:00 - 1:00 PM ET

About PACT

- An NHLBI-funded initiative committed to the advancement of effective cell therapies
- PACT supports the development of novel somatic cell therapy products by providing production assistance to the cell therapy community, as well as educational training via web seminars and at meetings
- PACT manufactures quality cell therapy products on behalf of investigators with funded clinical trials requiring support in product development and approval.
- PACT's educational training focuses on three general areas: translational development/scale-up and manufacture of cell therapy products; and quality assurance and regulatory issues.

PACT Members



The PACT Group provides education, leadership and production assistance to the cell therapy community through federally-funded contract manufacturing of therapeutic cell products.

Today's Education Web Seminar

Allison Hubel, PhD
University of Minnesota
Department for Laboratory Medicine and Pathology

Q & A Session

Presentation Slides

The presentation slides for this web seminar are available publicly on the main page at

www.pactgroup.net

For prior web seminars choose

"Educational Material=>Web Seminars"

Web Seminar Description

The presenter will outline approaches to the area of Good Manufacturing Practice specifically for facilities involved with products for cellular therapies. This web seminar will focus on troubleshooting cryopreservation protocols with emphasis on systematic methods of finding the source of cell therapy issues.

Web Seminar Objectives

- Learn what to do when a cryopreservation protocol goes wrong
- Develop systematic procedures in cyropreservation of cell therapy products
- Identify issues in post-thaw assessment of cell therapy products

Faculty Disclosure Information

The Accreditation Council for Continuing Medical Education (ACCME) is the governing body that accredits AABB to provide continuing medical education credits for physicians. In accordance with the ACCME *Standards for Commercial Support*, all faculty for this event have signed a conflict of interest form in which they have disclosed any significant financial interests or other relationships with the industry relative to the topics they will discuss during this program. Such disclosure allows you to better evaluate the objectivity of the information presented in the lectures. Please report any undisclosed conflict of interest you may perceive on the evaluation form.

Thank You.

Faculty Disclosure Information

Faculty	Disclosure	Nature of Relationship	Manufacturer/Provider
Allison Hubel	None	non-PACT member	University of Minnesota
Acacia Baker	None	PACT Member	The EMMES Corporation
Lisa Davis	None	PACT Member	The EMMES Corporation
David Styers	None	PACT Member	The EMMES Corporation
Debbie Wood	None	PACT Member	The EMMES Corporation

PACT Updates

PACT is accepting applications for cell therapy manufacturing. Investigators are encouraged to apply Please visit the website: www.pactgroup.net

PACT is supported with federal funds from:

National Heart, Lung, and Blood Institute, National Institutes of Health, Department of Health and Human Services

Administrative Center-The EMMES Corporation Contract Number: N01-HB-7166

Baylor College of Medicine Contract Number: N01-HB-37163

The University of Minnesota Contract Number: N01-HB-37164

The University of Pittsburgh Contract Number: N01-HB-37165

National Heart Lung and Blood Institute

35 minute presentation

Cryopreservation: trouble shooting

Allison Hubel, Ph.D
University of Minnesota

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Clinical context for cryopreservation

Cryopreservation is typically used for a variety of cell types and can be combined with liquid storage.

Cryopreservation permits

- Coordination with patent care regimes
- Completion of safety testing and quality control testing.
- Genetic diversity of cells available
- Transportation to/from collection/administration site.

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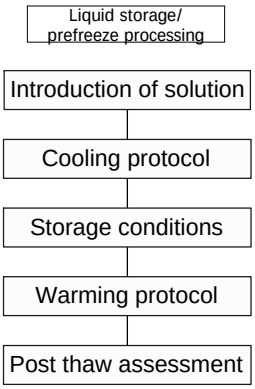
Components of a Cryopreservation Protocol



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Categories of problems

Problems obvious to operator

- Failure during controlled rate freezing
- Delayed latent heat

Problems not obvious to operator

- Poor post thaw viability

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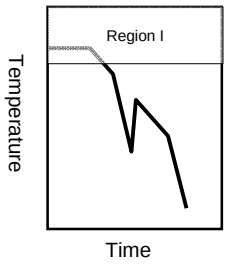
Failure during CRF

Mechanisms of failure

- Running out of LN2
- Failure of solenoid

Region I:

- Remove from CRF and place in back up freezer
- Viability of sample should be unaffected



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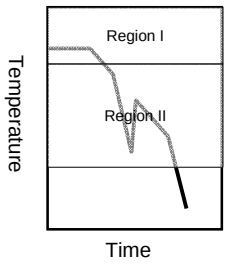
Failure during CRF, cont.

