Core Dynamics Corporate Overview

Small incubator – type company with focus on early feasibility prove-out of new applications for the MTG devices.

- Founded in 1995
  - Founded by Dr. Amir Arav
  - 2001 First whole organ cryopreservation and transplantation (ASRM Award)
  - 2002 Freeze-dried WBC
  - 2004 Successful spin-off of veterinary and IVF applications
  - 2006 First human cartilage transplants
- R&D based in Ness Ziona, Israel
  - 20 research scientists, engineers and support personnel
- Management
  - Wide experience in the fields of medicine, medical devices, cryopreservation and cell biology.

Whole Organ Cryopreservation

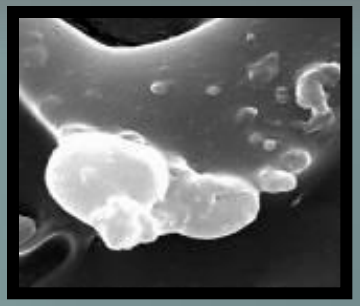
Freeze Dried WBC

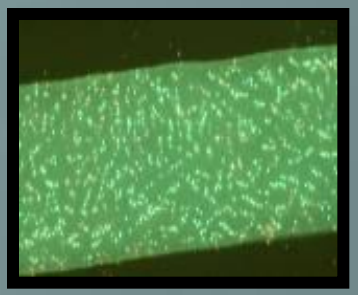
Cartilage Plug

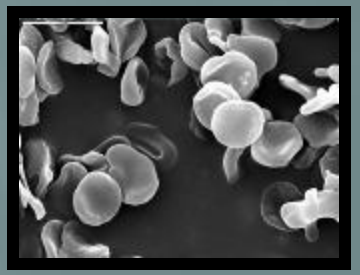
Current Application Focus Areas

Portfolio of project work is spread across preservation of several cell types.

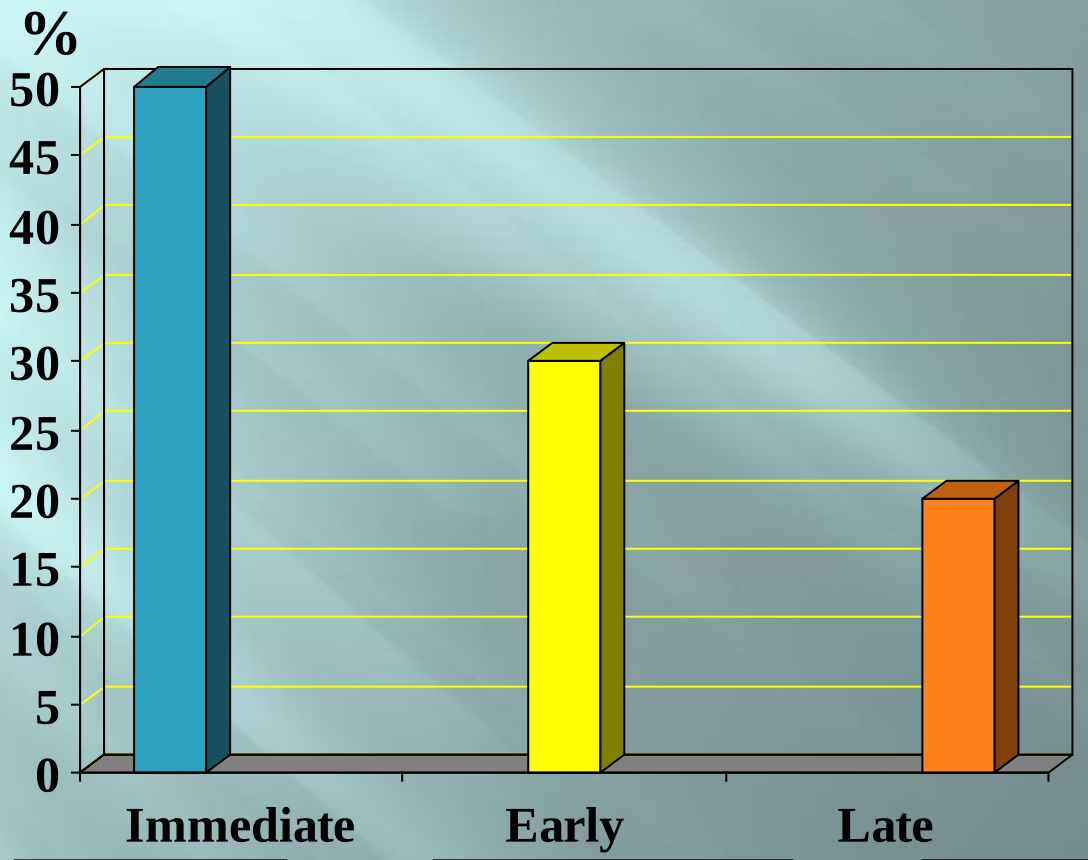
- Cell Therapy
  - Stem Cells: Hematopoetic, Mesenchymal, others
- Orthopedics
  - Cryopreserved viable allograft tissue
  - Cartilage Repair: Dowels, hemicondyles
  - Bone repair
- Transfusion Medicine
  - Cryopreserved Red Blood Cells
  - Freeze Dried RBC
  - RBC Reagents







When do Civilian Casualties Die ?



1 hour

2-4 hours

Weeks

Trunkey

Scientific American

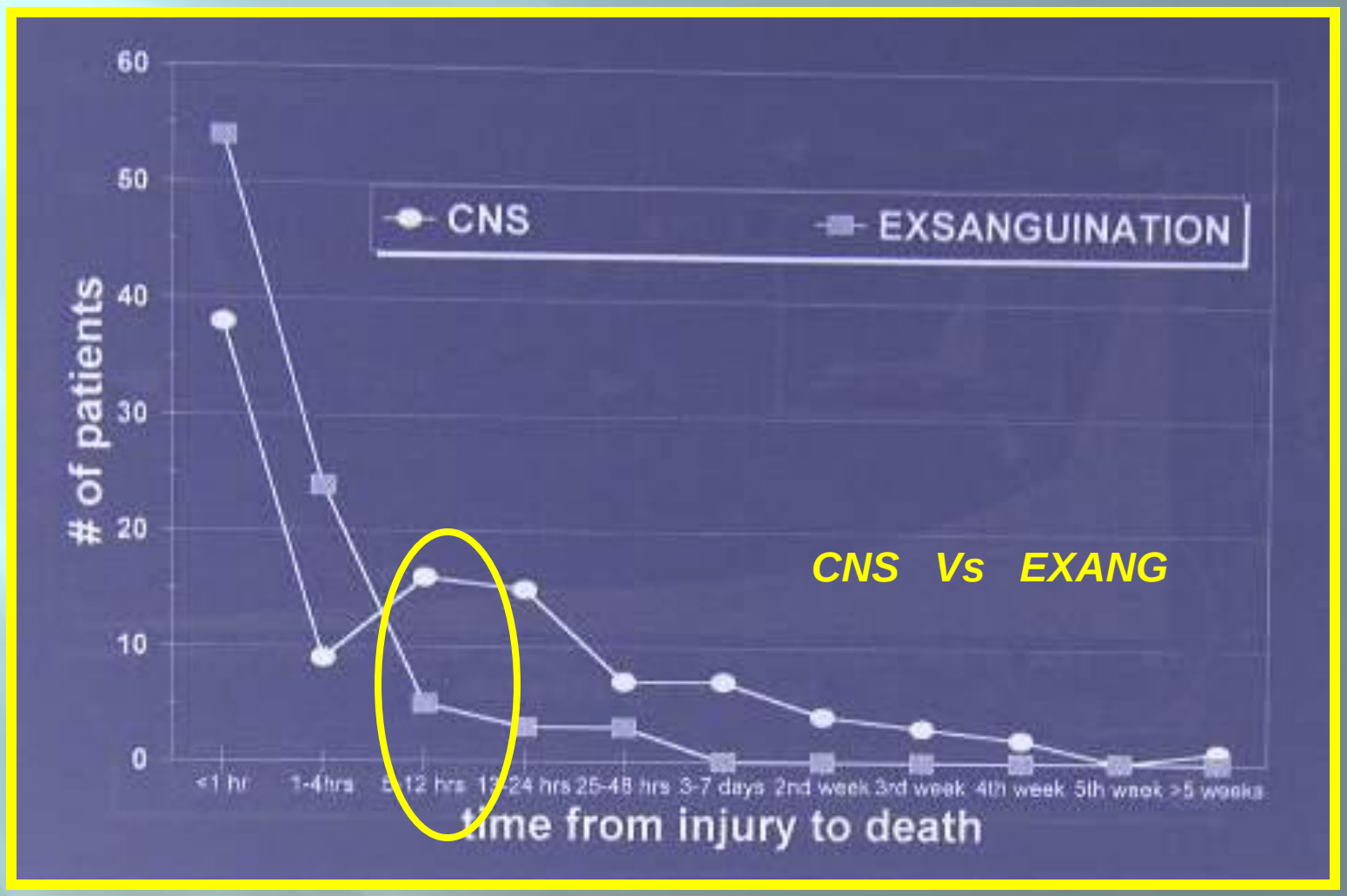
249:28-35, 1983

The Golden Hour

R A Cowley 1982

According to M. Stein

The golden hour

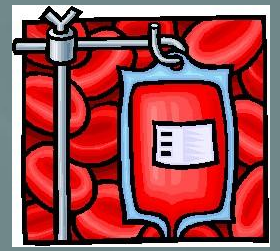


Sauaia et al, J Trauma, 1995

Current Practice for RBC storage

- **Liquid storage at 2-8˚C (refrigerator)**
  - Shelf life 35- 42 days, then discarded
  - >99% of blood stored liquid

- **Freezing at – 80˚C (Mechanical Freezer)**
  - Shelf life for years
  - Less then 1% frozen each year
  - Primarily for rare types



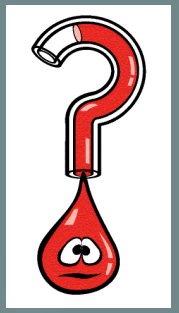
Problems with Current Storage Methods

Liquid Stored Blood

- short storage time leads to inefficiencies

Frozen stored Blood

- Bulky, costly, monitored freezers
- Not “available” in acute situations
- Not easily transportable
- Lost of 10-20% of the cells



Current RBC Freezing

- Addition of high glycerol concentrations (19 or 40%)
- 40-60 minute post thaw to wash blood to remove glycerol using costly automated equipment
- High cost/unit (approximately 2x cost of liquid stored blood).

Freeze drying RBC

- Storage for years
- 95% weight loss
- Can be store at room temperature

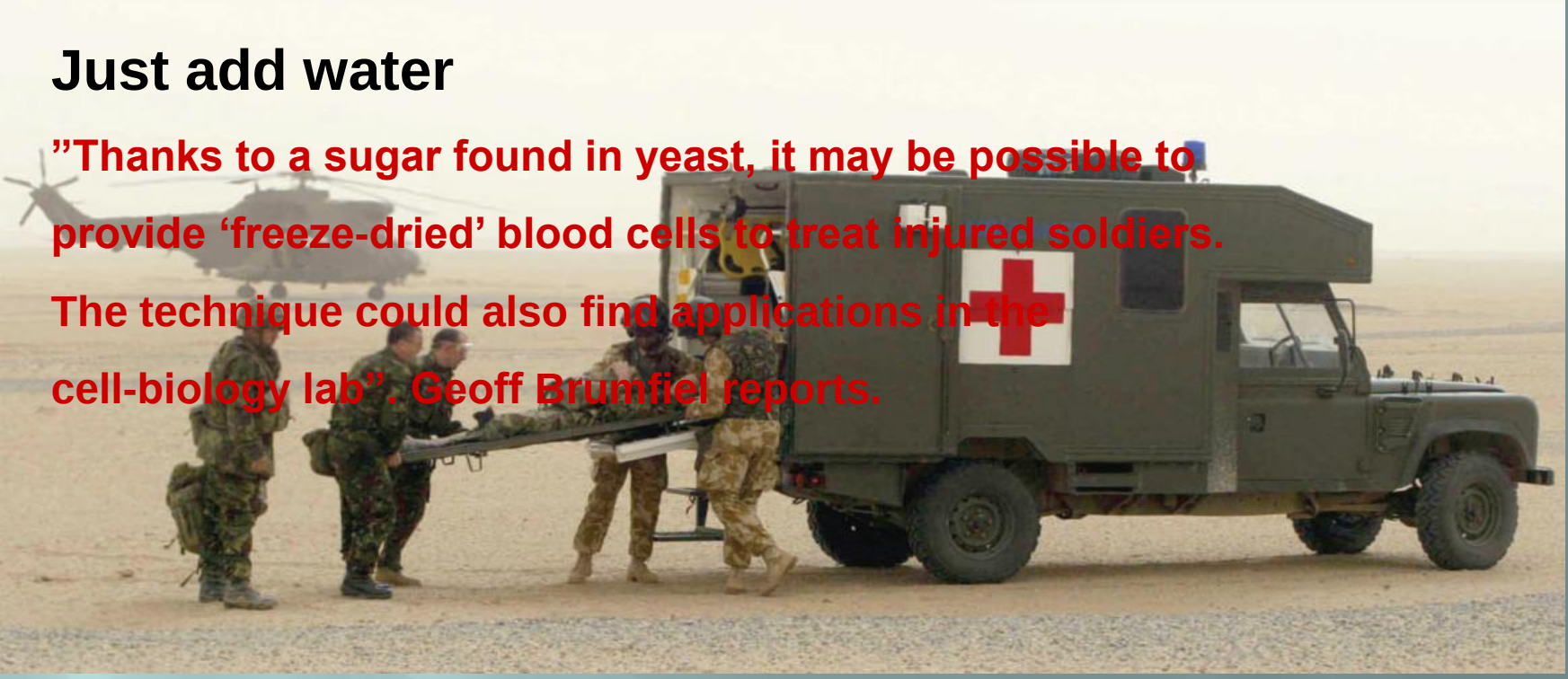
Freeze-dried RBC

news feature From Dr. Crowe Lab

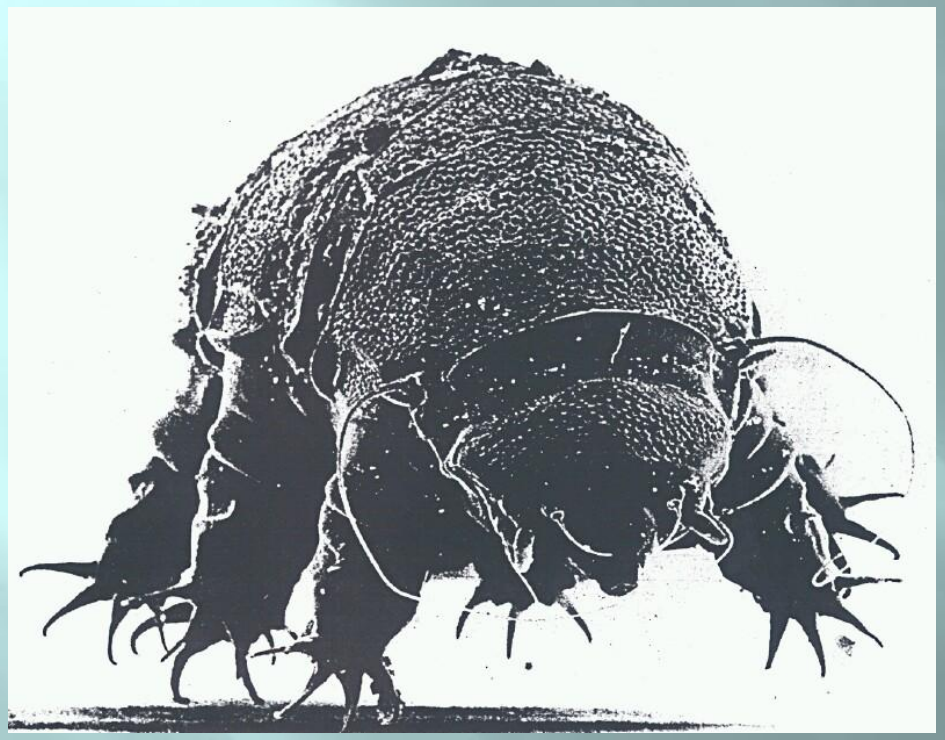
NATURE|VOL 428 | 4 MARCH 2004 | www.nature.com/nature

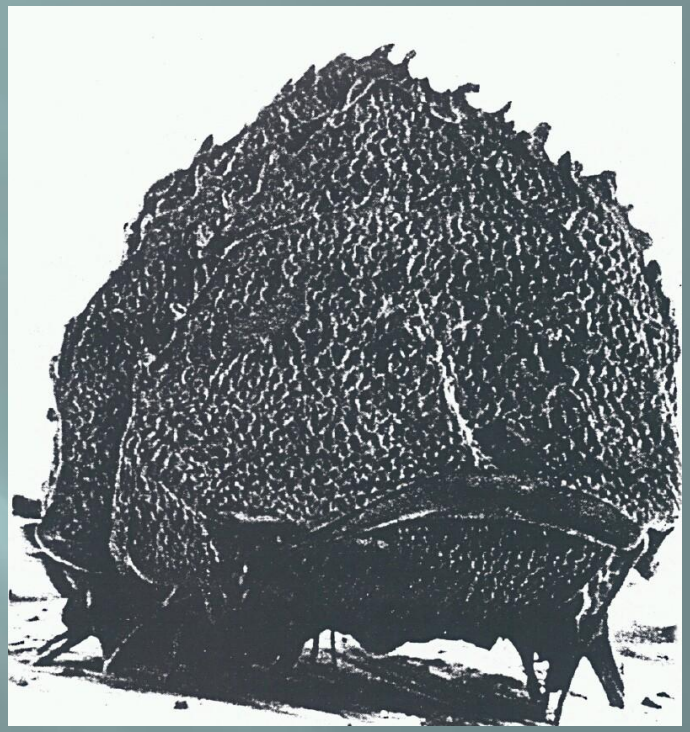
Just add water

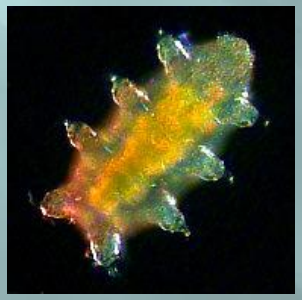
”Thanks to a sugar found in yeast, it may be possible to provide ‘freeze-dried’ blood cells to treat injured soldiers. The technique could also find applications in the cell-biology lab”. Geoff Brumfiel reports.



