

中国制冷学会小型制冷机及低温生物医学会议

生命系统的整体冻存与复活：未来技术畅想

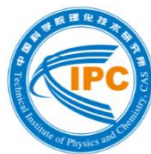
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中国科学院理化技术研究所低温工程学重点实验室

& 清华大学医学院生物医学工程系

*** E-mail: jliu@mail.ipc.ac.cn**

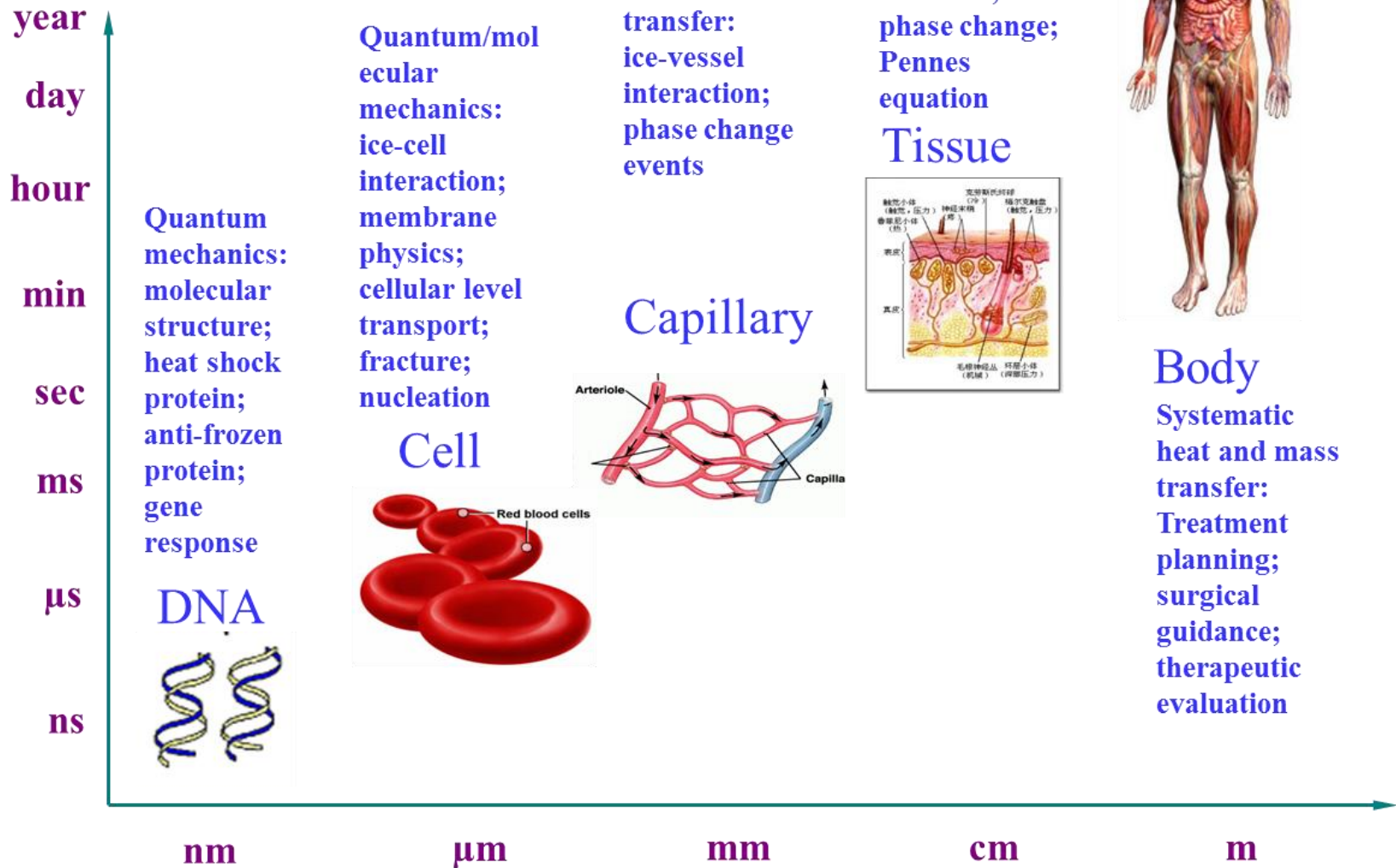
青岛·2018年8月10日



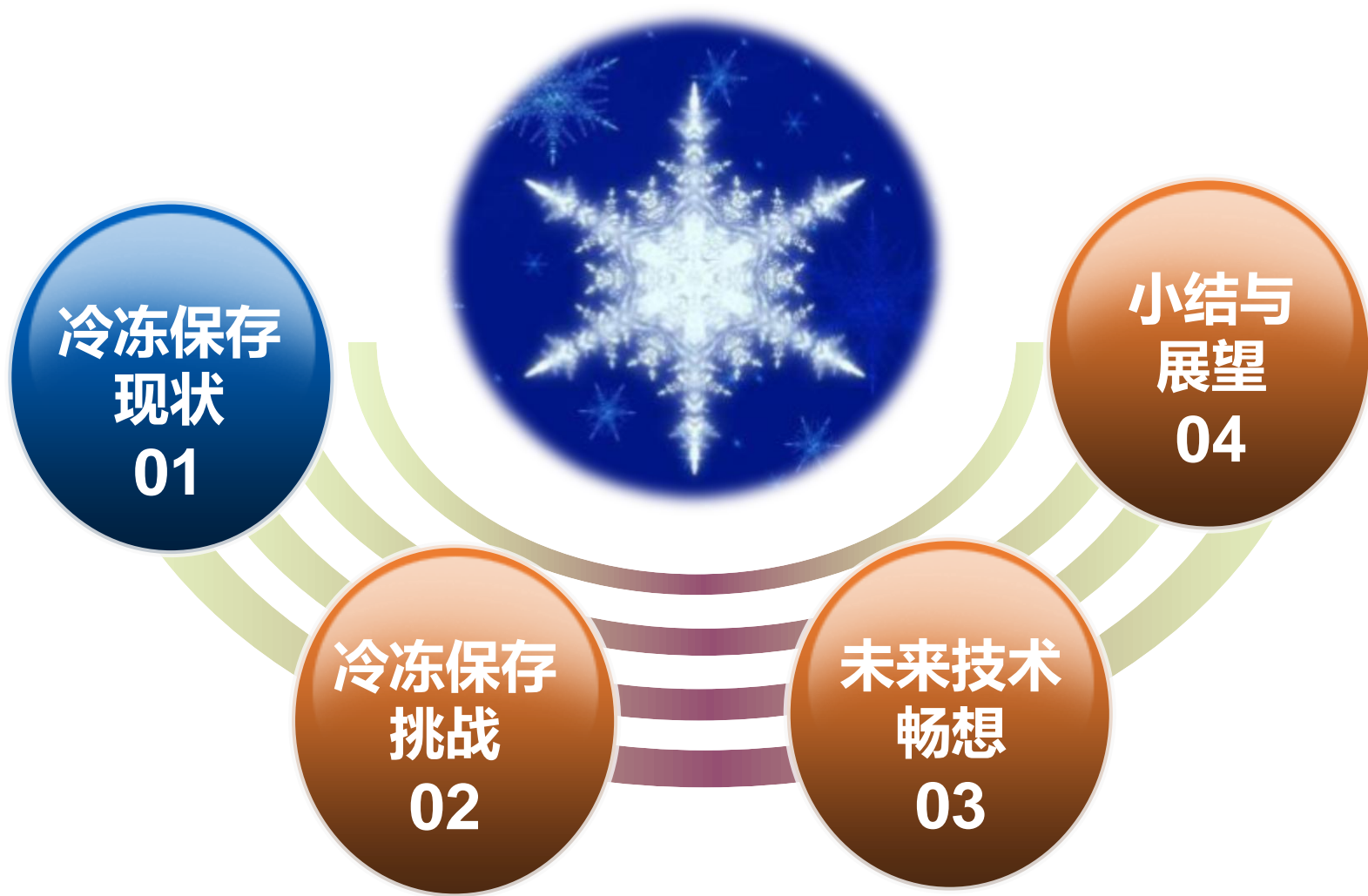
生命系统低温保存涉及的主要学科



生命系统低温保存



报告内容



生命系统低温保存



医药冷链主要对象

药品

组织器官

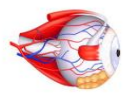
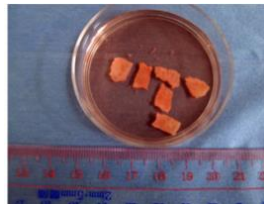
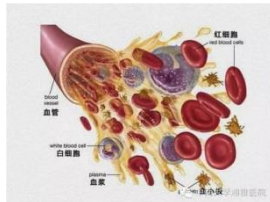
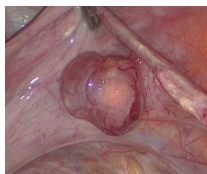
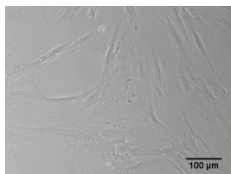
疫苗

