Innovative Technologies for Cryopreservation of Cancer-Relevant Samples for Biobanking

BIOBANKS

Sant Feliu, 1-7 Nov 2008

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EUROPEAN SCIENCE FOUNDATION

SETTING SCIENCE AGENDAS FOR EUROPE

BioBank

βιος = Life Bank= Store

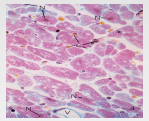
Store of Life

Most of tissues in BioBanks are DEAD!

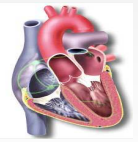
CryoPreservation

Preservation of Life by Cold

ALL cells



A FEW tissues



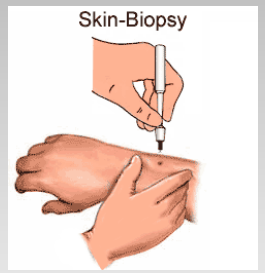
NO organ

Current Biopsy Preservation
vs.
Preservation of Functionality

Biopsy

⇩

Quenching in LN2



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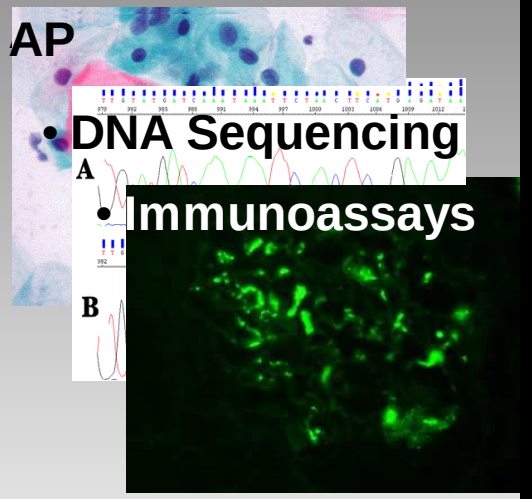
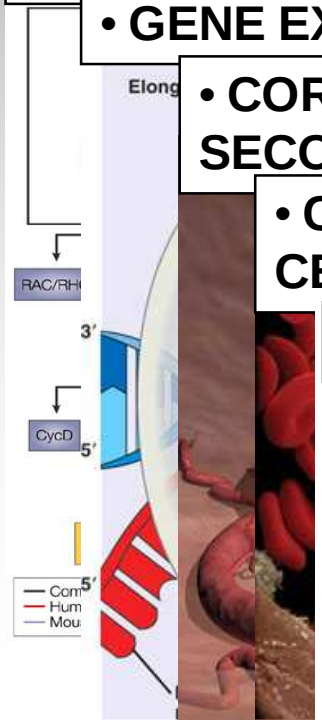
- AP
- DNA Sequencing
- Immunoassays