Lessons from Nature: From Anhydrobiosis to Freeze-Drying







Active Tardigrade

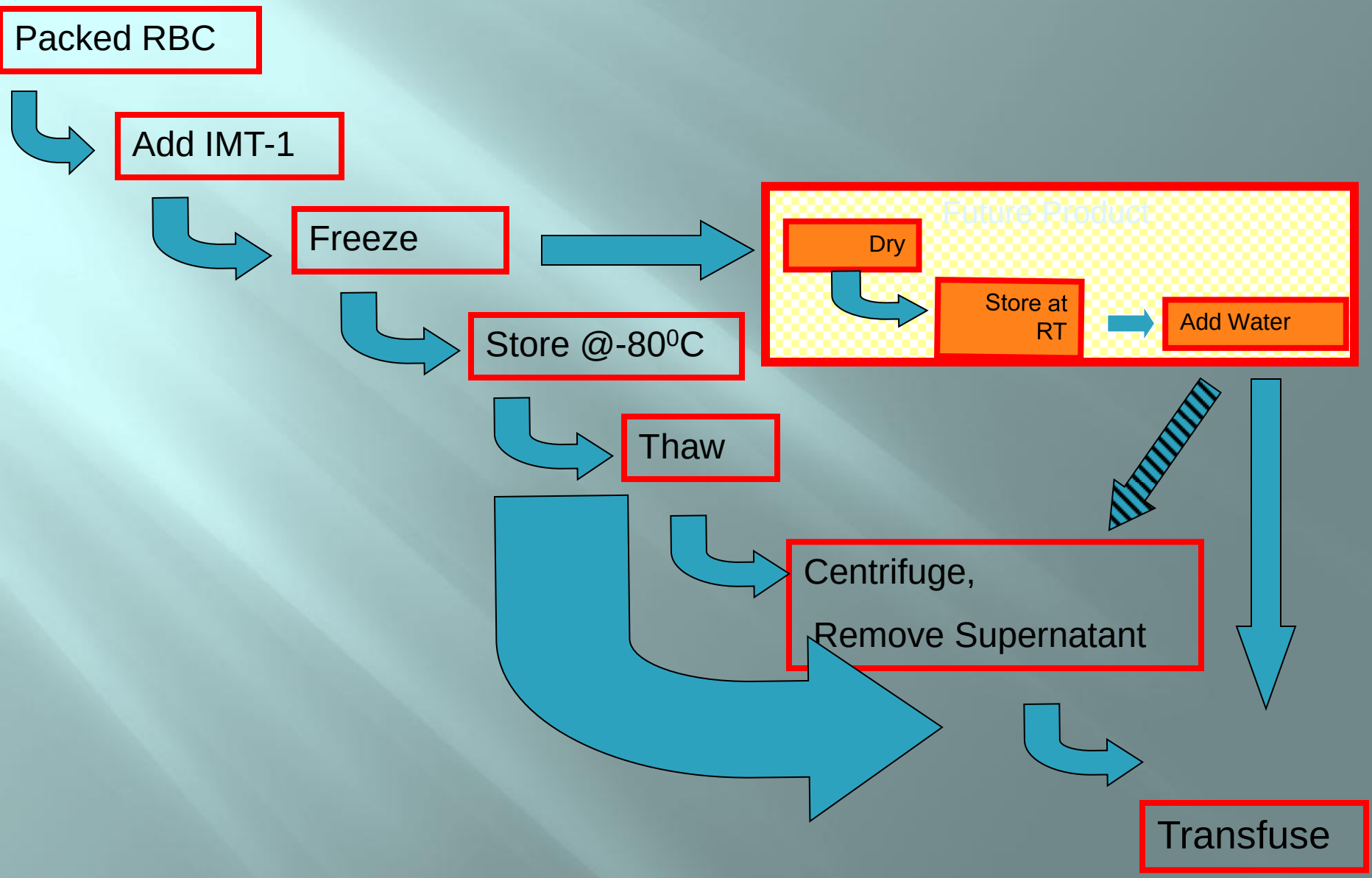
Dried Tardigrade

Crowe and Cooper. 1971. Scientific American

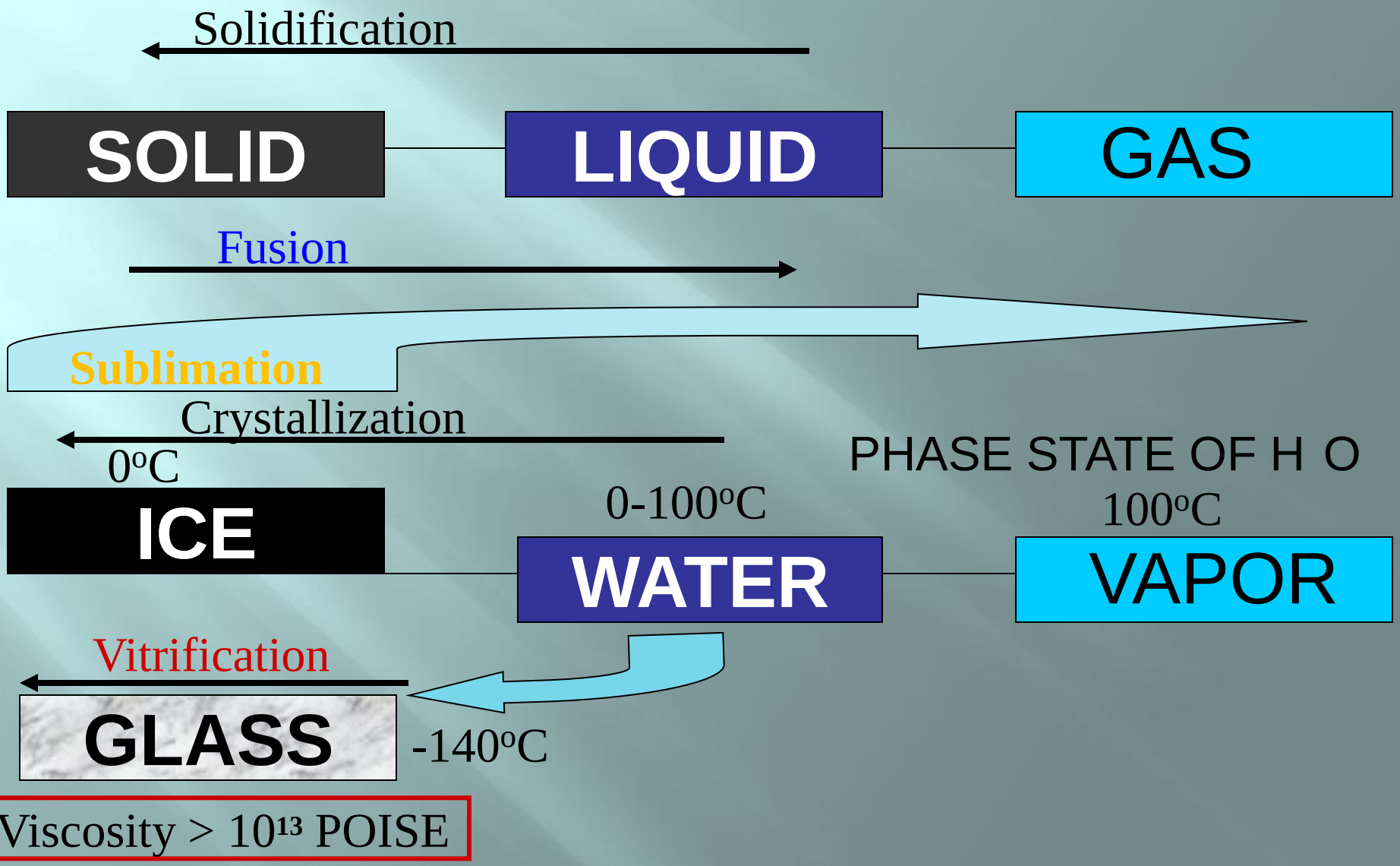
Core Dynamics Mission

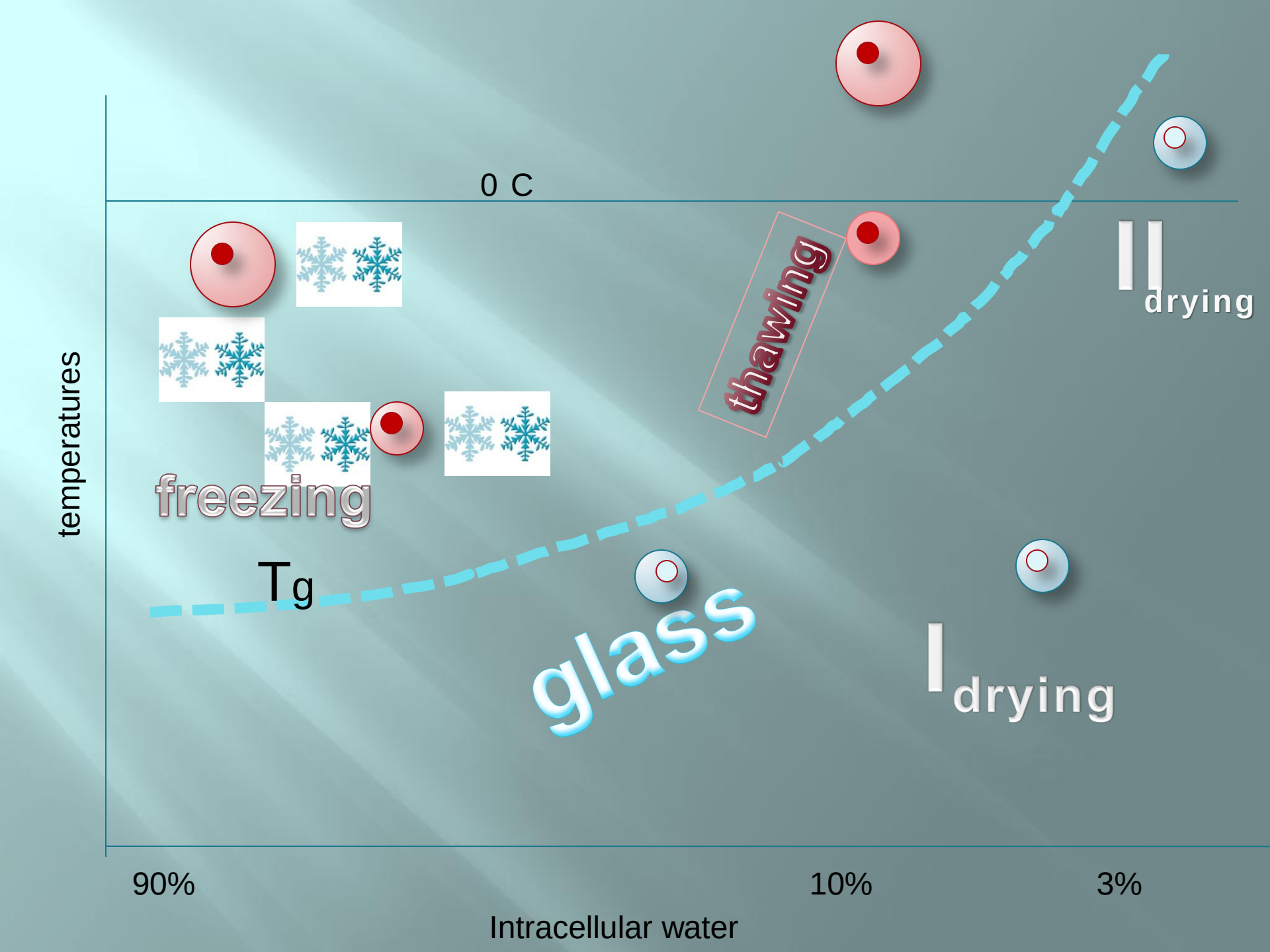
RBC in the battlefield

Blood Freezing and Lyophilization



Phase transition




temperatures
0 C
thawing
II drying
freezing
Tg
glass
I drying
90%
10%
3%
Intracellular water

Freezing of RBC

The effect of temperature on size of unfrozen channels

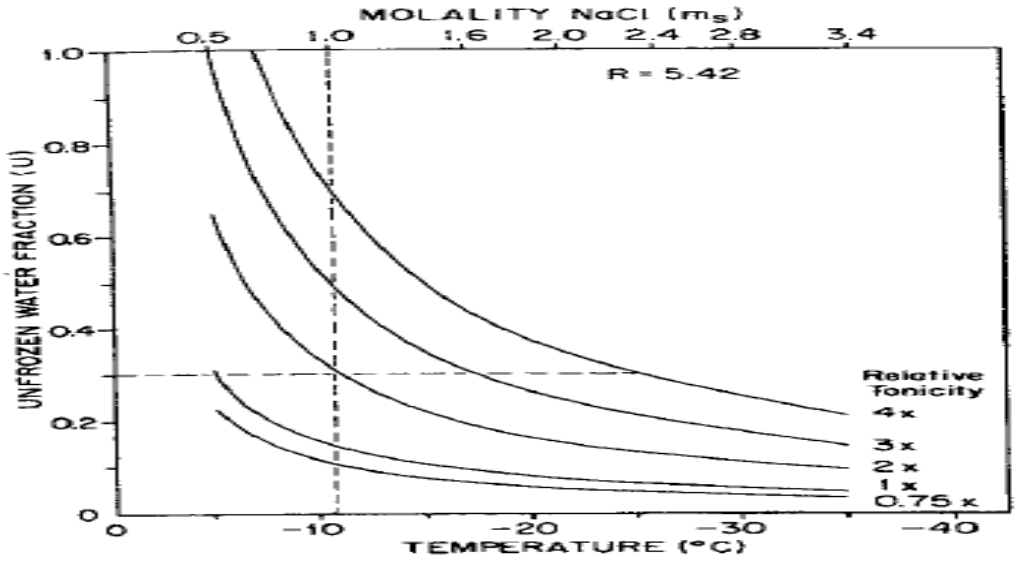


FIG. 4. U vs temperature for glycerol—NaCl—water solutions in which $R = 5.42$. The initial concentration of NaCl ranges from 0.75 to 4× isotonic. Details on the composition of the solutions prior to freezing are given in Table 1. The upper abscissa shows the molality of NaCl (m_s) that is present in the unfrozen portions of the solution at the indicated temperatures. The vertical dotted line is an example of conditions yielding constant m_s and variable U. The dotted horizontal line illustrates conditions yielding constant U and variable m_s. Modified from Mazur *et al.* (9).