血液制品

细胞

组织

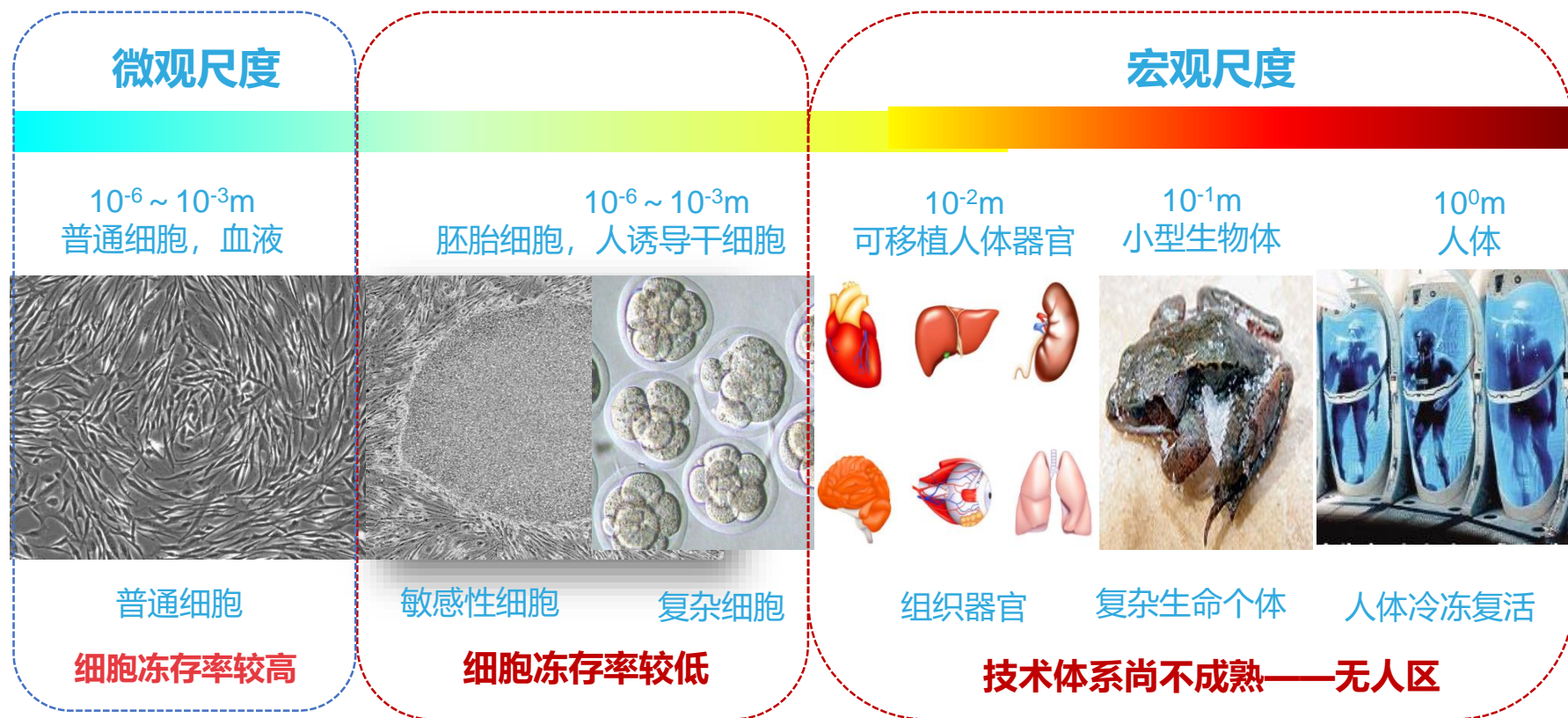
器官



生命系统低温保存现状

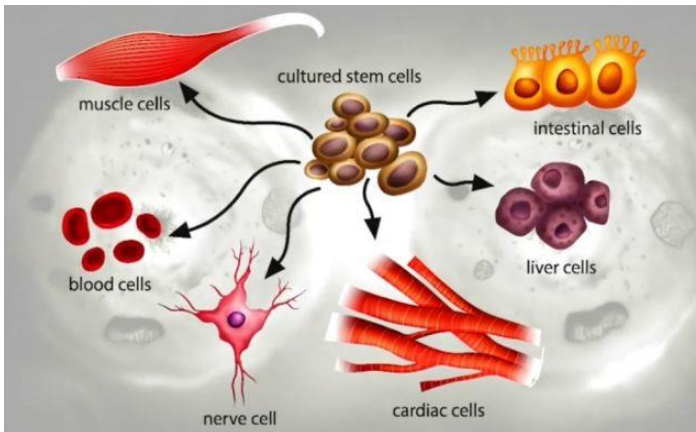
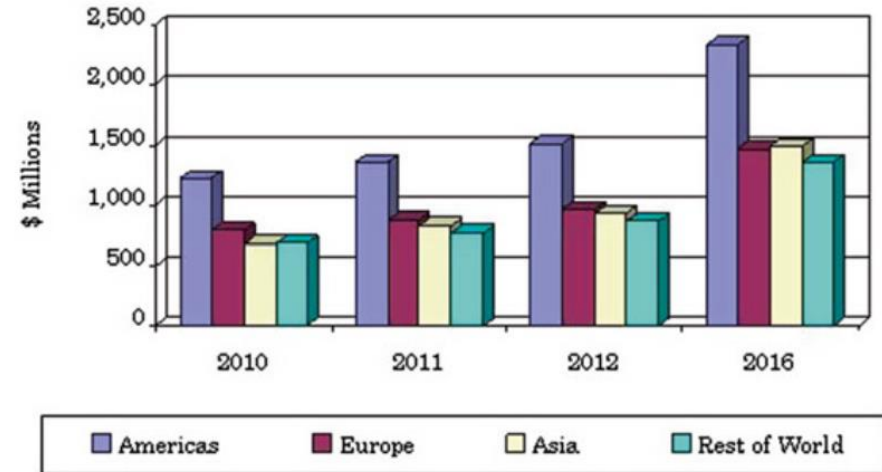
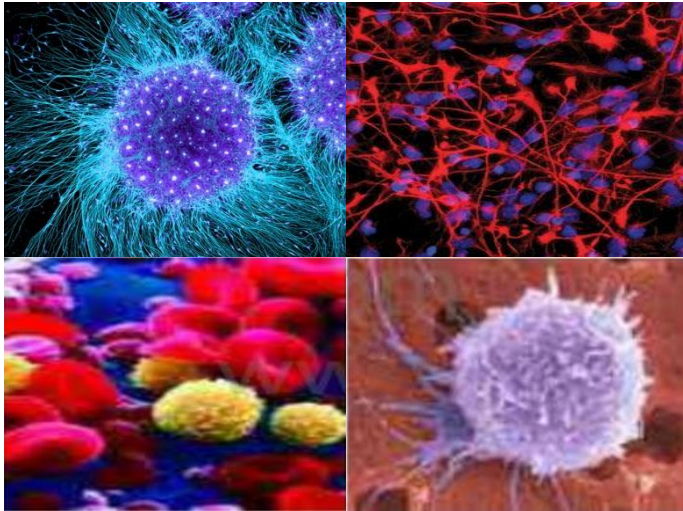


低温冷冻保存是生物材料长期保存的唯一可行途径

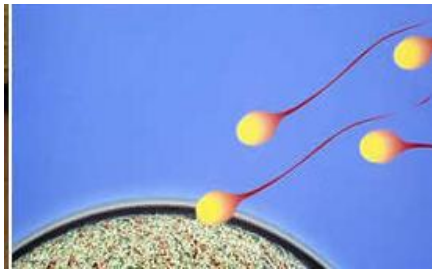
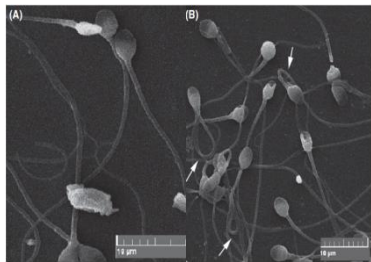