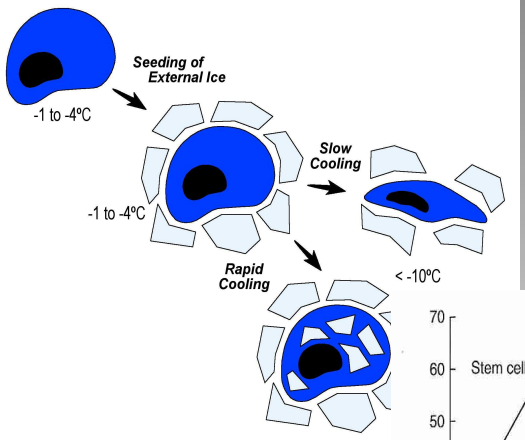
Preservation of Functionality

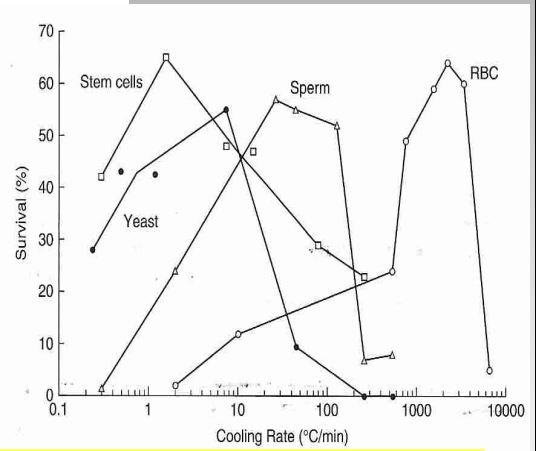
- MOLECULAR PATHWAYS
- GENE EXPRESSION
- CORRELATION WITH SECONDARY TUMORS
- CIRCULATING TUMOR CELLS (CTC)
- DRUGS INTERFERENCE
- PROTEOMICS
- DIAGNOSIS
- PROGNOSIS
- .....



Conventional Cryopreservation

Slow Freezing

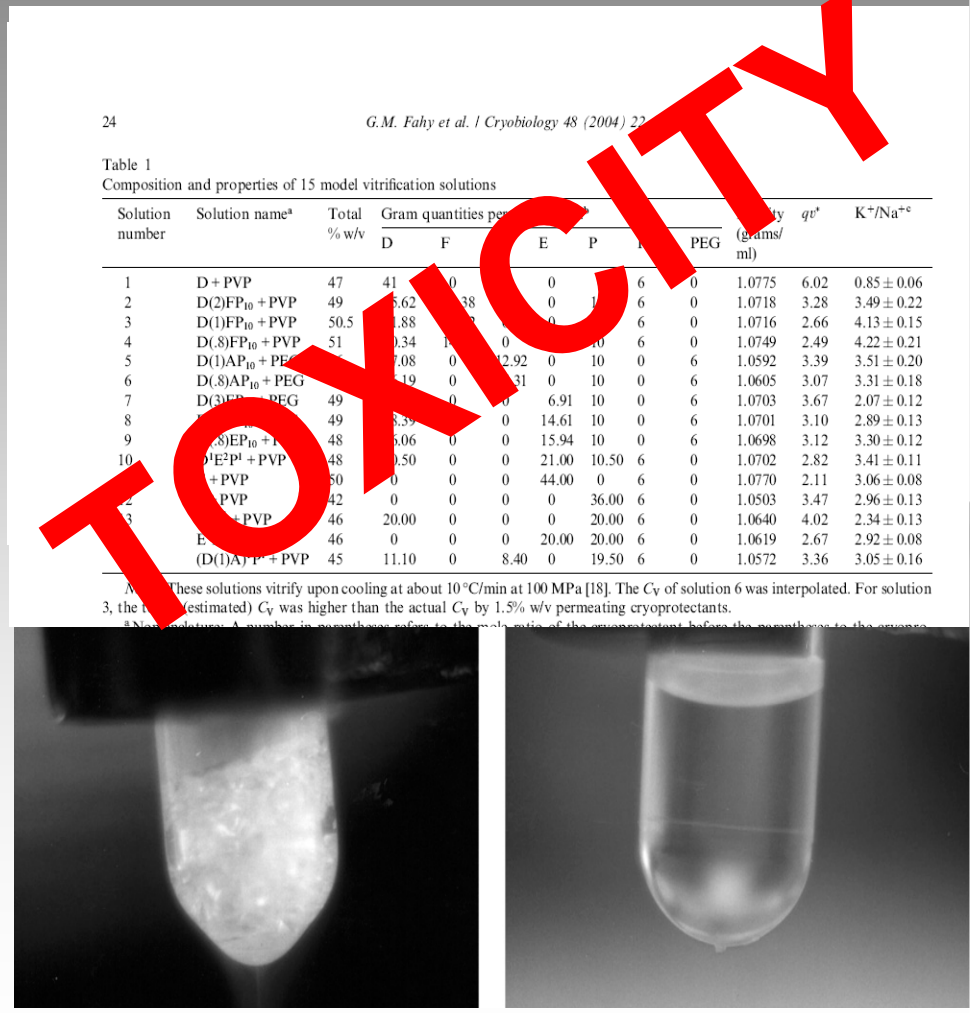




DRAWBACKS

- Isolated cells in suspension
- Seeding of ice
- Controlling cooling rate
- No tissues
- Osmotic and Mechanical Stress
- Low recovery rates in some cell types