Mazur et al., 1983

The effect of unfrozen channels on RBC survival

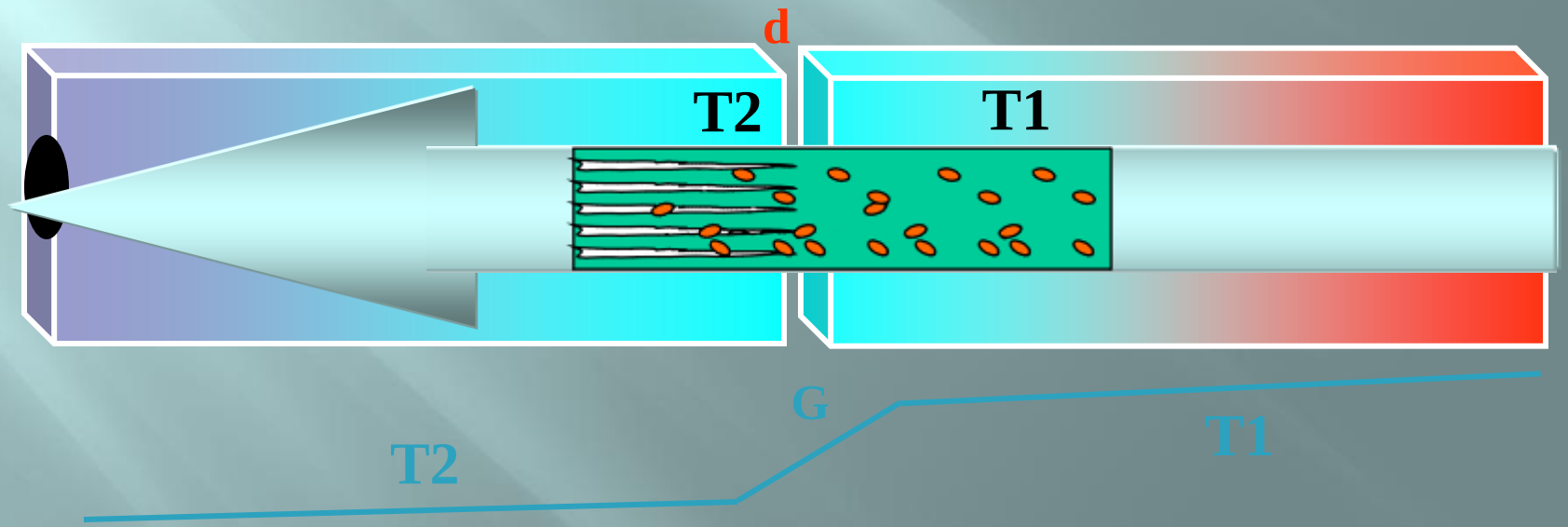
TABLE 2

Survival of Human Erythrocytes Suspended in Various Glycerol–NaCl–Water Solutions with $R = 5.42$, Frozen at 0.6°C/min to Various Subzero Temperatures, and Thawed Slowly[a]

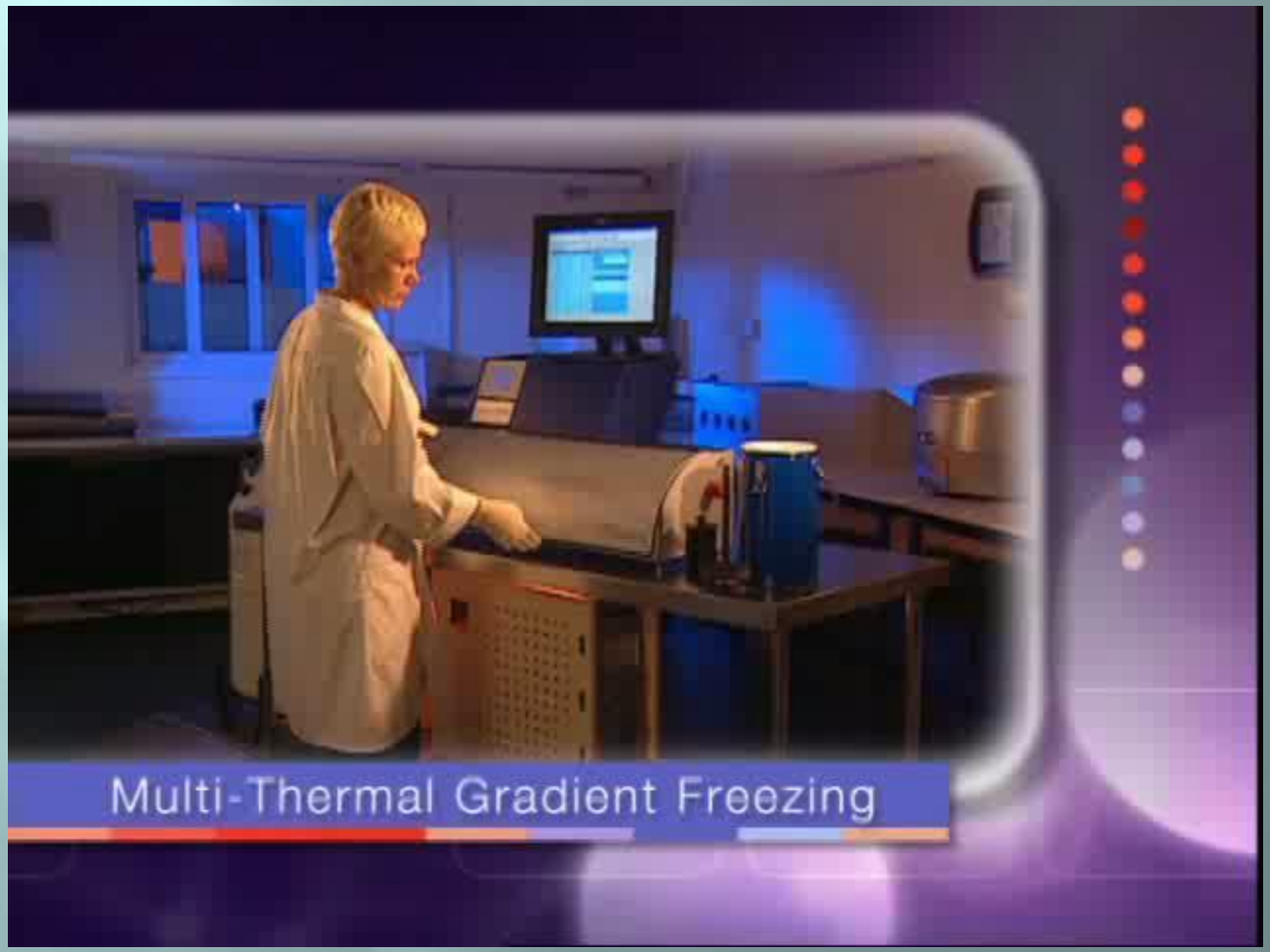
Condition	Relative NaCl tonicity	Temperature (°C)	Molality NaCl (m_s)	Fraction unfrozen (U)	N	Absolute survival Mean (%)	Absolute survival SE	Normal survival Mean (%)	Normal survival SE
R5–0.75×–G3									
1	0.75	−3.6	0.36	0.300	6	85.6	1.2	100.0	1.4
2	0.75	−7.6	0.72	0.150	6	86.8	1.9	101.4	2.2
3	0.75	−10.7	1.00	0.109	6	78.1	2.1	91.2	2.4
4	0.75	−15.2	1.45	0.075	6	50.2	1.3	58.7	1.5
5	0.75	−16.8	1.60	0.068	6	40.6	1.4	47.4	1.6
6	0.75	−21.5	2.00	0.054	6	25.1	1.7	29.3	2.0
7	0.75	−26.0	2.40	0.045	6	19.2	1.0	22.4	1.1
8	0.75	−29.8	2.80	0.039	6	12.8	0.4	14.9	0.5

The Technology Directional Freezing

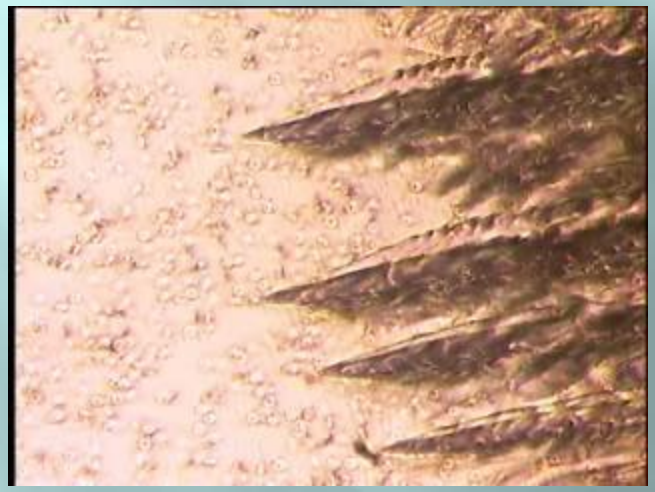
- Core Dynamics directional freezing technology
  - Accurate control over ice crystal propagation
  - Avoids mechanical damage and toxicity