现代低温冷冻保存提出诸多重大理论及技术挑战

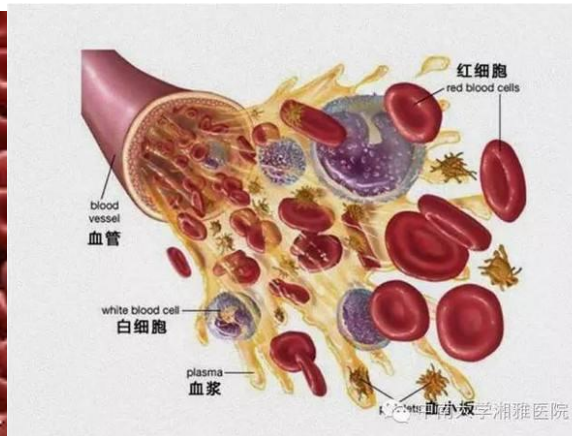
冷冻现状：干细胞



冷冻现状：生殖细胞



冷冻现状：血液组织

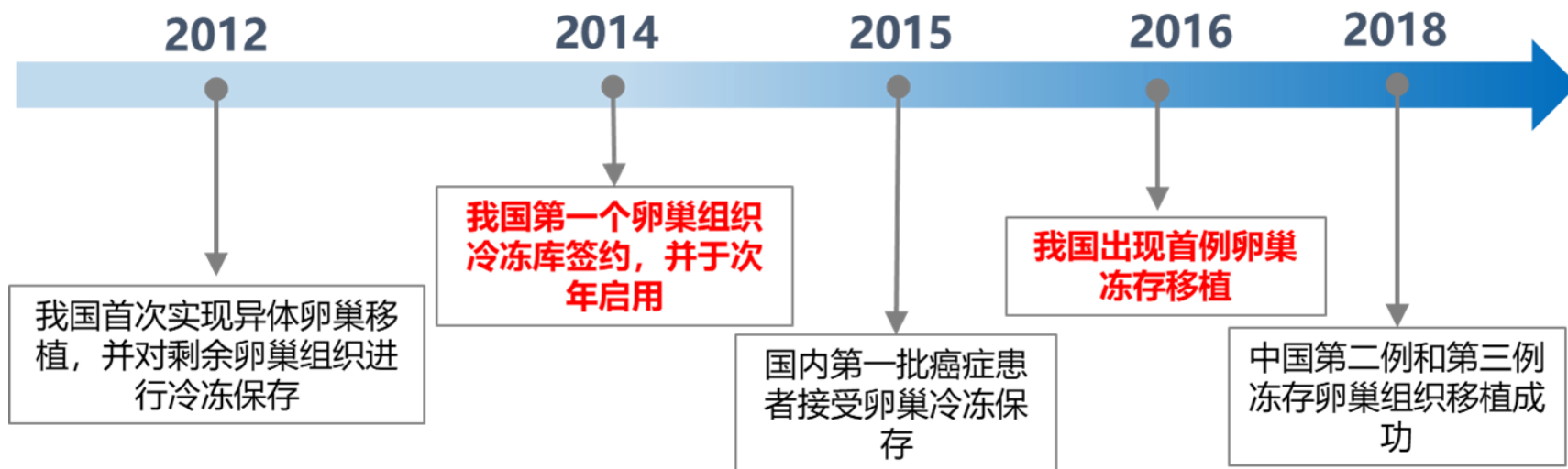
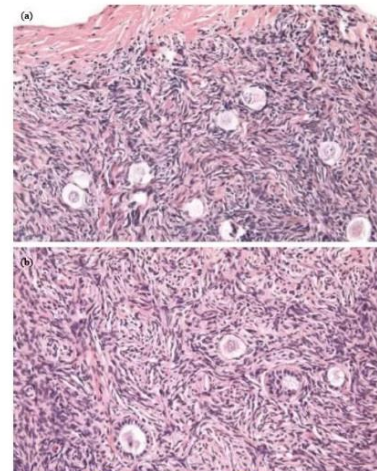