Region II (T_{nuc} to -40 C)

- Remove sample and place in -80 C freezer
- High probability that sample has been damaged
- Region of greatest activity
  - Solidification
  - Biological



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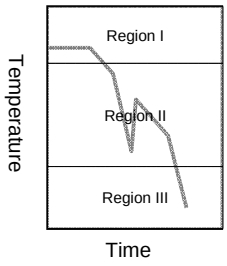
Failure during CRF, cont.

Region III (-40 C to T_{end})

- Place in LN2 or -80 C freezer
- Minimize warming during transfer
- Sample unlikely to be affected



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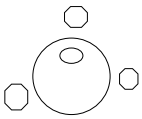
"Delayed" Latent heat

Nucleation and growth of ice releases the latent heat of fusion. The temperature at which this occurs, T_{nuc} is important.

Material Science
•Concentration of extracellular solution
•Ice crystal growth characteristics



Biology:
•Permeability of cell to water
•Membrane phase characteristics

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Relationship between T_{nuc} and IIF

- IIF is an accepted mechanism of damage.
- Cooling rate, T_{nuc} and cell type influence fraction of cells with IIF.

Toner, 1992

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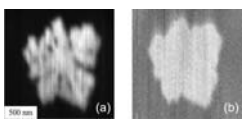
Methods of seeding sample

- "Automatic seeding"
  - Clinical protocols.
- Manual seeding
  - IVF protocols.
- Uncontrolled seeding.
  - Both clinical and laboratory settings.
- None of the methods really controls T_{nuc} for all samples being frozen
- Temperature history for all samples is not known



Bottom line: a solution for the problem does not yet exist

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Technology in development

- CRF with controlled nucleation for each vial
- Wireless temperature measurement for each vial

QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.

(Petersen, Cryobiology, 2006)

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Poor post thaw viability

Caveat: Previous studies have established feasibility of freezing cells

Presumption: flaws in implementation of the protocol

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‘Debugging’ a protocol

Starting point:

- Each protocol element
  - Prefreeze processing
  - Cryopreservation solution introduction
  - Segments of CRF protocol
  - Storage
  - Warming
  - Post thaw assessment

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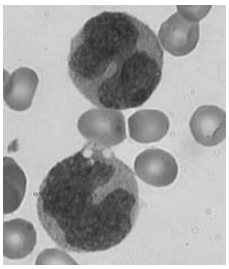
Prefreeze processing

Protocols should minimize stress, oxygen/nutrient deprivation, etc.

Measure: Screen for early markers of apoptosis, biochemical markers of stress, etc.

Modify: reduce duration of liquid storage, develop storage solution, etc.



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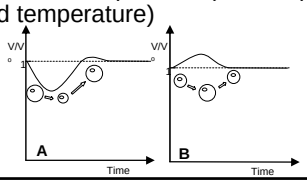
Cell processing for preservation

- Preservation requires the use of specialized solutions.
- Introduction and removal of the solutions can result in cell death.

Measure: cell losses after introduction and removal of solution (without freezing)

Modify: introduction protocol (multistep, gradual, reduced temperature)



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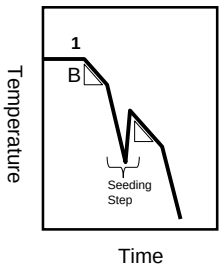
Controlled rate freezing

Segment One:

Measure: temperature in sample and compare to chamber temperature.

Modify: hold time to permit sample temperature to track chamber



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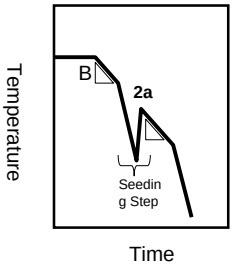
Controlled rate freezing, cont.

Segment 2a: seeding step

Measure: temperature at which sample seeds; viability of cells

Modify: seeding protocol to increase seeding temperature



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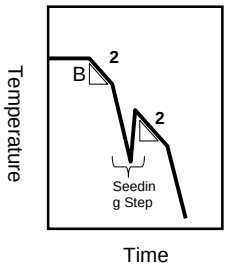
Controlled rate freezing, cont.