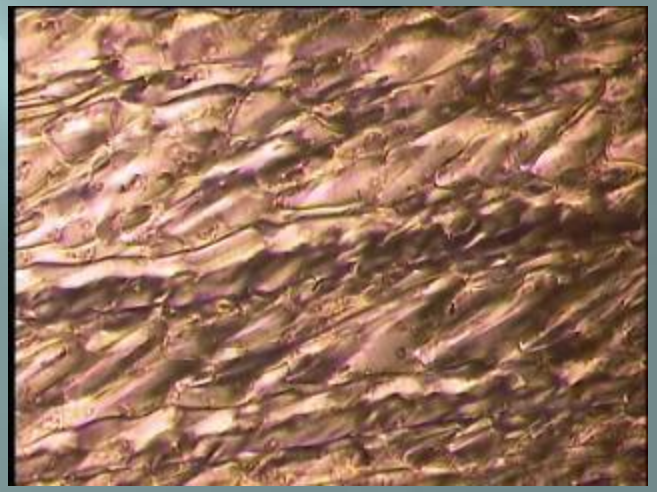


Multi-Thermal Gradient Animation

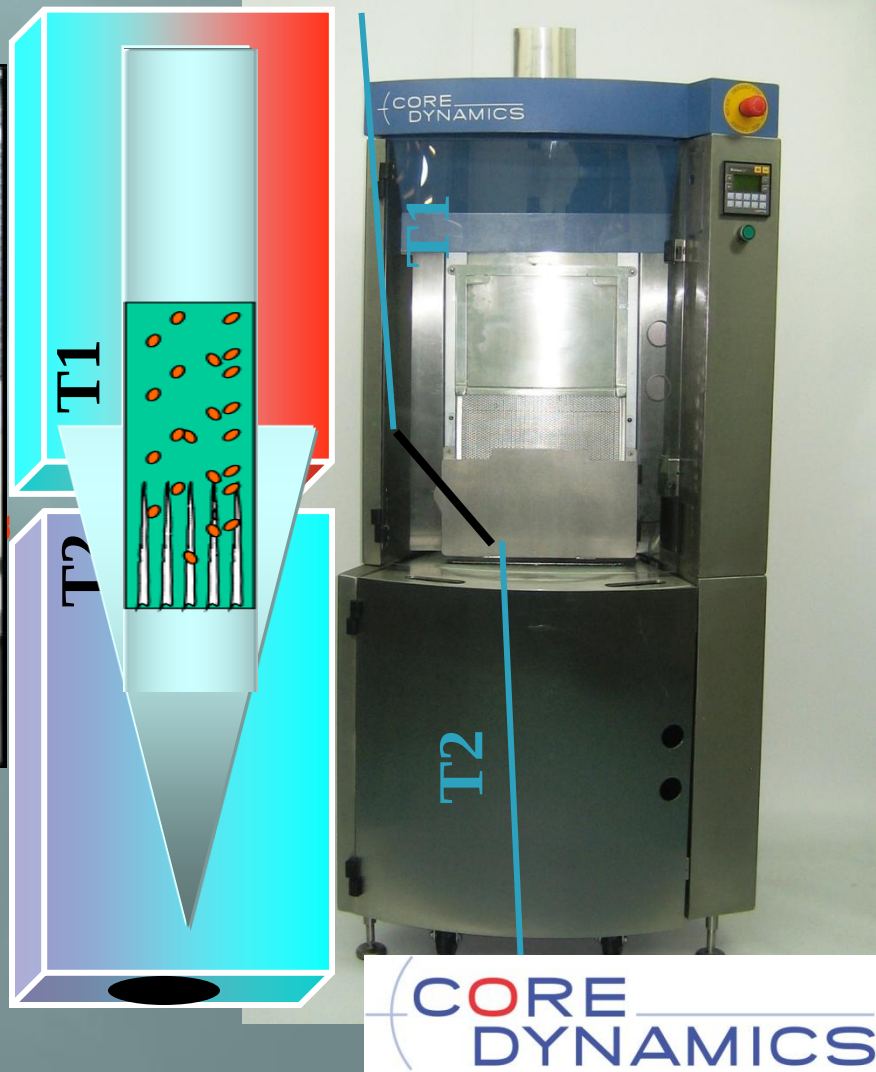


Directional freezing of RBC





The directional freezing device

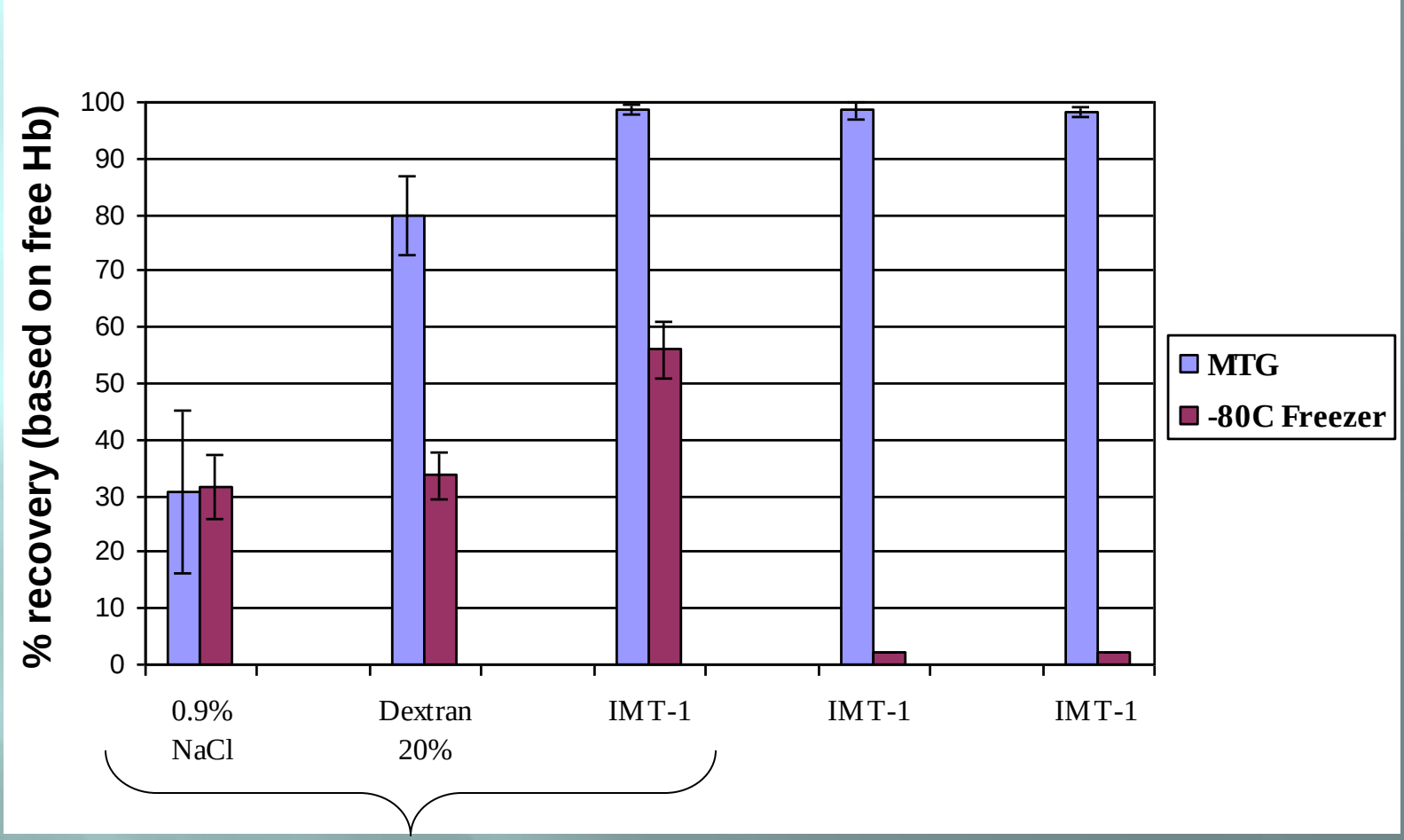


CORE DYNAMICS

Ice Thawing Device DTD

CORE DYNAMICS

RBC Scale Up

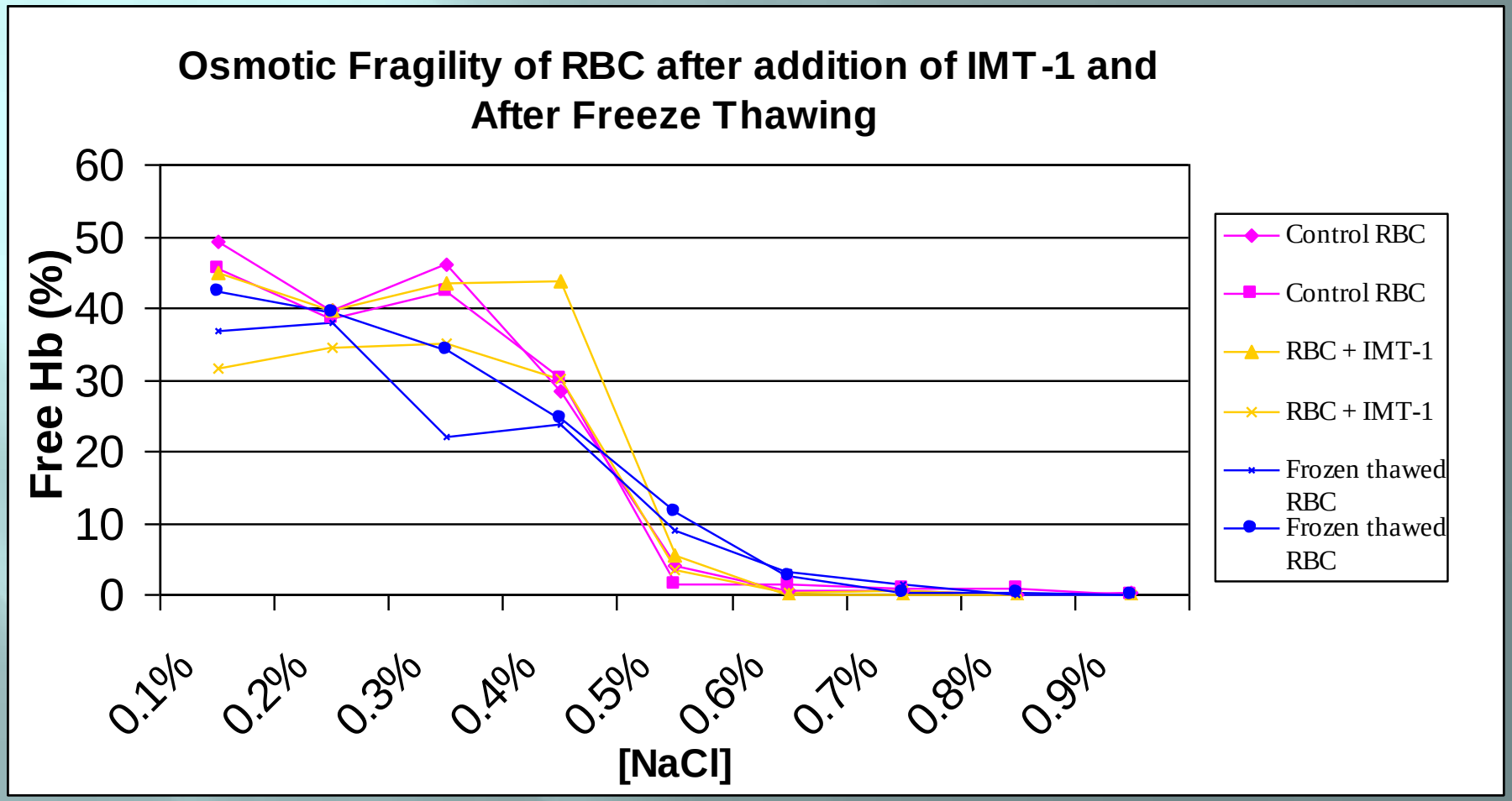


2.5ml sample (n=10)

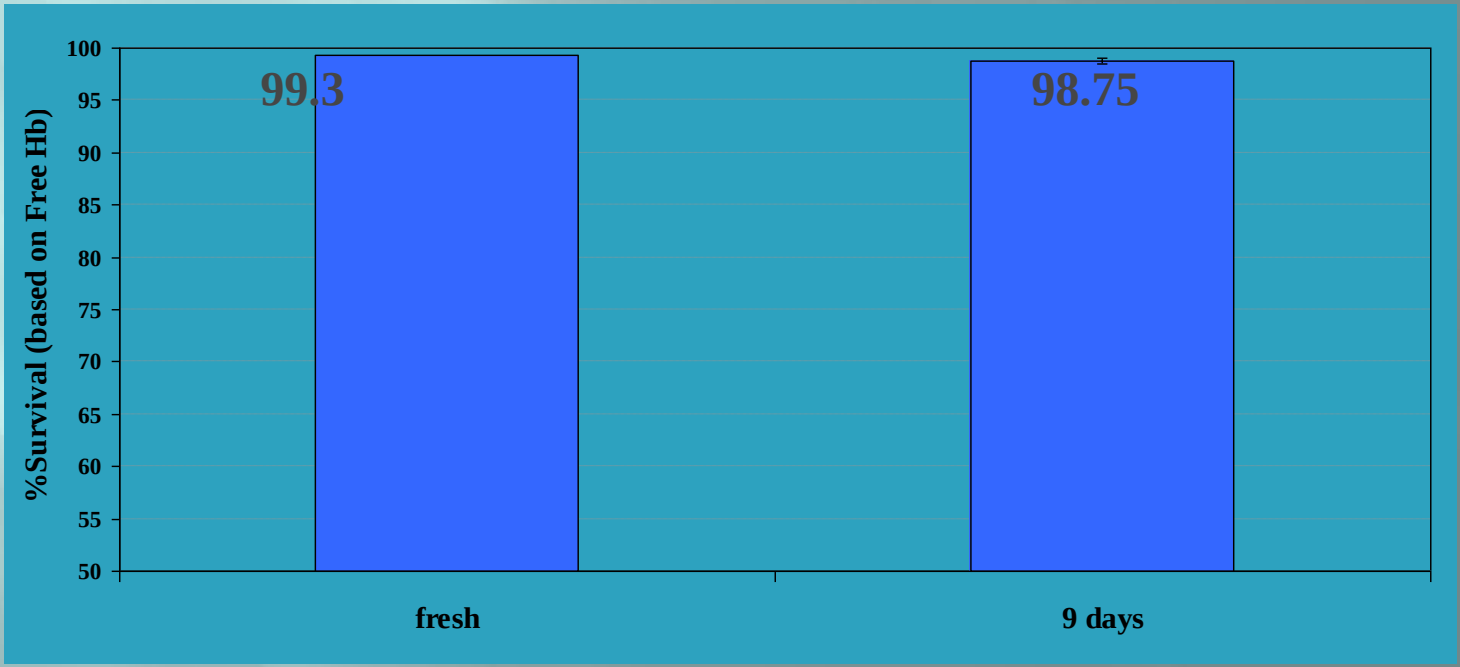
100ml sample (n=9)

500ml sample (n=5)

Osmotic fragility of frozen blood



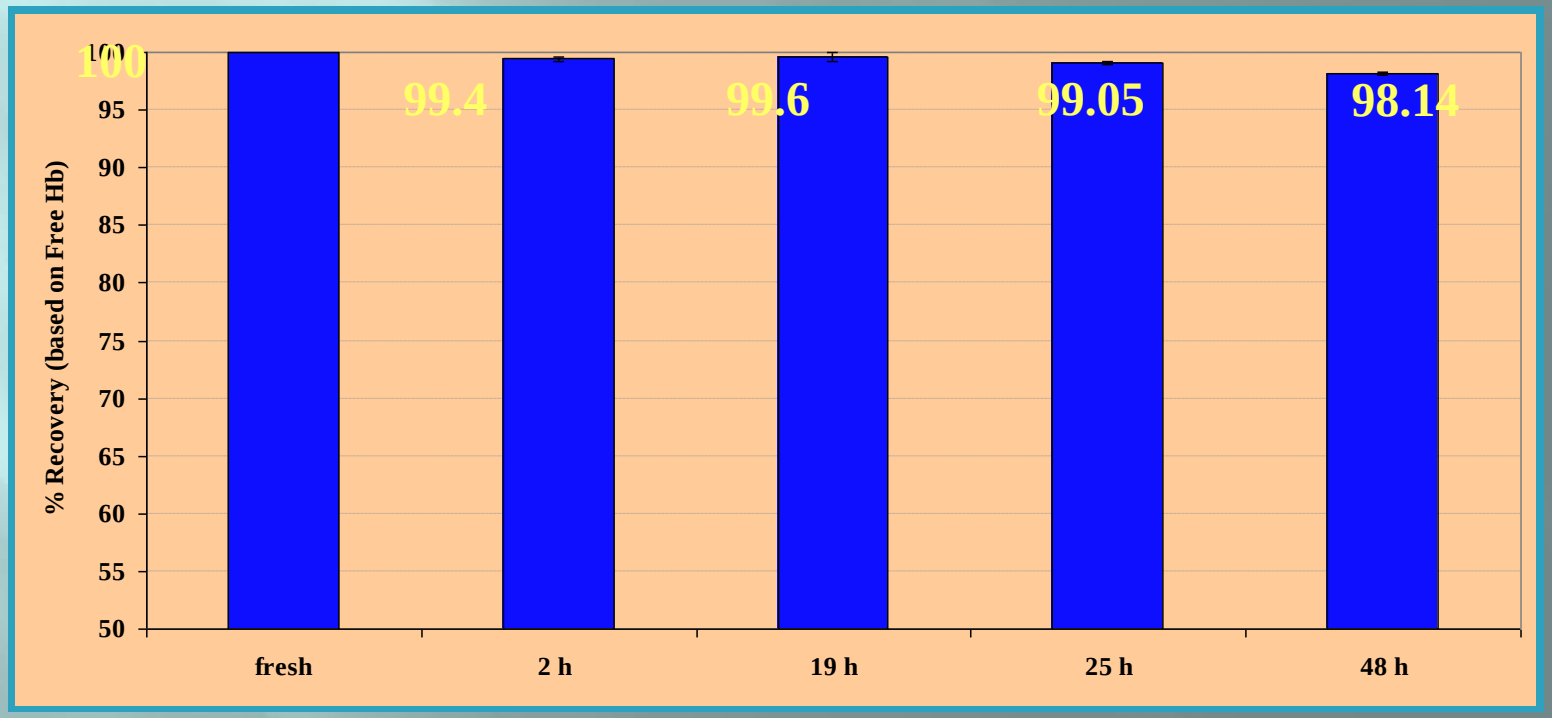
Dry Shipper Storage for 9 days of 10 units of blood



- 300 ml

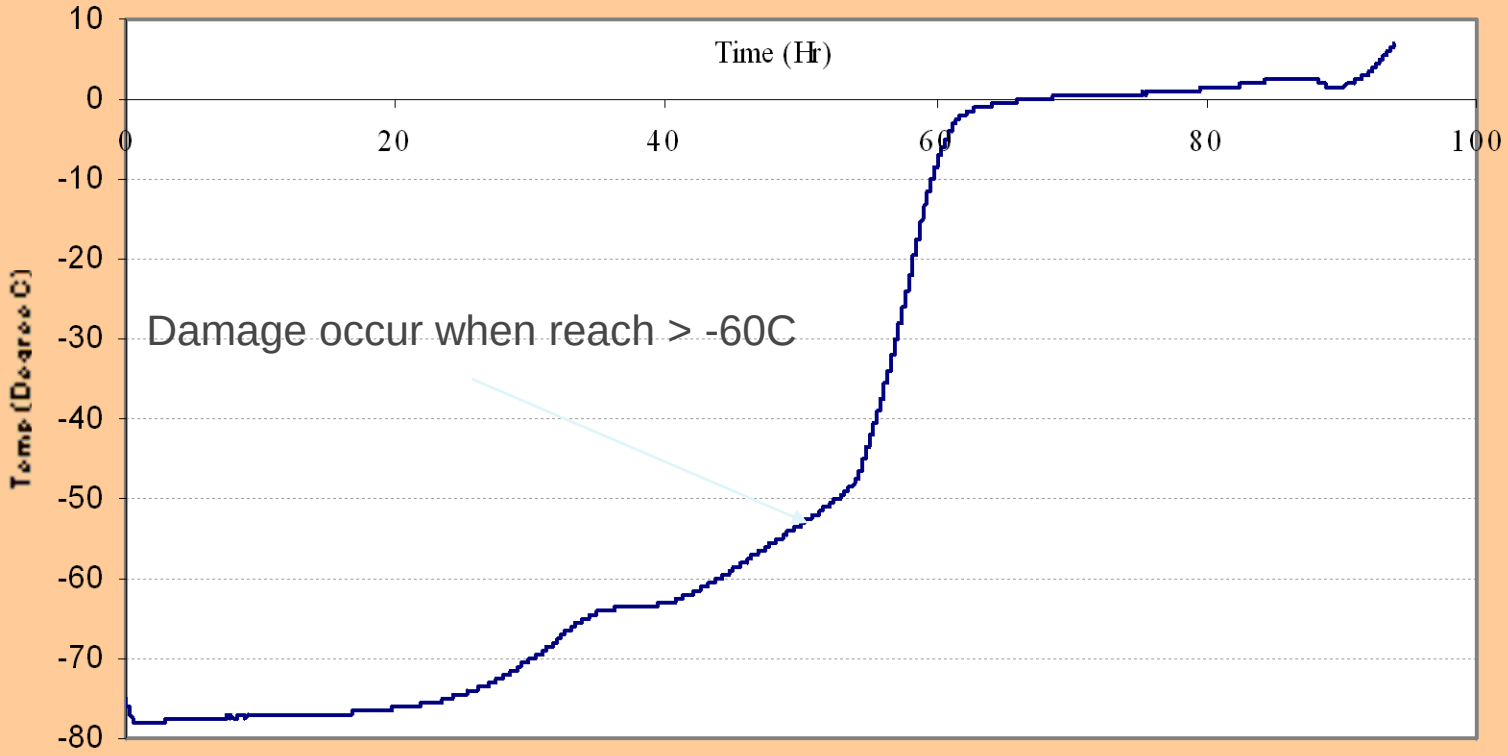
Dry Ice Storage for 48h using 2kg DI/blood unit

% Survival (based on Free Hb)