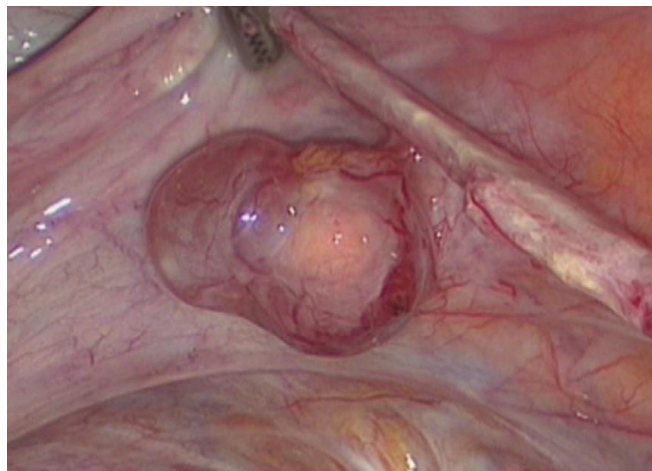


www.nipt.com

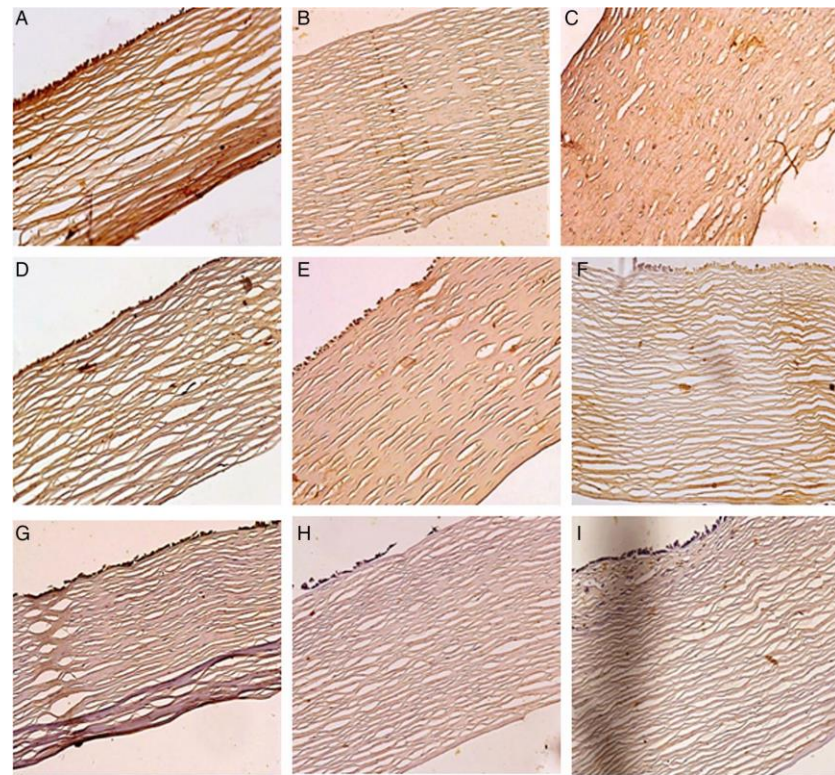
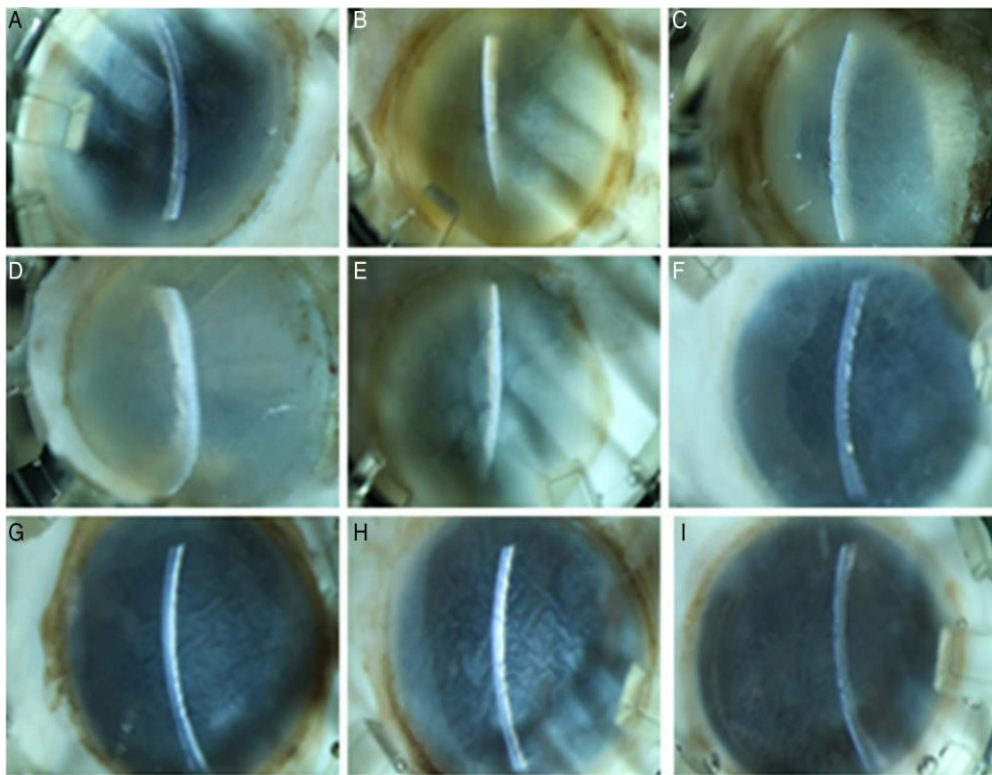
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冷冻现状：卵巢组织