Vitrification



24 G.M. Fahy et al. / Cryobiology 48 (2004) 22

Table 1
Composition and properties of 15 model vitrification solutions

Solution number	Solution name[a]	Total % w/v	Gram quantities per									
			D	F		E	P		PEG	(grams/ml)	qv^*	K^+/Na^+[c]
1	D + PVP	47	41	0		0		6	0	1.0775	6.02	0.85 ± 0.06
2	$D(2)FP_{10}$ + PVP	49	5.62	38		0		6	0	1.0718	3.28	3.49 ± 0.22
3	$D(1)FP_{10}$ + PVP	50.5	1.88			0		6	0	1.0716	2.66	4.13 ± 0.15
4	$D(.8)FP_{10}$ + PVP	51	0.34	1		0		6	0	1.0749	2.49	4.22 ± 0.21
5	$D(1)AP_{10}$ + PE		7.08	0		2.92	0	10	0	6	1.0592	3.39	3.51 ± 0.20
6	$D(.8)AP_{10}$ + PEG		4.19	0		1.31	0	10	0	6	1.0605	3.07	3.31 ± 0.18
7	D(3)F	PEG	49	0		0	6.91	10	0	6	1.0703	3.67	2.07 ± 0.12
8			49	8.39		0	14.61	10	0	6	1.0701	3.10	2.89 ± 0.13
9	.8)EP	+	48	6.06	0	0	15.94	10	0	6	1.0698	3.12	3.30 ± 0.12
10	$^1E^2P^1$ + PVP		48	0.50	0	0	21.00	10.50	6	0	1.0702	2.82	3.41 ± 0.11
	+ PVP		50	0	0	0	44.00	0	6	0	1.0770	2.11	3.06 ± 0.08
	PVP		42	0	0	0	0	36.00	6	0	1.0503	3.47	2.96 ± 0.13
	+ PVP		46	20.00	0	0	0	20.00	6	0	1.0640	4.02	2.34 ± 0.13
	E		46	0	0	0	20.00	20.00	6	0	1.0619	2.67	2.92 ± 0.08
	(D(1)A)	+ PVP	45	11.10	0	8.40	0	19.50	6	0	1.0572	3.36	3.05 ± 0.16

These solutions vitrify upon cooling at about 10 °C/min at 100 MPa [18]. The C_V of solution 6 was interpolated. For solution 3, the (estimated) C_V was higher than the actual C_V by 1.5% w/v permeating cryoprotectants.

TOXICITY

Our Technologies

Quartz Capillaries & Slush

SLOW FREEZING

Advantages

- Low cpa concentration
- Low toxicity

Disadvantages

- Control Cooling rate
- Need of Seeding
- Long exposition to cpa
- Very cell-type dependent
- Expensive equipment

VITRIFICATION

Advantages

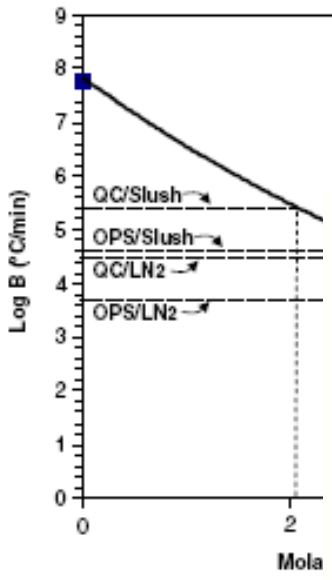
- No control of cooling rate
- No seeding
- Low sensitive to the cell type
- Cheap equipment