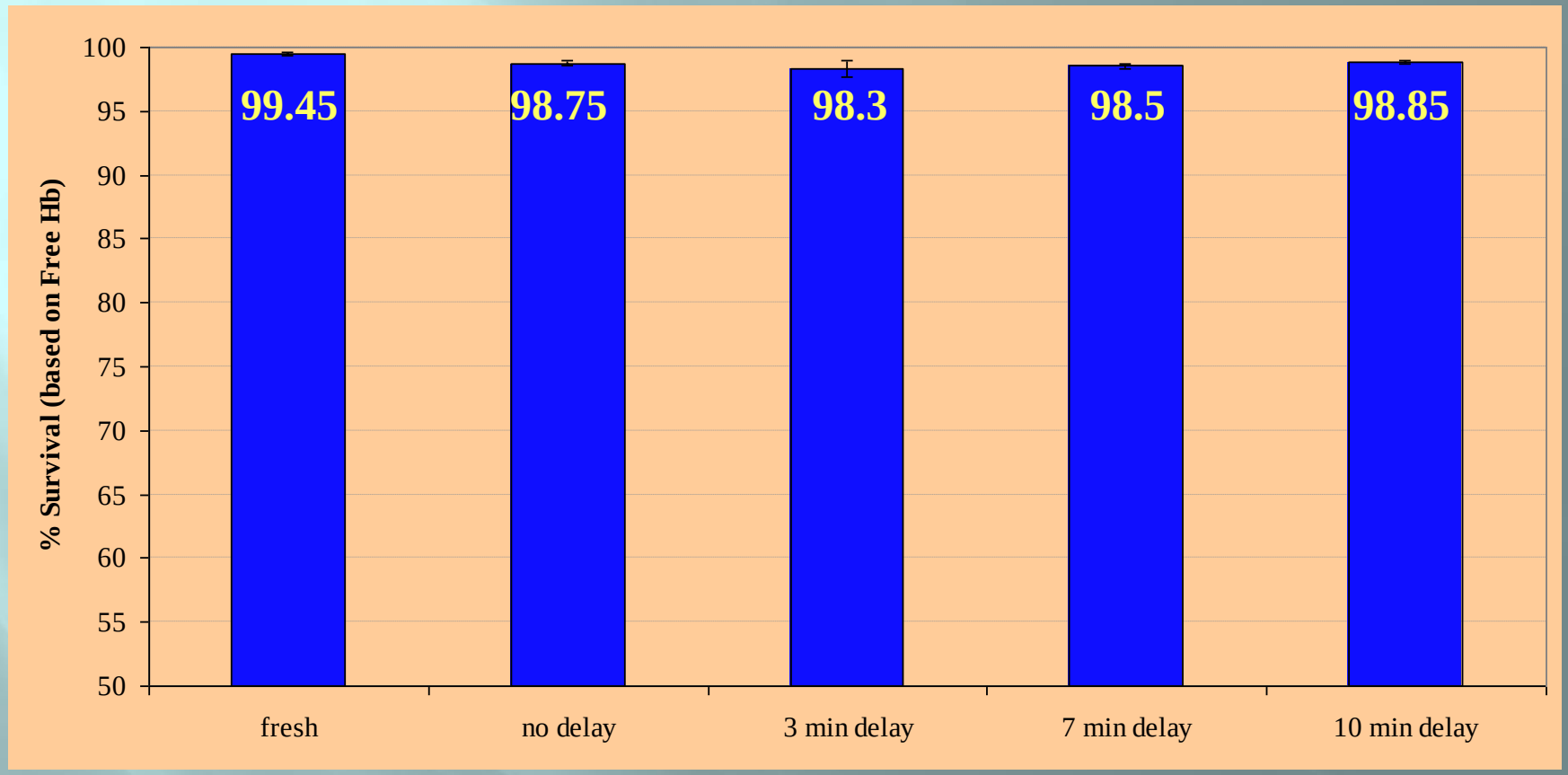


- 500 ml

Dry Ice Temperature Recording



Effect of delay Thawing

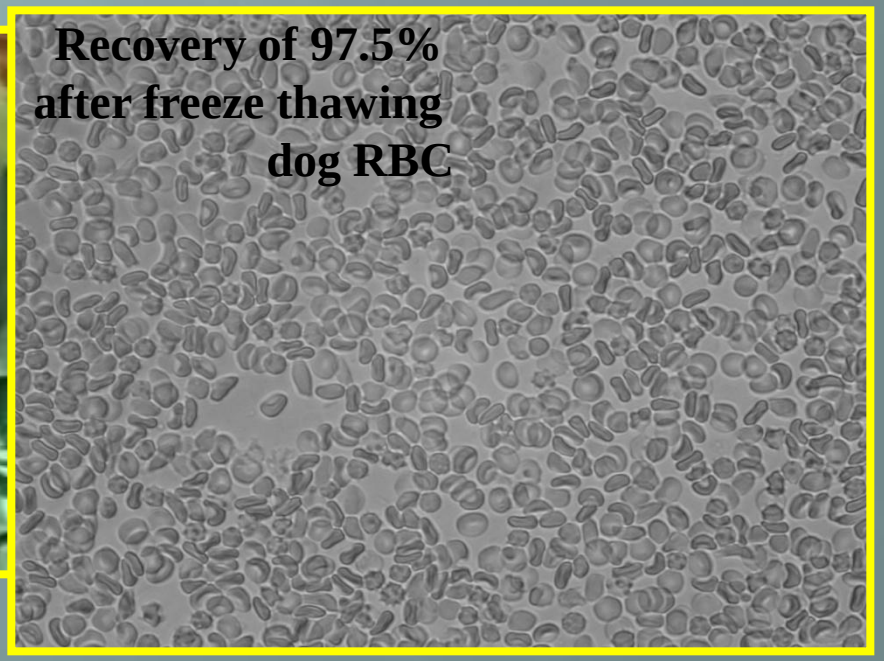


•500 ml

Freezing Short Dated RBC Units

- 500ml

In Vivo RBC-Cr^{51} Survival



80% of RBC found circulating 24 hr after transfusion

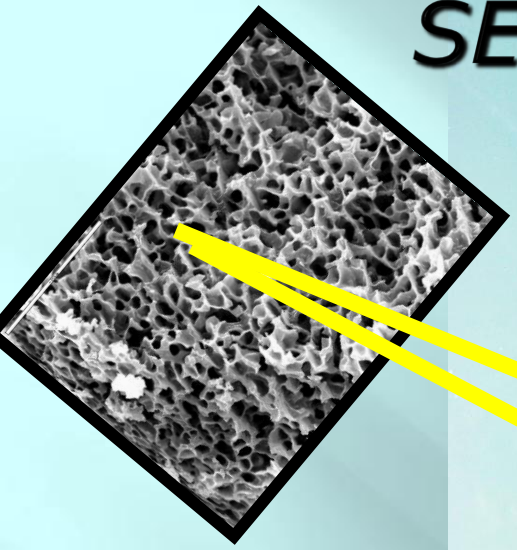
Core Dynamics Vision

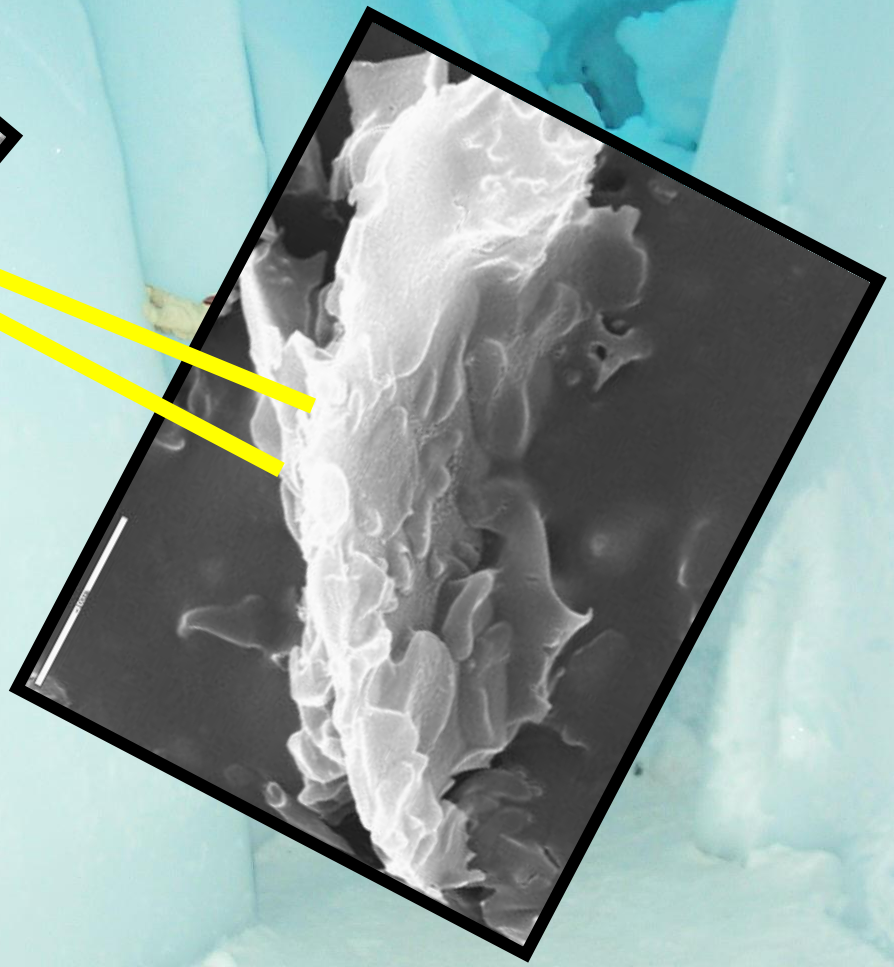
Sterile Water

Dried Blood

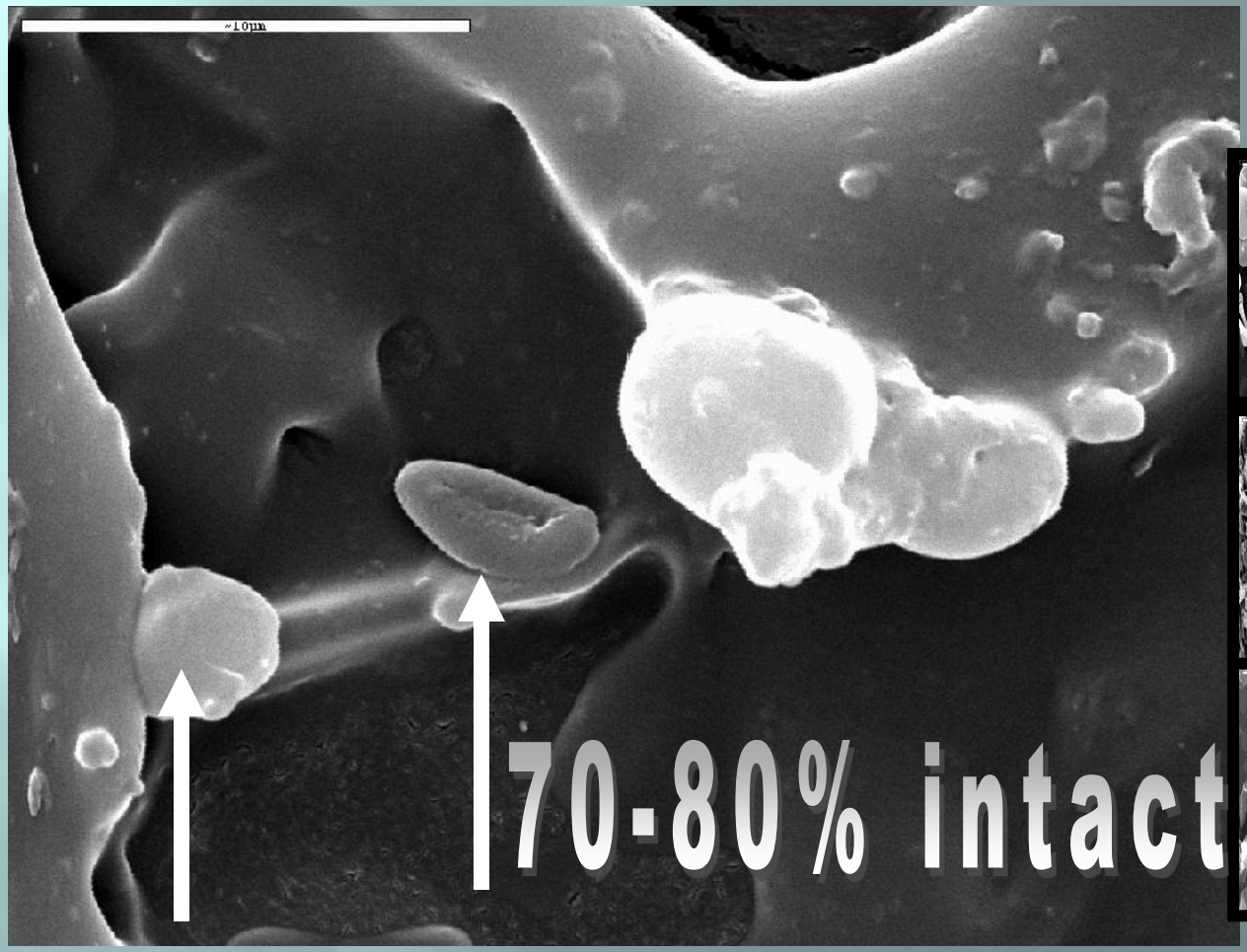
Bed-side Filter

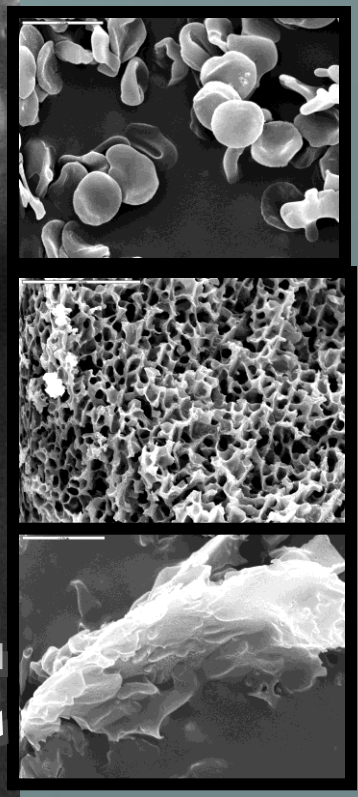
SEM of RBC's with IMT-1





FREEZE DRYING BLOOD



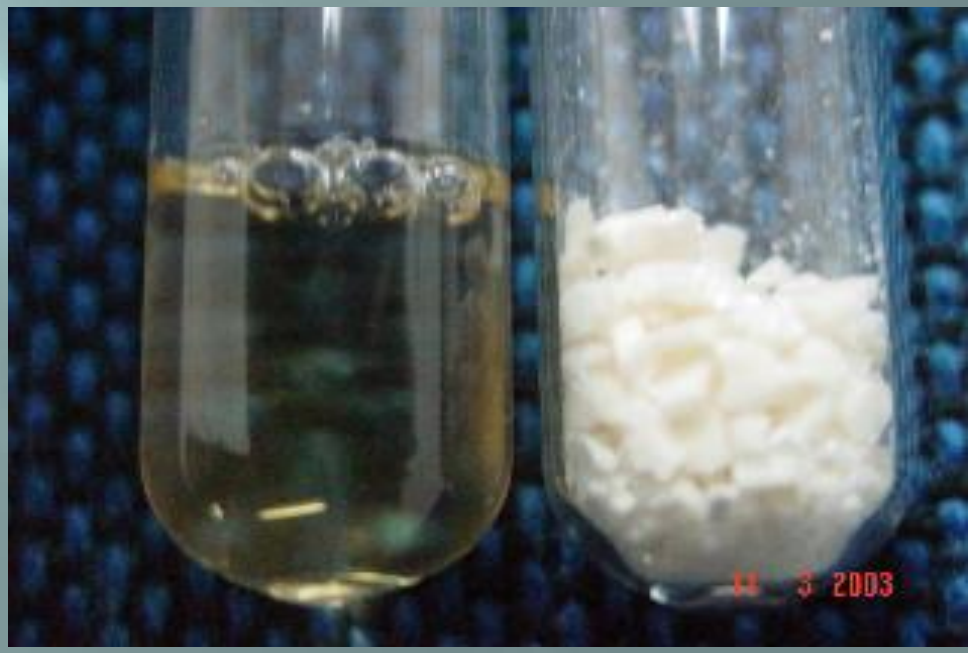


Leukocytes and an erythrocytes that were frozen with 50% fetal calf serum and 0.1M Trehalose, after lyophilization. Magnified X5000

Freeze drying of RBC

- Stabilization – *IMT solution*
- Freezing without Cryoprotectants – *MTG machine*
- Sublimation - *low temp lyophilizer*
- Storage- Vacuum sealed
- Re-hydration- filtration and concentration (F&C)