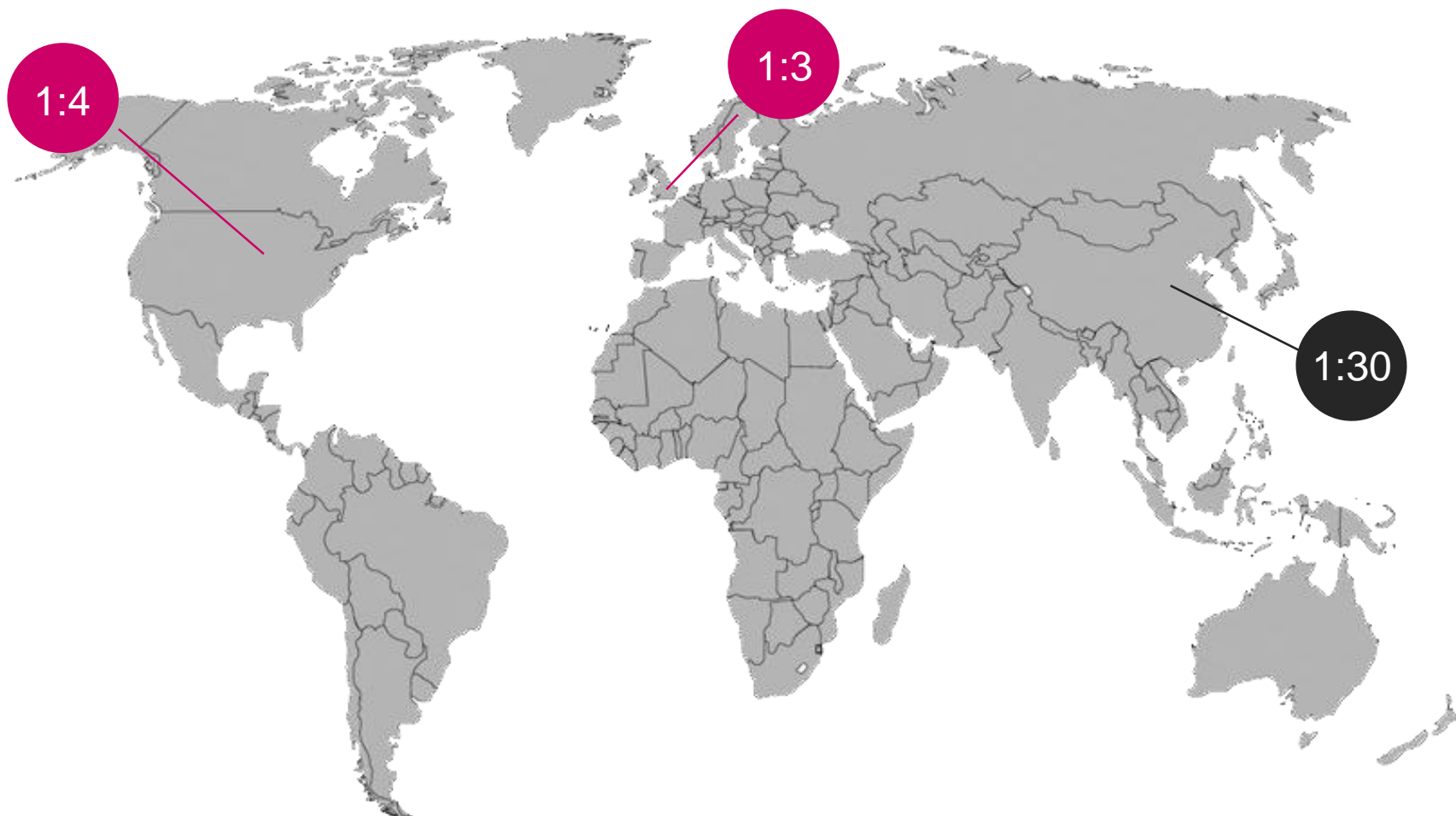


冷冻现状：角膜组织



角膜在冷冻状态下最长保存期为6~12个月

冷冻现状：器官



每年仍有半数的心脏和肺脏捐赠器官被丢弃

冷冻现状：器官



医药冷链

医药冷链主要对象

器官移植医学中的冷藏问题

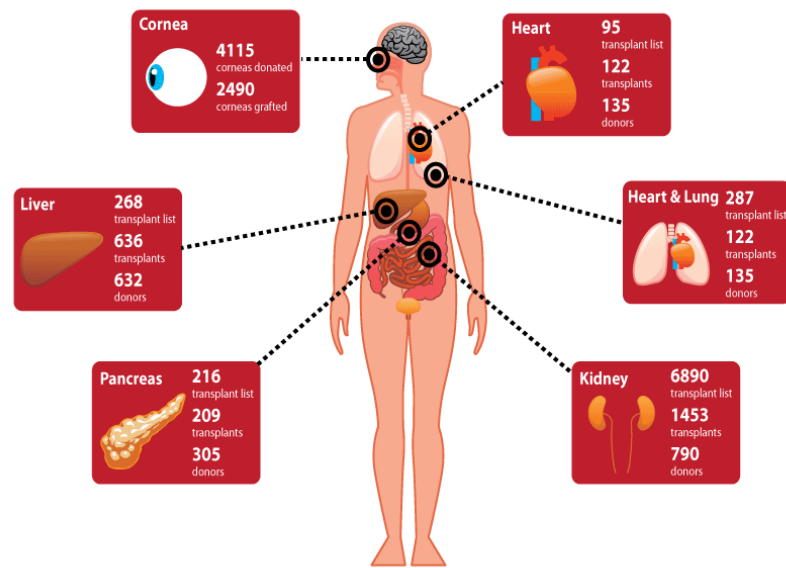


NG Healthcare EU

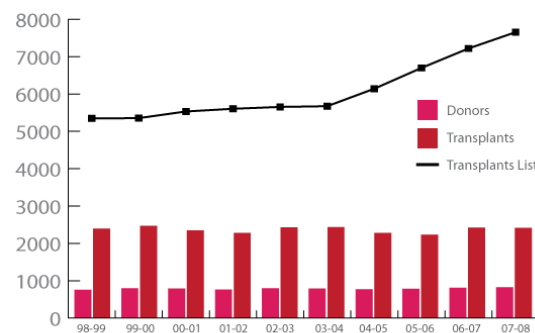


Organ Donation in the UK

After a year of concerted effort to boost UK organ donation rates, the number of people agreeing to donate their organs after death is growing. But it is still at a slower rate than it's required to be.