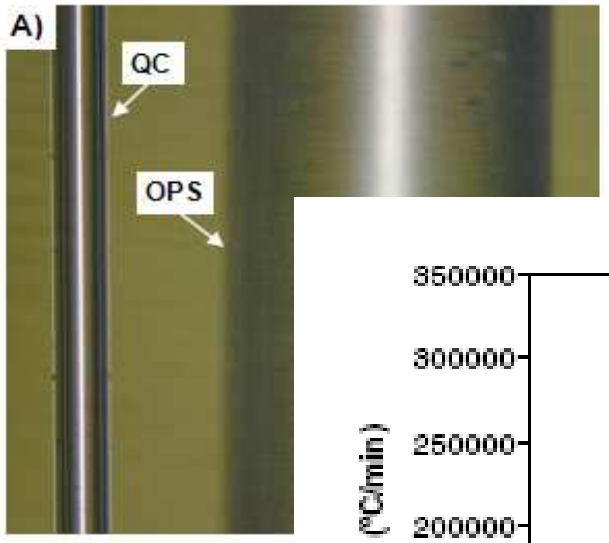
Disadvantages

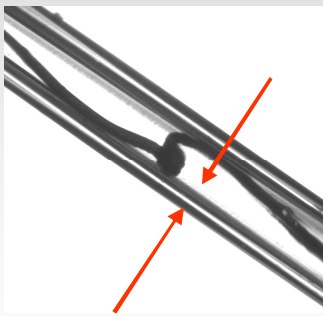
- High cpa concentration
- High toxicity