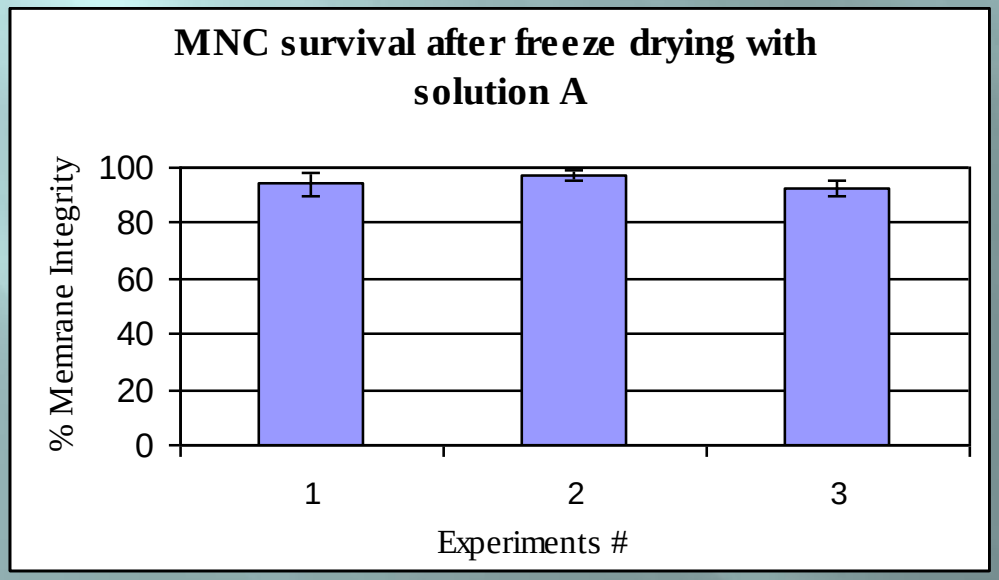
DRIED LEUKOCYTES

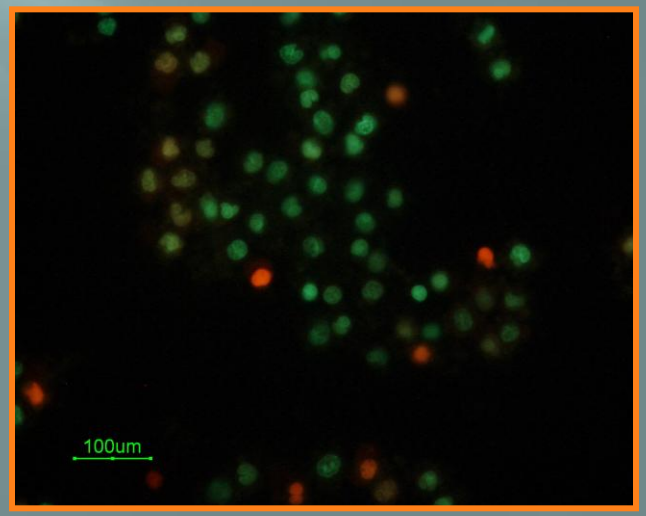


MNC AND CD 34+ CELL AFTER RECONSTITUTION

Determination of CD34+/CD45+ cell number within the MNC population (FACS)

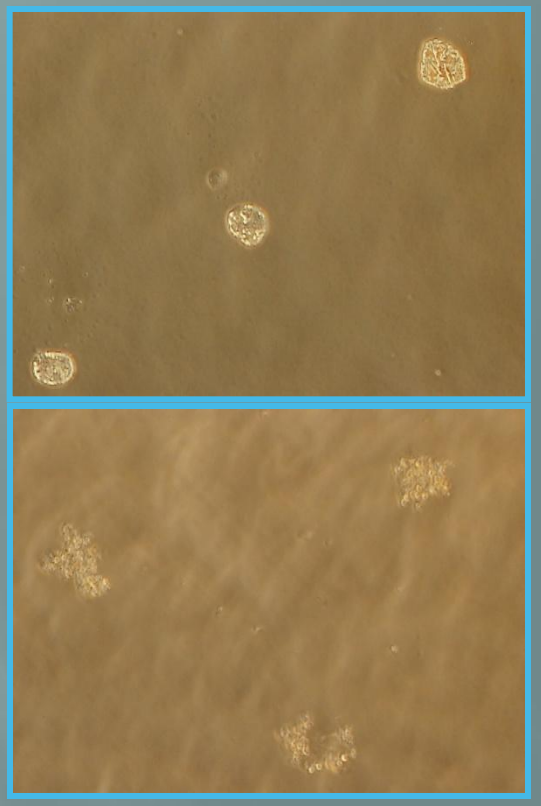
Sample	Total WBC (10^6/ml)	%CD34	CD34 Total (cell number/ml)
Fresh UCB	5.8	0.21	12400
Fresh MNC	2.3	0.68	15600
Lyo 1	2.5	0.69	17300
Lyo 2	2.5	0.70	17600





CFU ASSAY OF FRESH AND REHYDRATED MNC DERIVED FROM HUMAN UCB

Sample	Well 1	Well 2	Total colonies	Type
Control	4 –E, 31-GM	6 –E, 18-GM	10 –E, 49-GM =**59**	CFU-E/GM
Lyo 1	2 –E; 18-GM	3 –E; 9-GM, 2 –Mix	5 –E, 27-GM 2-Mix =**34**	CFU-E/GM/Mix
Lyo 2	1 –E, 23-GM	1 –E, 16-GM	2 –E, 39-GM =**41**	CFU-E/GM
Lyo 3	4 –E; 22-GM	12-GM, 2 –Mix	4 –E, 34-GM 2-Mix =**40**	CFU-E/GM/Mix
Lyo 4	1 –E; 20-GM 1 –Mix	1 –E; 10-GM 2 –Mix	2 –E, 30-GM, 3-Mix =**35**	CFU-E/GM/Mix
Lyo 5	6-GM, 4 Mix	16-GM, 2 Mix	22 –GM, 6-Mix =**28**	CFU-GM/Mix

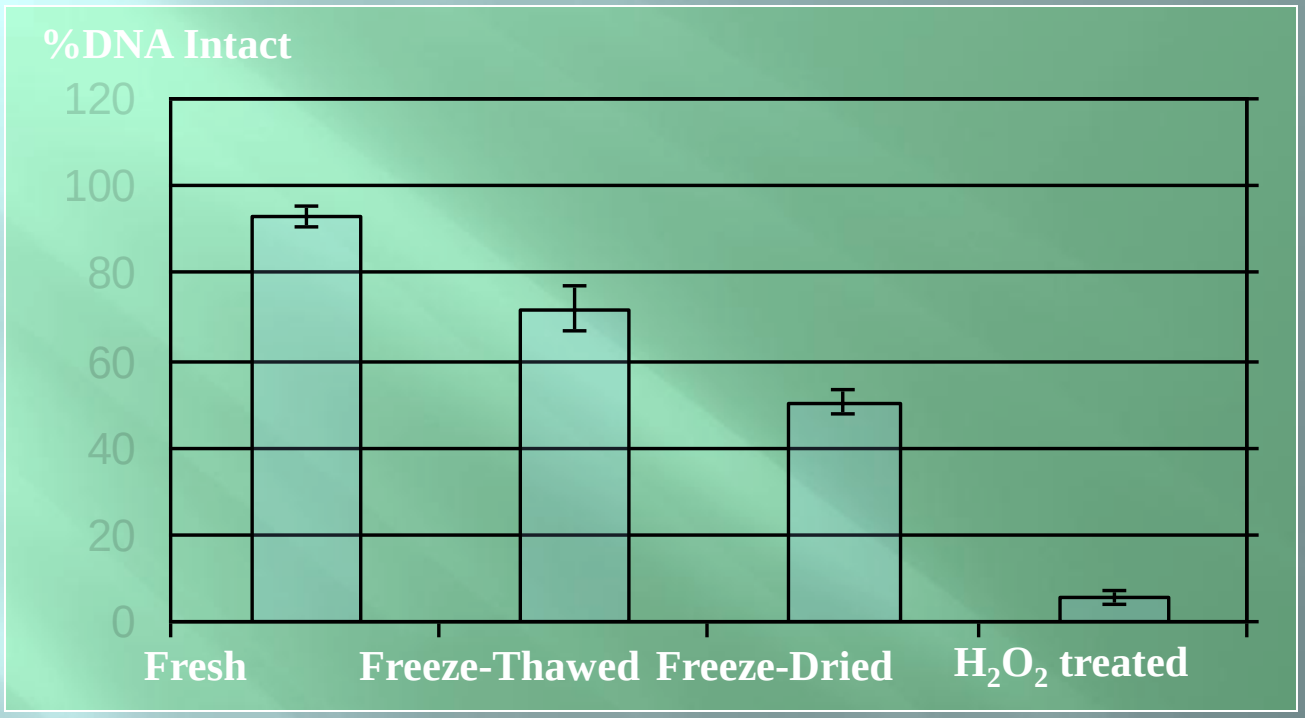


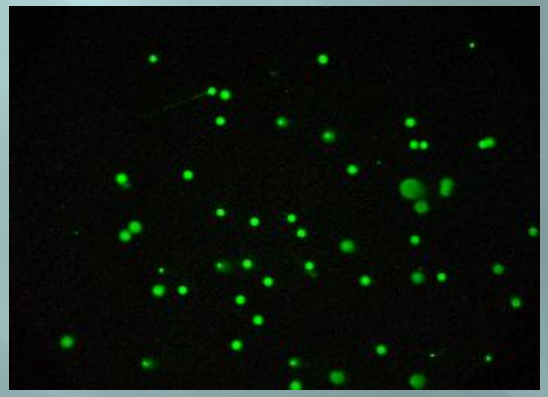
CFU-E = Growth of erythrocyte colonies

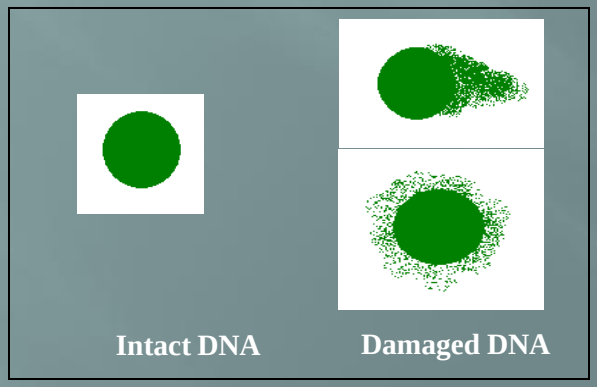
CFU-GM = Growth of granulocytes and macrophage colonies

CFU-Mix = mixture of CFU-GM and CFU-GM colonies

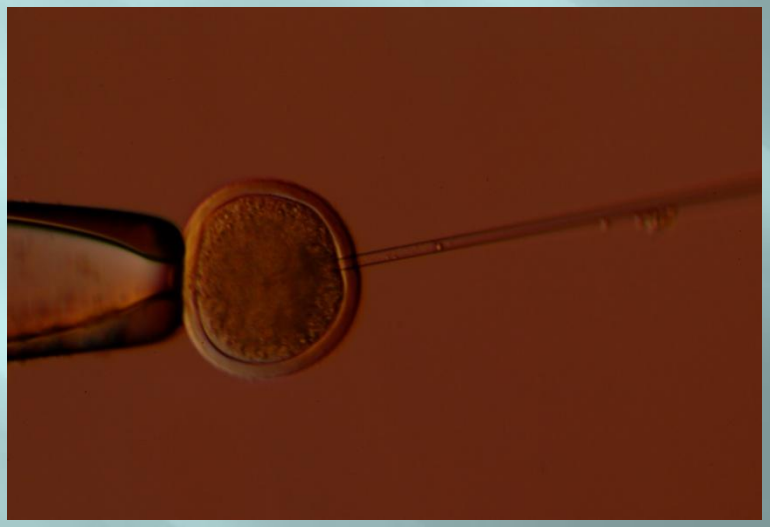
DNA INTEGRITY AFTER DIFFERENT TREATMENTS

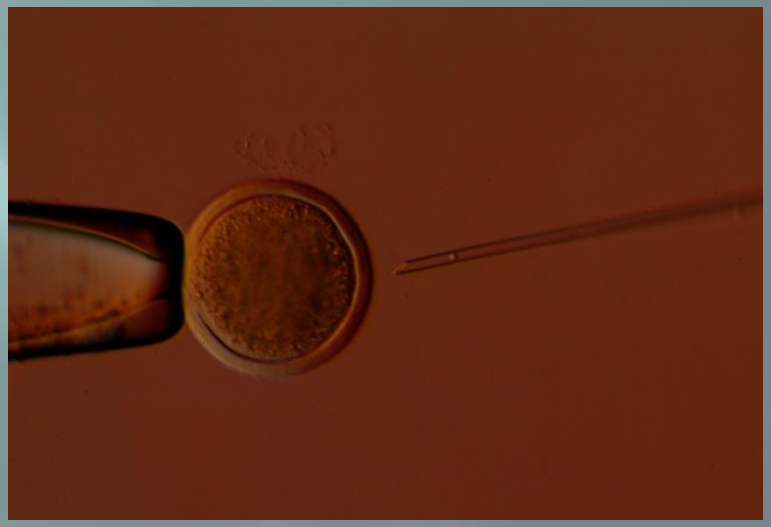






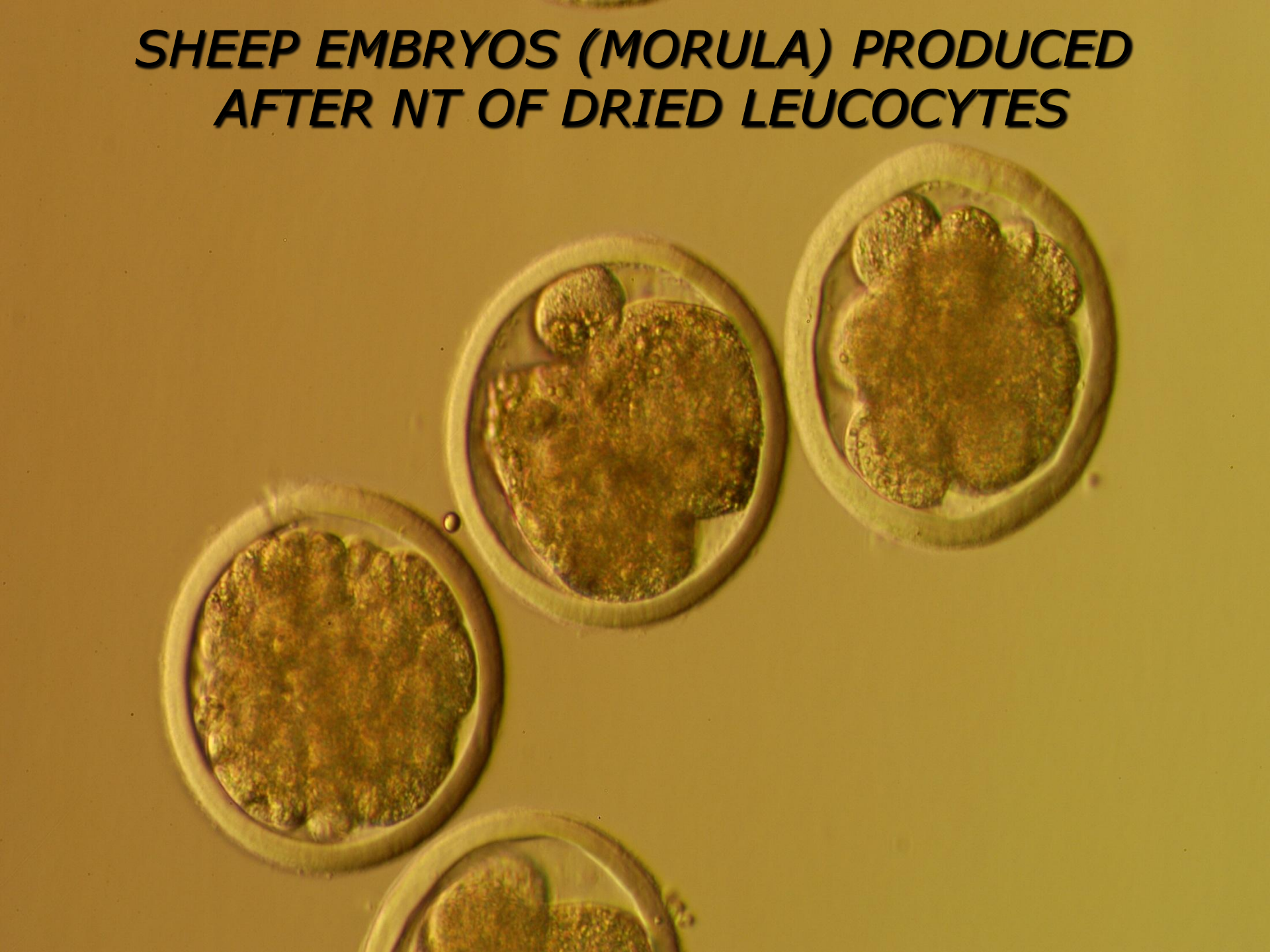
NUCLEAR TRANSFER WITH DRIED CELLS





Loi et al., PLOS ONE 2008

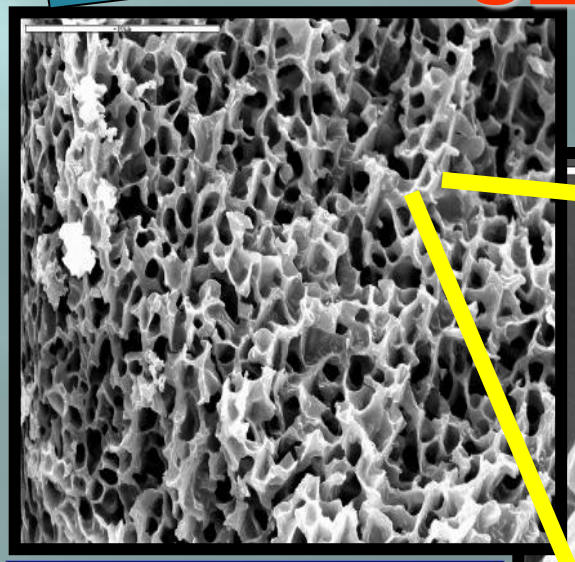
SHEEP EMBRYOS (MORULA) PRODUCED AFTER NT OF DRIED LEUCOCYTES