Total Transplants and Donors



Statistics for 2008 - 2009



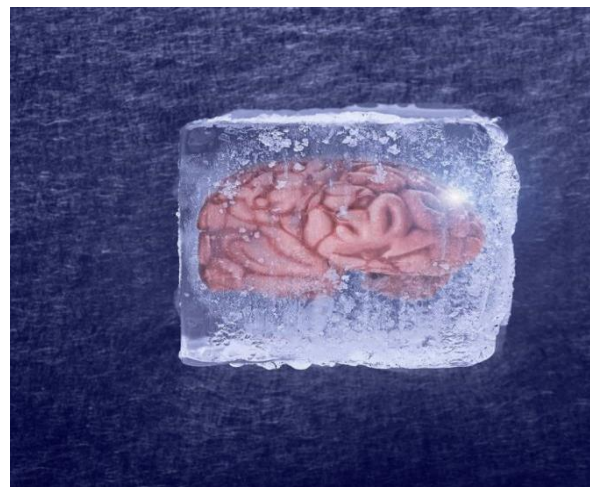
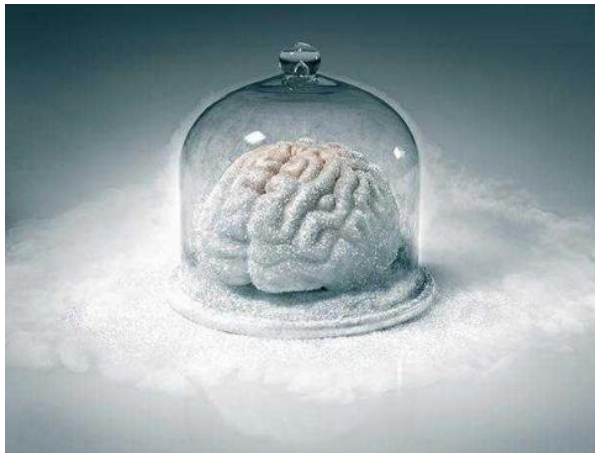
Source: NHS Transplant Activity in the UK

冷冻现状：大脑



醛稳定化冷冻保存法

脑组织冻存



活体大脑

唤醒大脑

再造身体



记忆存储

冷冻现状：动物体



Ice worm



Snow flea



Ice fish



American bees



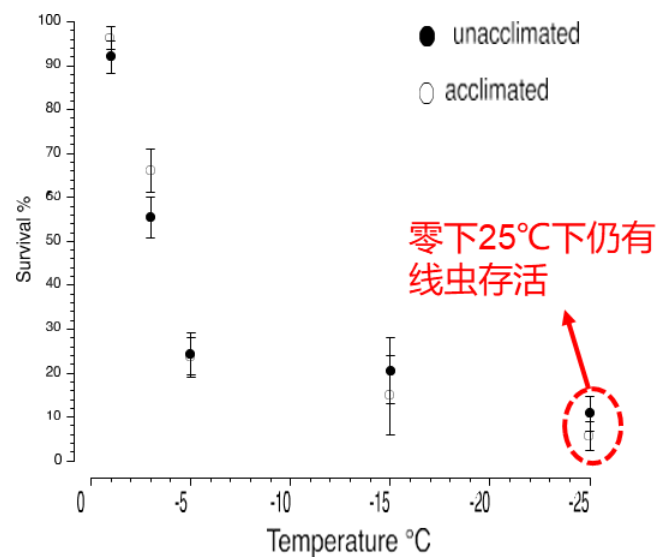
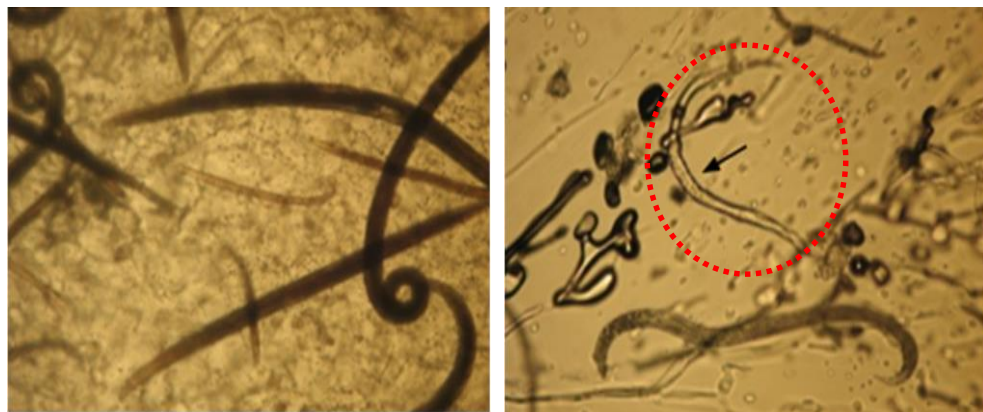
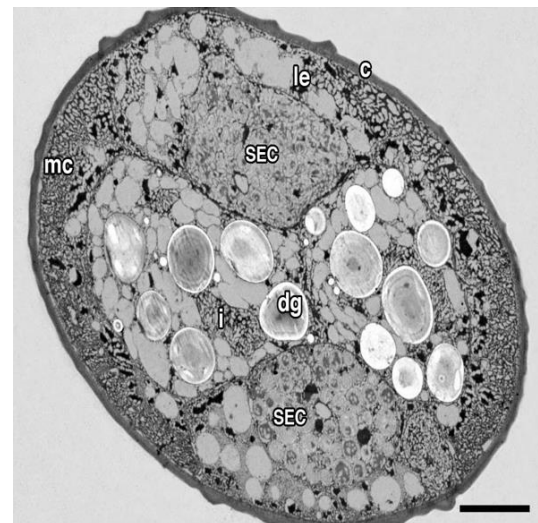
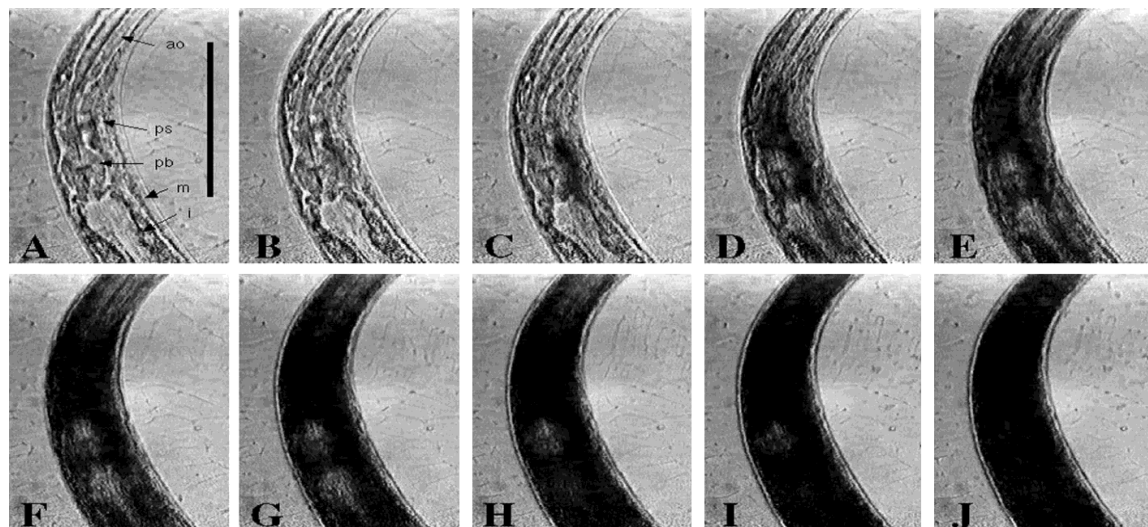
Sea turtle