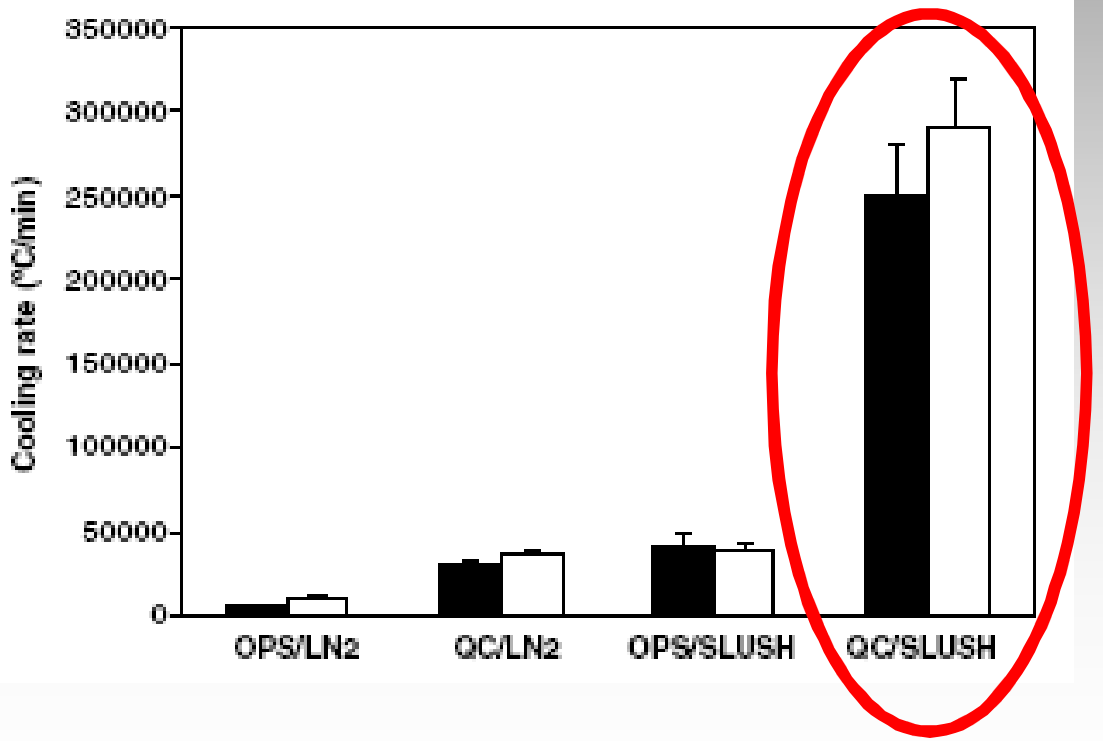
Equilibrium Vitrification

Quartz Capillaries & Slush







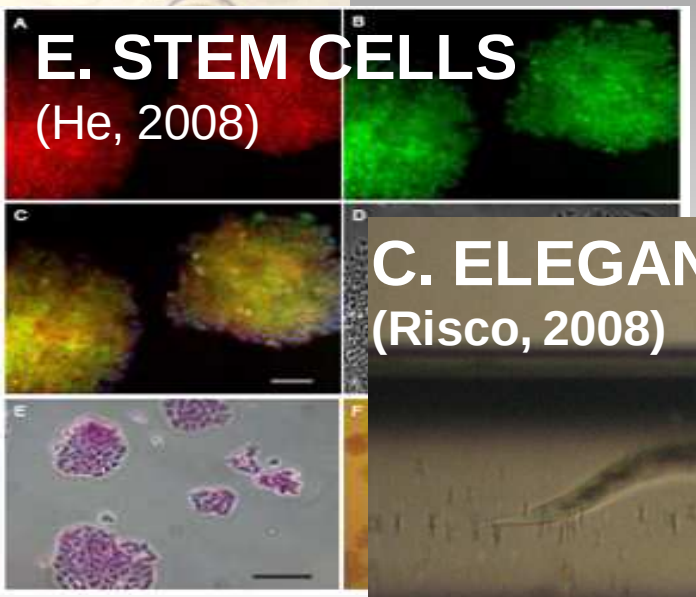


Source: Thermal performance of quartz capillaries for vitrification. Ramon Risco et al. Cryobiology 55 (2007) 222–229

Quartz Capillaries & Slush

Succesful Applications:

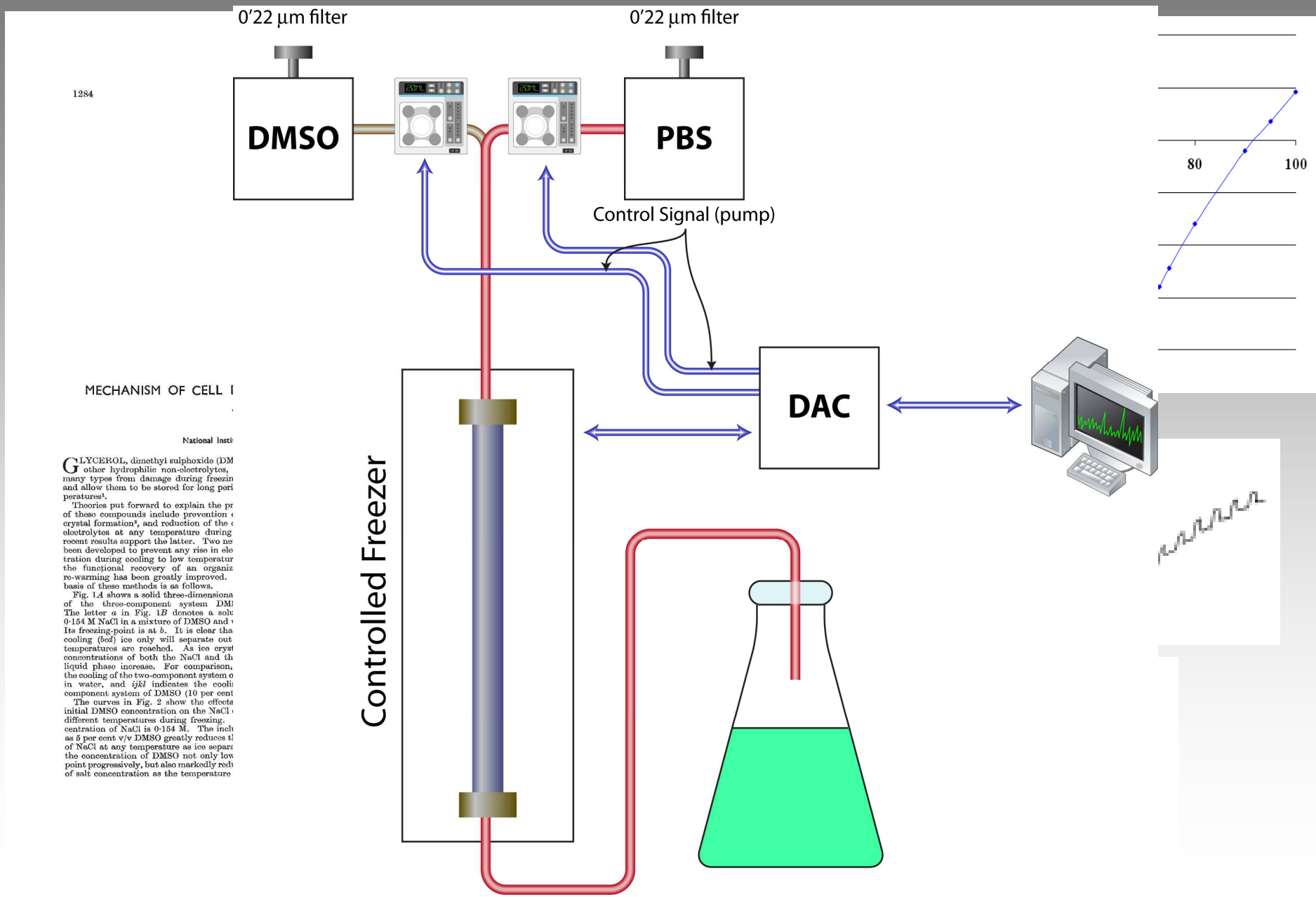
OOCYTES
(Risco, 2008)