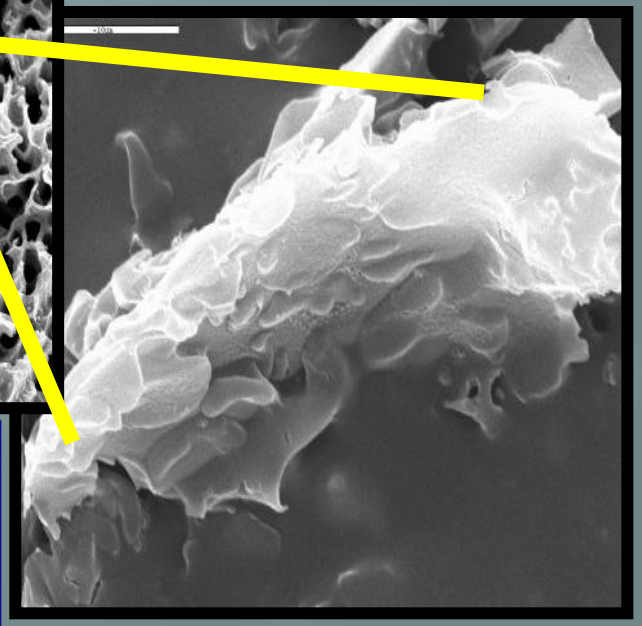


FREEZE DRYING – IN VITRO

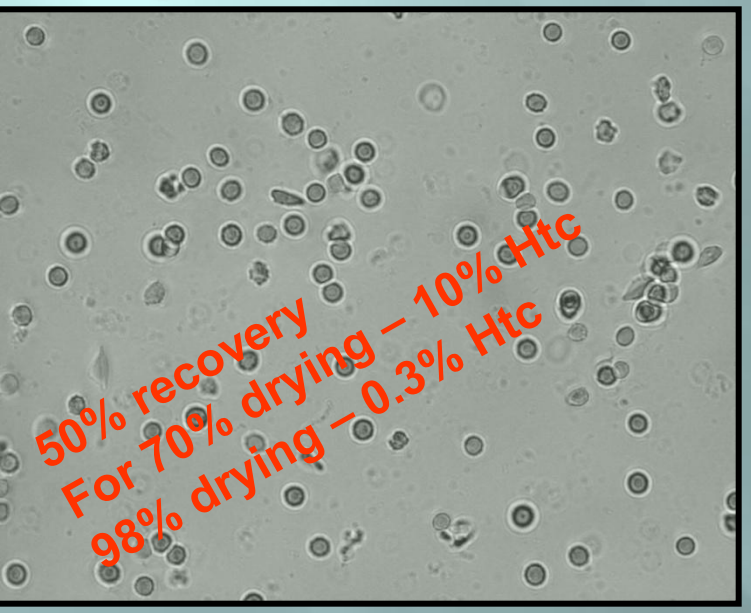
Water

SEM

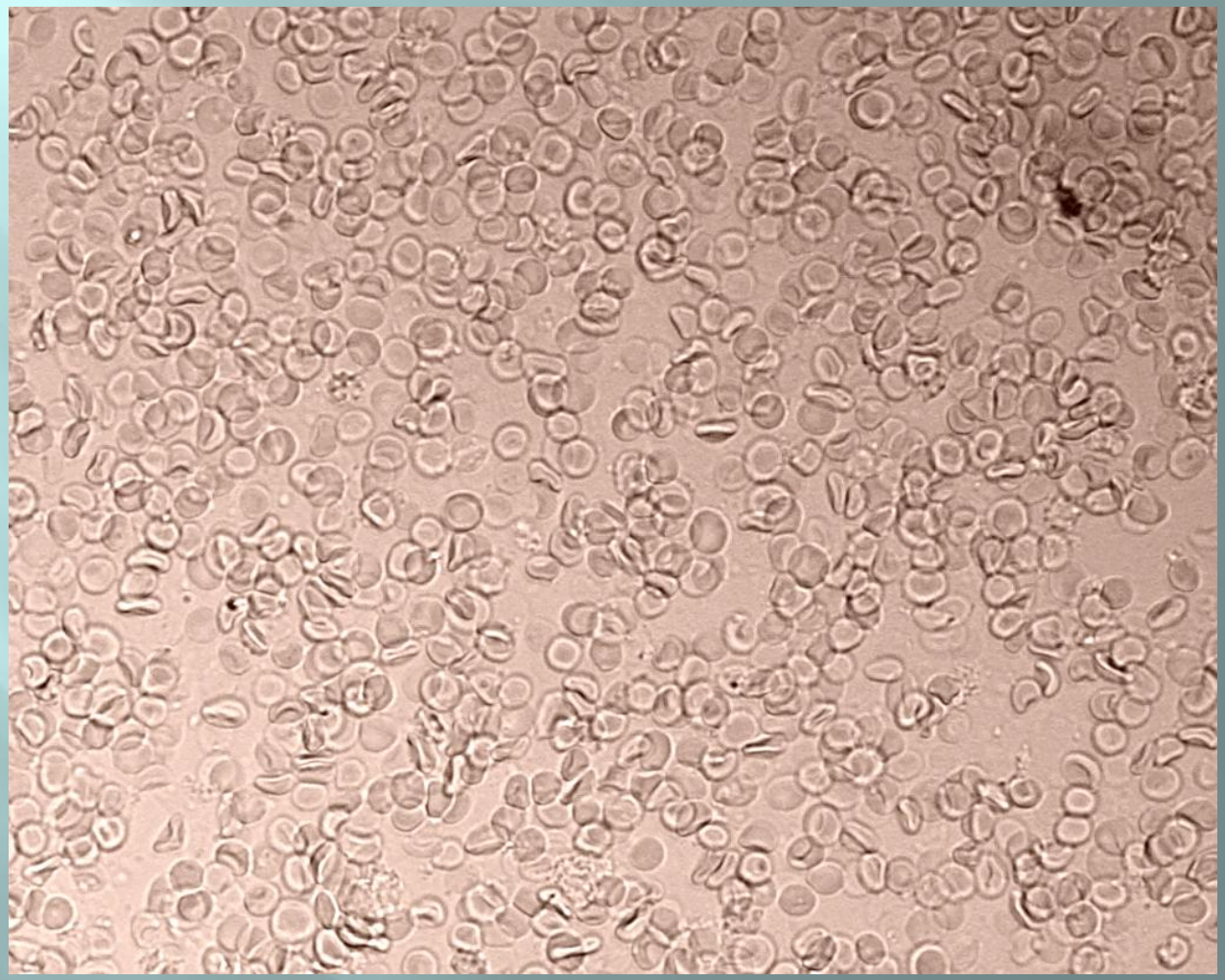




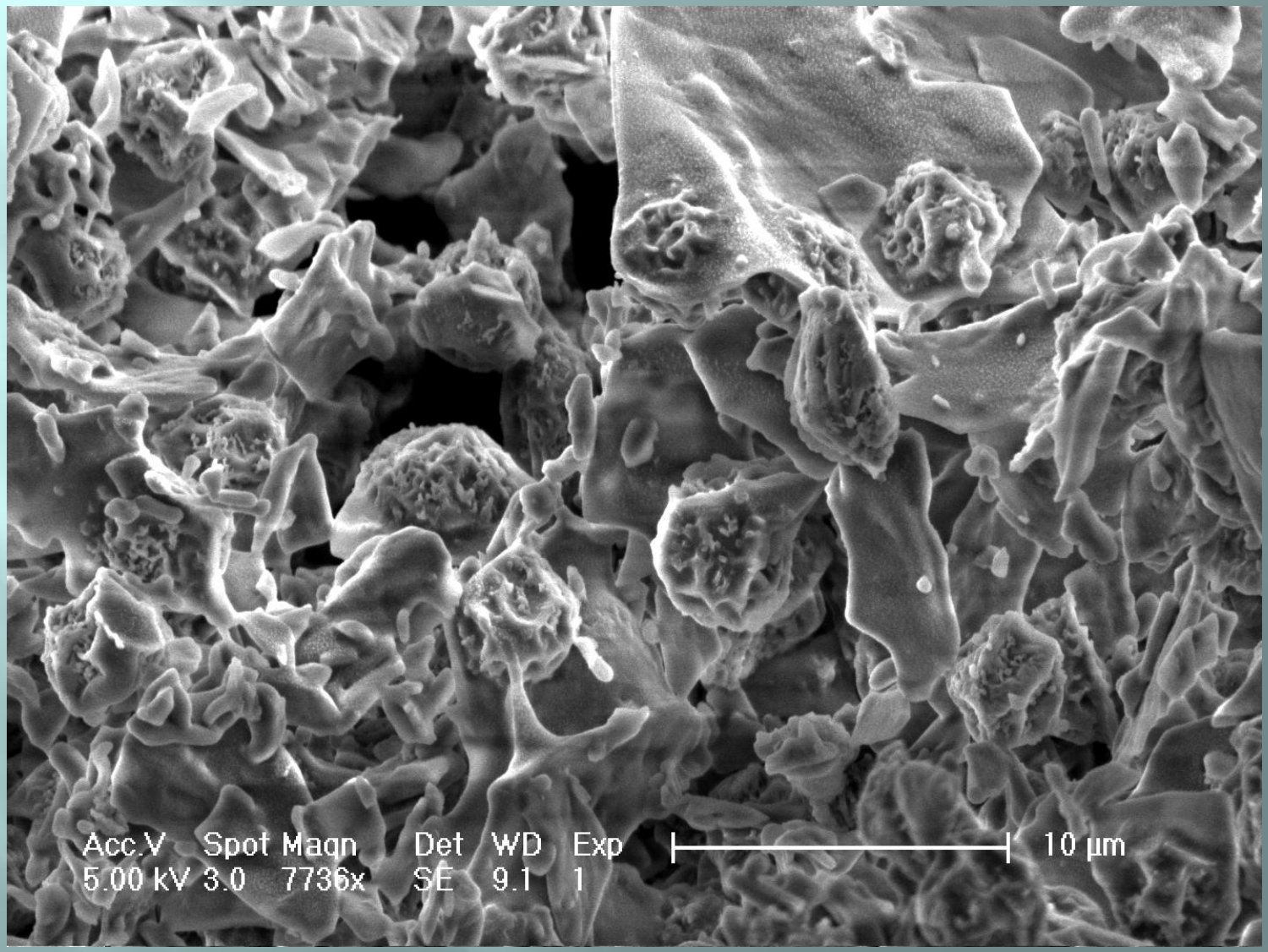
Light Microscope



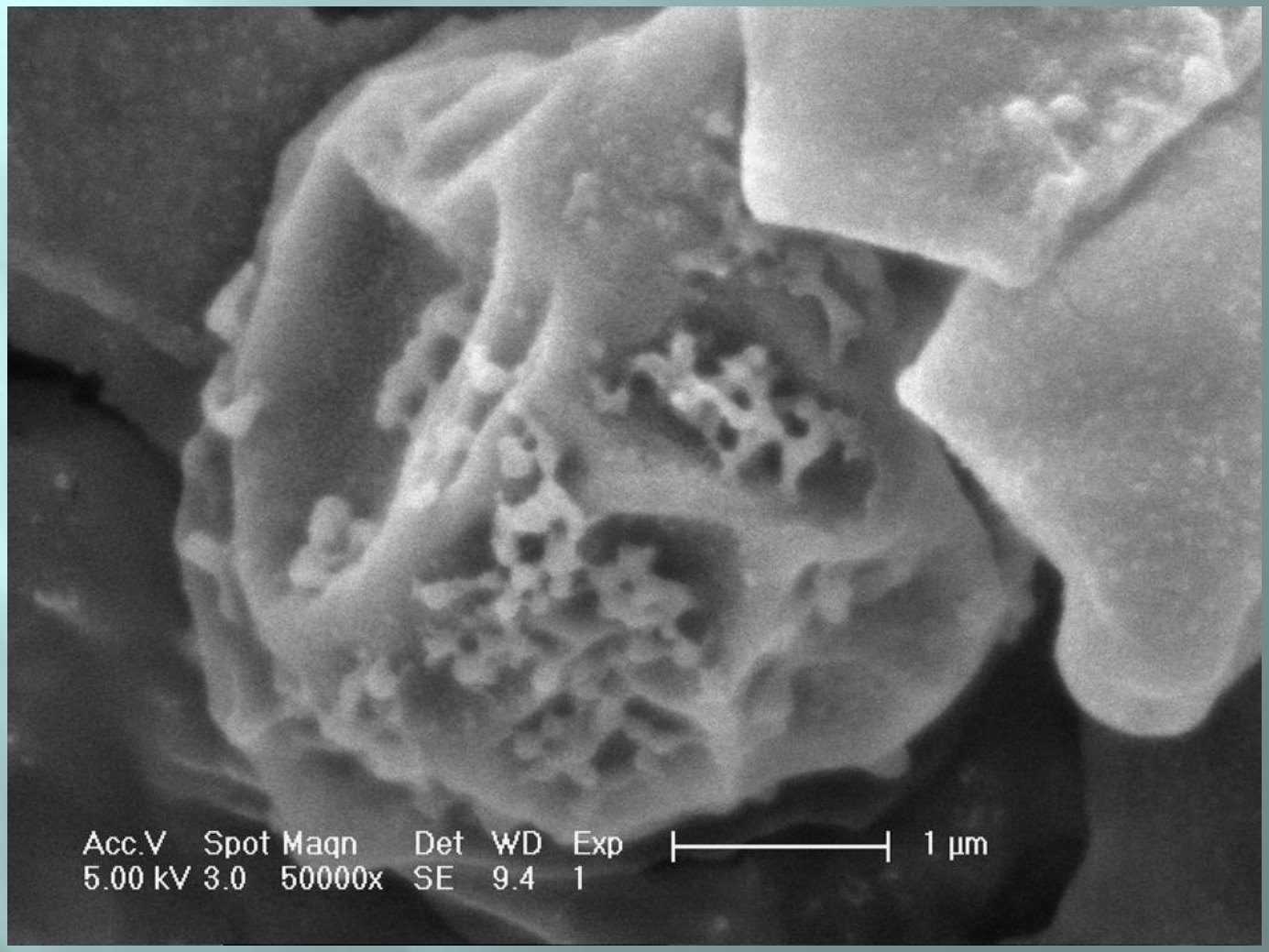
FD RBC MAINTAINING MORPHOLOGY AFTER REHYDRATION