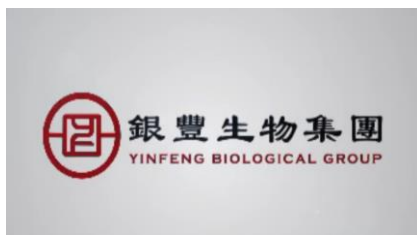
Tree frog

冷冻现状：动物体

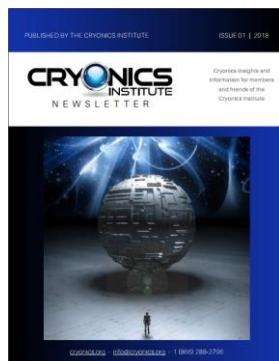
线虫



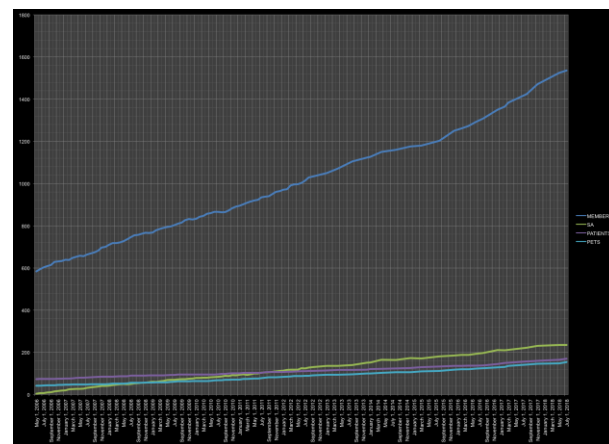
冷冻现状：人体



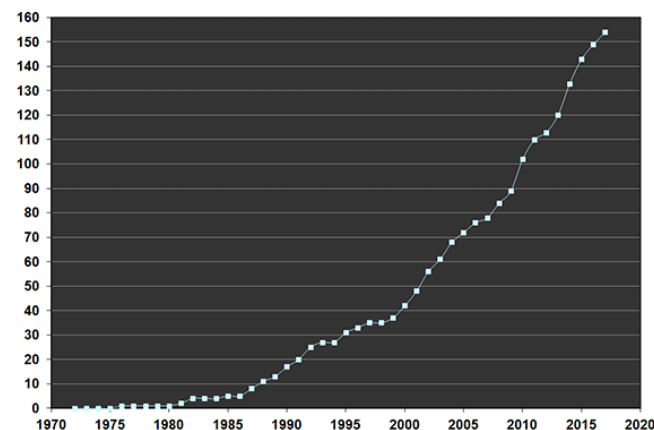
将人体冷冻商业化的公司



关注人体冷冻技术的杂志



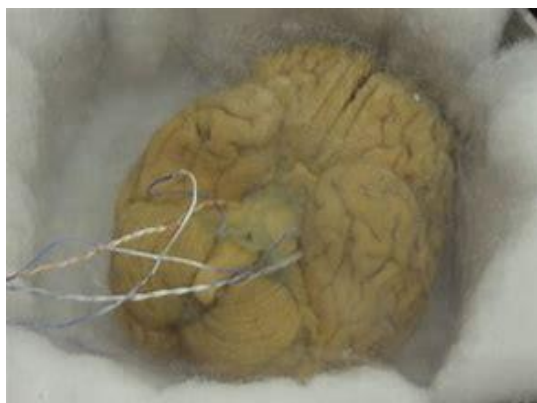
CRYONICS



ALCOR