E. STEM CELLS
(He, 2008)

C. ELEGANS
(Risco, 2008)

Equilibrium Vitrification



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MECHANISM OF CELL I

National Insti

GLYCEROL, dimethyl sulphoxide (DM
other hydrophilic non-electrolytes,
many types from damage during freezin
and allow them to be stored for long peri
peratures[1].

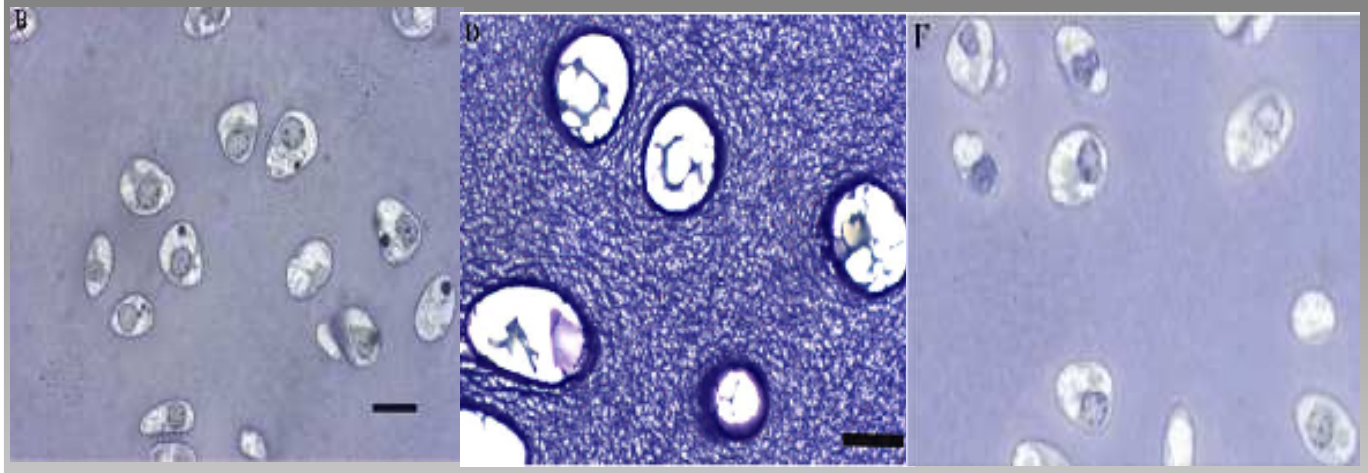
Theories put forward to explain the pr
of these compounds include prevention
crystal formation[2], and reduction of the
electrolytes at any temperature during
recent results support the latter. Two ne
been developed to prevent any rise in ele
tration during cooling to low temperatur
the functional recovery of an organiz
re-warming has been greatly improved.
basis of these methods is as follows.