SEM images



SEM images



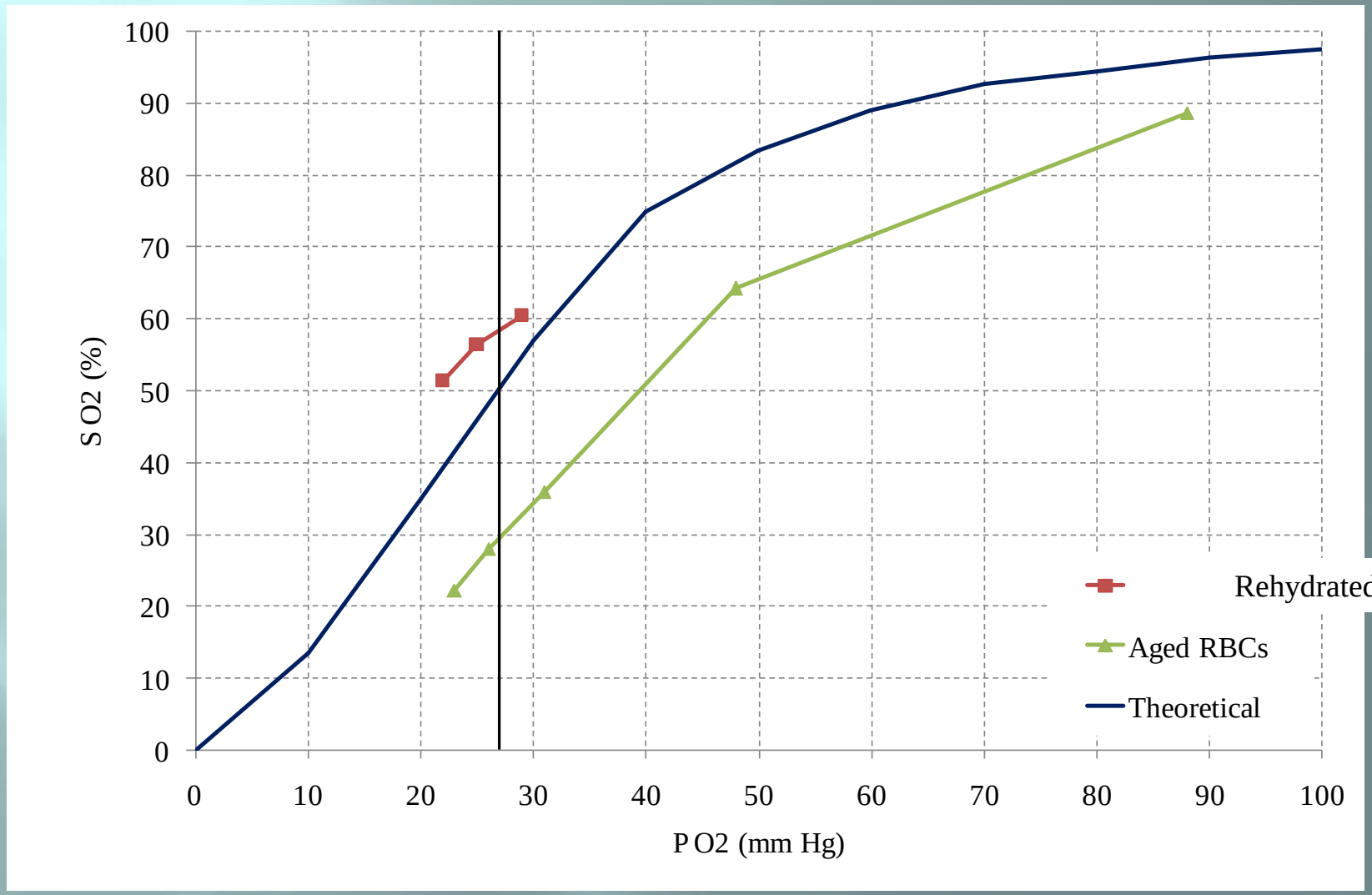
ATP AND 2,3-DPG CONCENTRATION

2,3-DPG	Unit A	Unit B
	µmol / g Hb	µmol / g Hb
Fresh	7.6 0.1	4.2 0.3
Hypother. 5 days	0.8 0.5	0.8 0.2
Rehydrated	6.2 0.9	6.1 1.2

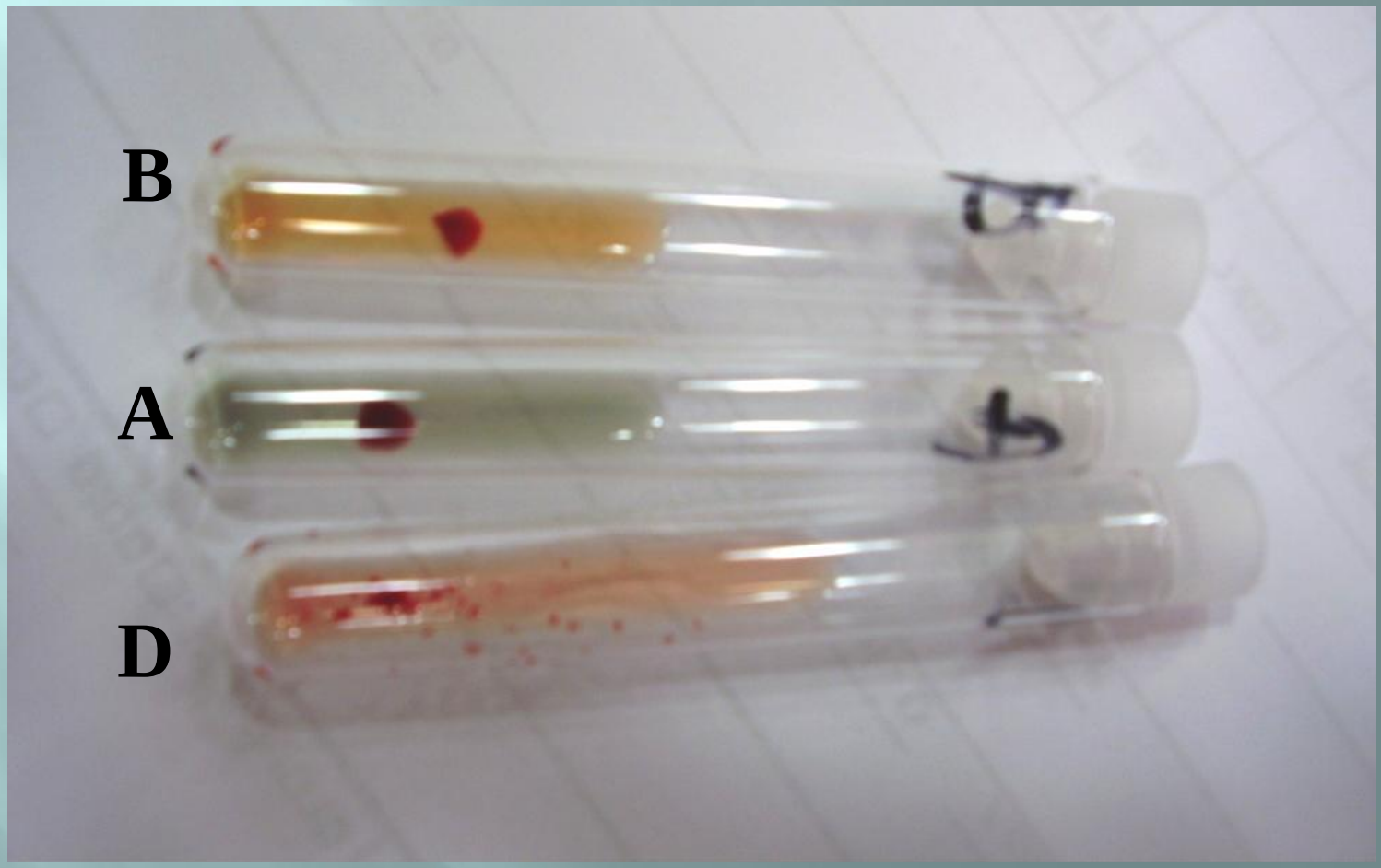
ATP	Unit A	Unit B
	µmol / g Hb	µmol / g Hb
Fresh	7.0 0.3	4.1 0.4
Hypother. 5 days	1.8 0.3	1.9 0.4
Rehydrated	2.7 1.5	2.0 0.3

1:10 dilution, 100 µl droplets, rehydration with dextran 40 kDa

OXYGEN-DISSOCIATION OF REHYDRATED RBCS



FD RBC – BLOOD REAGENT OPPORTUNITY



FREEZE DRIED BLOOD REAGENTS: RABIN MEDICAL CENTER

A full panel:

Fresh RBCs

} Rehydrated RBCs From the same unit

	A	B	D	C	$\overline{c}$	E	e	k
Fresh	+4	+4	+3	+3	+4	-	+4	+3
Rehydrated	+4	+4	+3	+3	+4	-	+3	+2
	Jk_a	Jk_b	Fy_a	Fy_b	M	N	S	$\overline{s}$
Fresh	-	+3	+3	-	+3	+1	-	+3
Rehydrated	-	+2	+2	-	+3	+1	-	+2

Performed at the transfusion service laboratory, Rabin Medical Center, Petach Tikva, under the supervision of Dr. J. Orlin, the blood bank Director, and Ms. Bruria Shalev, the lab manager.

DEVELOPING DRIED BLOOD FOR THE BATTLEFIELD

Technical Challenges/Impacts

Increasing dried RBC viability from 50% to 80%➢

Increase Hct from 0.5% to 30%➢

Up scaling volume from 2.5ml to 250ml➢

FDA approval of IMT freezing solution➢

Freeze dry in a close system➢

FDA approval of Freeze Dried Blood Product➢

PRE-CLINICAL WITH IMT -1

Genotoxicity – (GLP)

- AMES study – None mutagenic (bacteria)
- MLA – Caused mutations (cell line)
- MNT – None mutagenic (mice in vivo)

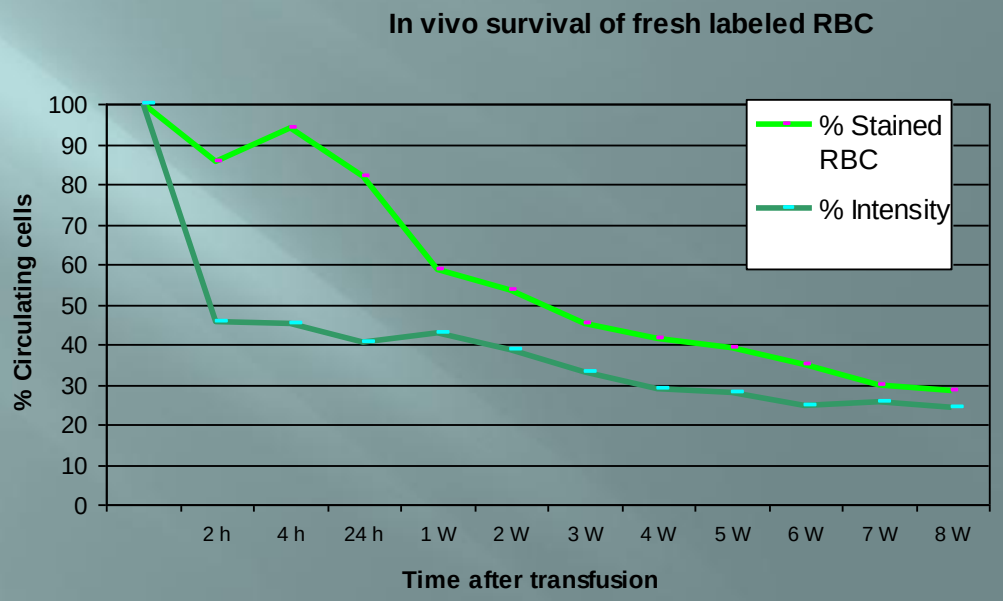
Toxicity –

- Escalating dose – Gross pathology: No abnormalities
  (Pigs) Histopathology: No abnormalities

- Repeated dose – No toxicity (Rabbits)

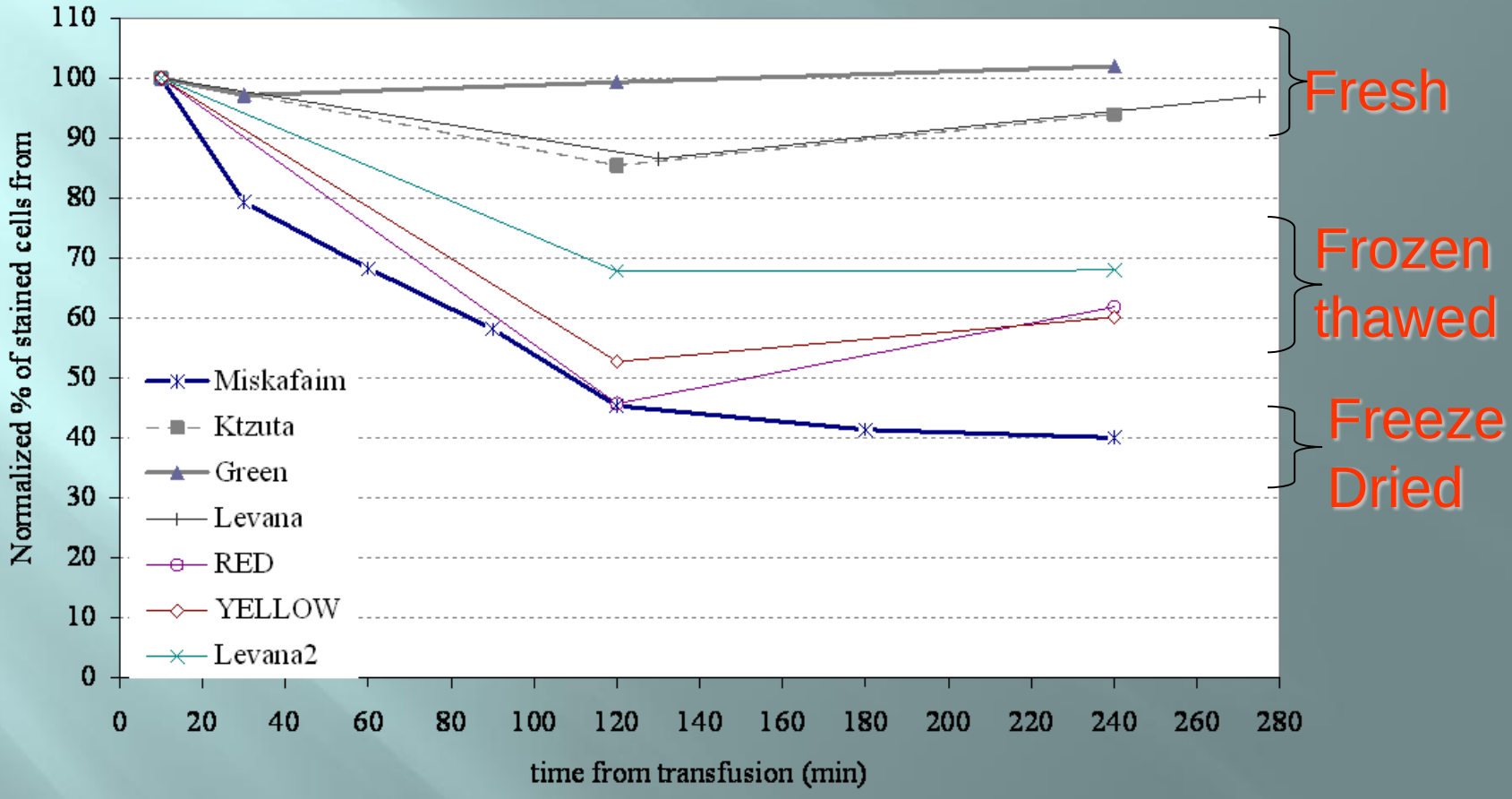
ANIMAL MODEL - WHY DONKEYS?

- Blood volume similar to human
- In vitro blood survival similar to human
- No need for sedation and tranquilizer



FREEZE DRYING – IN VIVO (DONKEYS)

FREEZE DRYING – IN VIVO (DONKEYS)



FREEZE DRYING

CORE DYNAMICS IN ISRAEL

CORE DYNAMICS