Segment two: cooling rate

Measure: cooling rate over high subzero temperatures

Modify: cooling rate to achieve improved survival



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Storage

Measure: Viability as a function of storage temperature

Monitor: temperature fluctuations in storage unit near sample.

Modify: reduce storage temperature and protocol for accessing repository

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Warming

Measure: warming rate for sample. Objective: B>200 C/min

Modify thawing protocol (increase agitation or bath temperature)

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Post thaw assessment

It is easy to perform post thaw assessment badly

Kansas Stem Cell Program Sued

October 2006

A group of patients has sued a Kansas City stem cell transplant program alleging that it used shortcut methods to prepare the cells, resulting in the deaths of one-fourth of 40 patients treated between 1998 and 1999. Those patients died within 100 days of the treatments from complications including hemorrhages, infections and the return of their cancer. In two years, half of the patients died -- a much higher death rate than other centers.

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Post thaw assessment

Assessing the viability of a frozen and thawed cell is not the same as assessing the viability of a cell

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Post thaw assessment

Freezing and thawing produces:

- Changes in membrane integrity
- Metabolic function
- Tendency toward apoptosis

Implying that:

- Membrane integrity tests ≠viability
- Viability may change with time post thaw

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Post thaw assessment, cont.

Helpful hints

- Do not use trypan blue
- Perform post measures at the same time point post thaw
- Prevent measurement bias (discussed in upcoming slides)
- Use multiple measures of post thaw assessment

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Post thaw assessment: measures

- Physical integrity (fluorescent dyes).
- Metabolic activity
- Mechanical activity (attachment, contraction)
- Mitotic activity (proliferation assay)
- Transplantation potential.

Method of assessment should depend upon the system (cell/tissue) of interest. It is common to need more than one assay.

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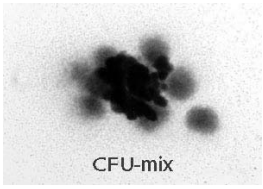
Assays for HSCs

- CFU
- CD34+ enumeration via flow cytometry
- These assays are performed on intact cells
- 'Recovery' > 100% frequently reported



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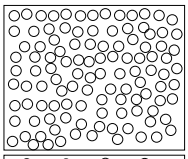
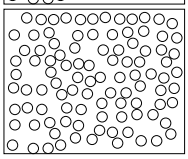
Measurement Bias

Prefreeze Assay
Total number of cells =100
Number of target cells =6
Frequency of target cells=7/100=7%



Post thaw Assay
Total number of cells =71
Number of target cells =5
Frequency of target cells=5/71=7%



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Measurement Bias, cont.

Current method of accounting

Recovery of target cell= frequency of target cell post thaw/frequency of target cell prefreeze= 0.07/0.07=100%!!!

⇨Measurement Bias

You have not accounted for the cells that were lost

Recovery of target cell=total number of target cells post thaw/total number of target cells prefreeze

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Summary

- Problems with cryopreservation can be both apparent and hidden from the operator
- Various strategies can be used to 'debug' problems with the protocol
- Post thaw assessment critical
- Properties of thawed cells vary from non-frozen cells
- Improper interpretation common
- Measurement bias is common

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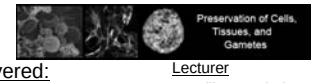
Professional short course



Topics covered:

- Liquid storage of cells and tissues.
- Fundamentals of cryopreservation
- Protocol development
- Quality systems
- Clinical cell cryopreservation
- Repository design
- Gamete preservation
- Tissue preservation
- Regulatory issues for cell/tissues

Lecturer

- Allison Hubel, University of Minnesota
- Charles Lee, University of North Carolina, Charlotte
- Ian Pope, CoreCryolab
- Ken Roberts, University of Minnesota
- Marilyn Waxberg, RCRI
- Alptekin Aksan, University of Minnesota
- David McKenna, University of Minnesota
- Diane Kadidlo, University of Minnesota

www.me.umn.edu/education/shortcourses/preservation

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Production Assistance for Cellular Therapies

Q & A Session

February 21, 2008

Speaker Contact E-mail

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Presentation Slides

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- Sign and fax roster to 240-306-2527
- Complete an online survey

http://www.surveymonkey.com/s.aspx?sm=cq0E4U_2fj7N9qR5j7KfV9Mg_3d_3d
(link above embedded in the reminder email sent Wednesday, February 20th)

Note: Please complete within 48 hrs of the program.

AABB Live Learning Center

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Thank you for attending!

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