Fig. 1A shows a solid three-dimensiona
of the three-component system DM
The letter *a* in Fig. 1*B* denotes a solu
0·154 M NaCl in a mixture of DMSO and
Its freezing-point is at *b*. It is clear tha
cooling (*bcd*) ice only will separate out
temperatures are reached. As ice cryst
concentrations of both the NaCl and th
liquid phase increase. For comparison,
the cooling of the two-component system o
in water, and *ijkl* indicates the cooli
component system of DMSO (10 per cent

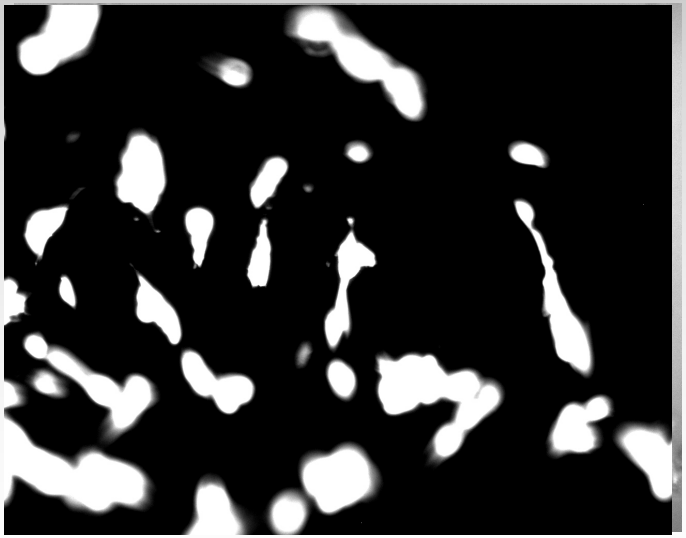
The curves in Fig. 2 show the effects
initial DMSO concentration on the NaCl
different temperatures during freezing.
centration of NaCl is 0·154 M. The incl
as 5 per cent v/v DMSO greatly reduces t
of NaCl at any temperature as ice separa
the concentration of DMSO not only low
point progressively, but also markedly redu
of salt concentration as the temperature

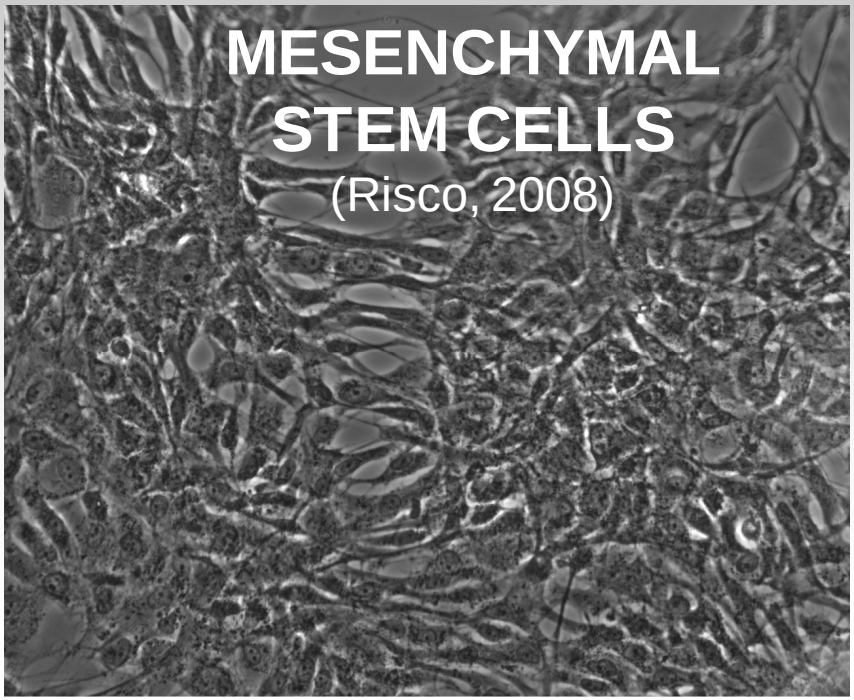
Equilibrium Vitrification

A. CARTILAGE



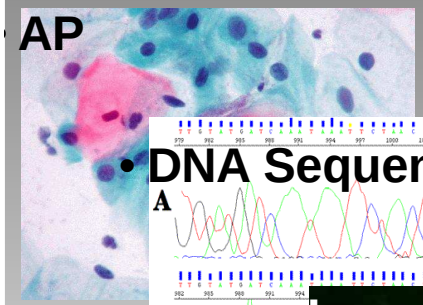
86.7% ± 1.8% incorporation of ^{35}S sulfate into GAGs (Pegg, 2007)



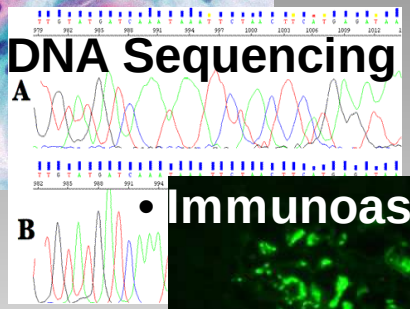


CONCLUSIONS

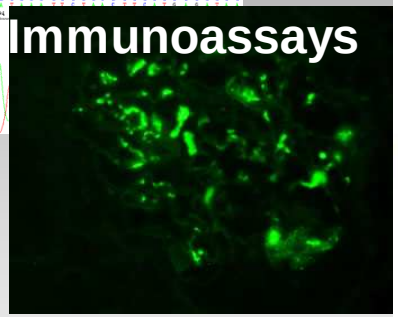
PRESENT STATUS OF BIOBANKING



• AP



• DNA Sequencing



• Immunoassays

CAPABILITIES OF NOWADAYS CRYOPRESERVATION TECHNOLOGIES

• MOLECULAR PATHWAYS

• GENE EXPRESSION

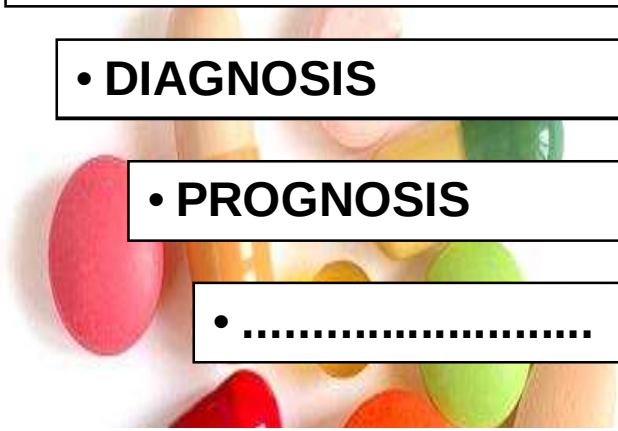
• CORRELATION WITH SECONDARY TUMORS

• CIRCULATING TUMOR CELLS (CTC)

• DRUGS INTERFERENCE

• PROTEOMICS

• DIAGNOSIS



• PROGNOSIS

• .....

Collaborators:

- Mehmet Toner (Harvard Uni.)
- Alberto Olmo (Univ. Seville)