接受冷冻保存的患者统计数据

冷冻现状：人体



国内首例神经系统冷冻保存



国内首例人体冷冻实验

常规的人体冷冻手术的实施步骤：



患者已宣布死亡



初始冷却并维持血液循环

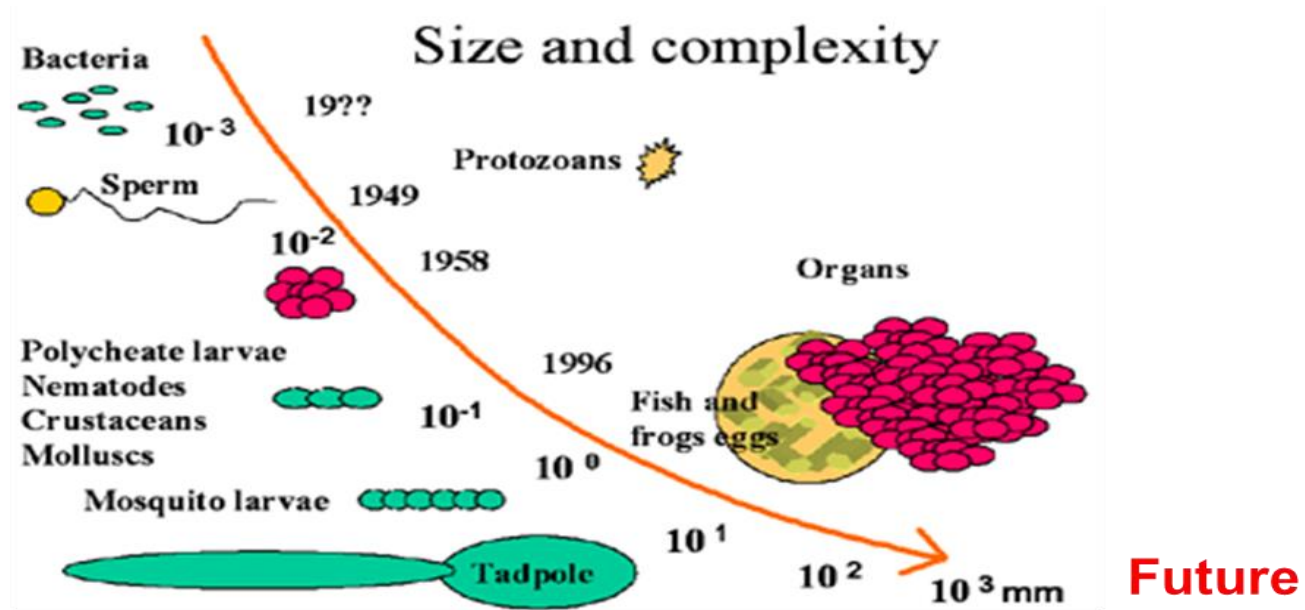


精准控制保护剂灌注和程序降温



进一步降温并储存至液氮中

复杂细胞/大器官/复杂生命个体/人体的冷冻保存

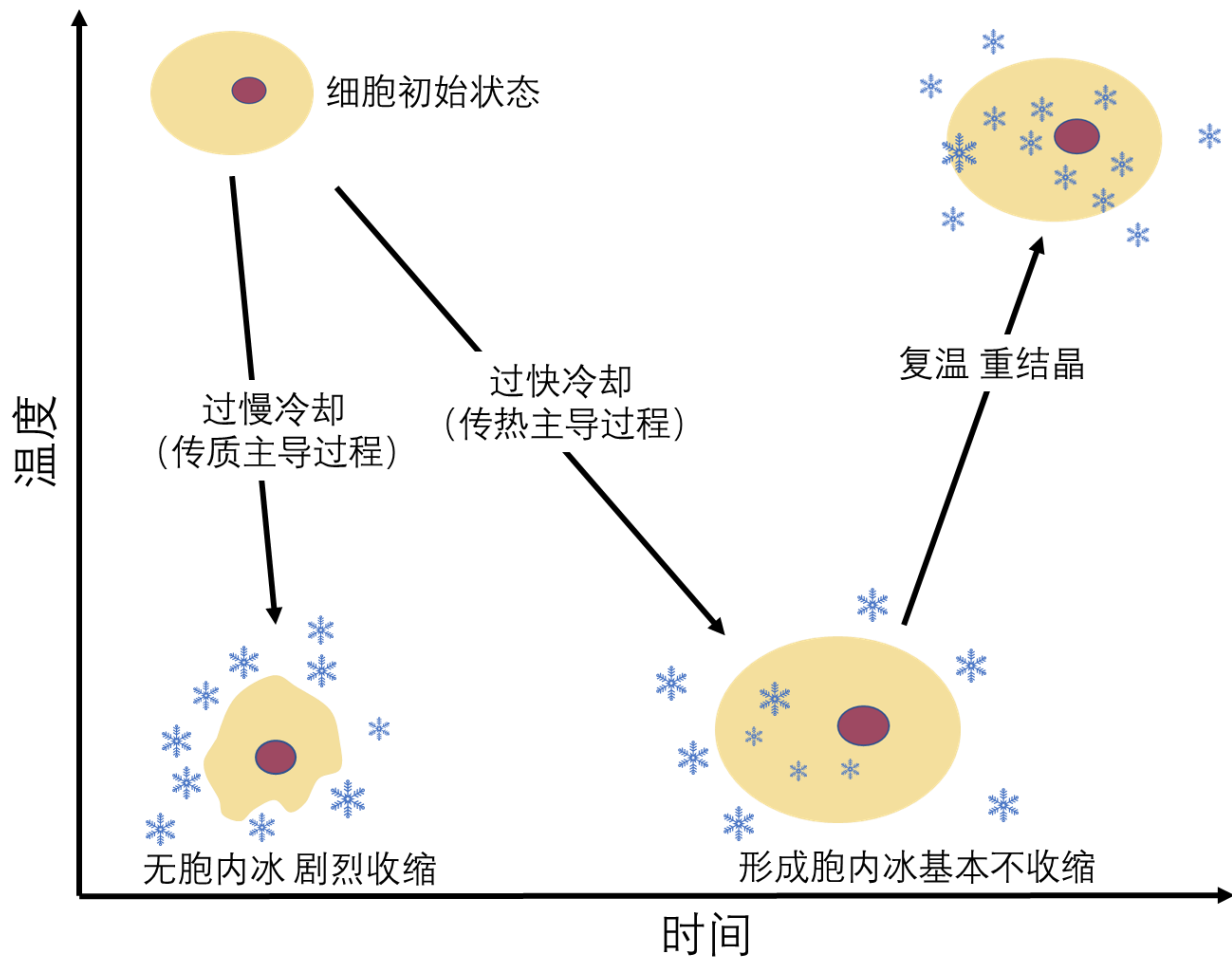
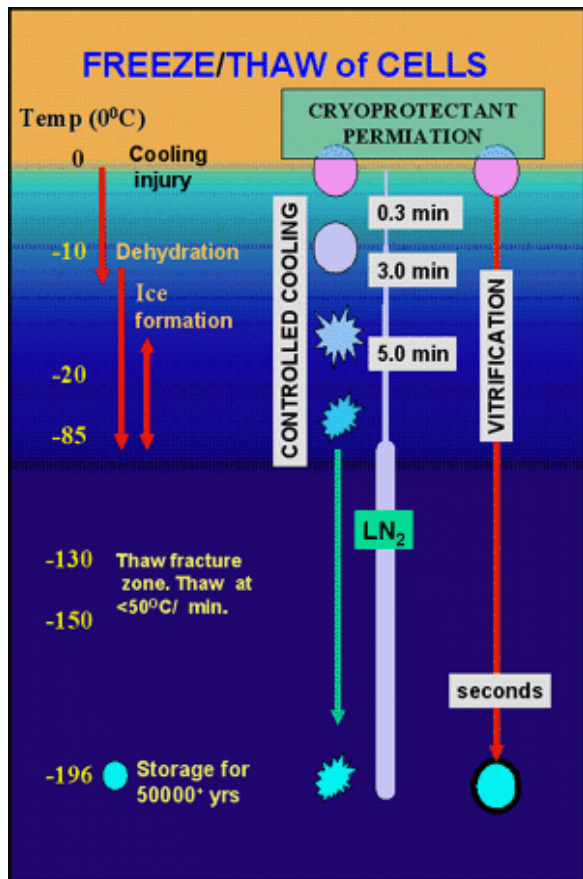


报告内容



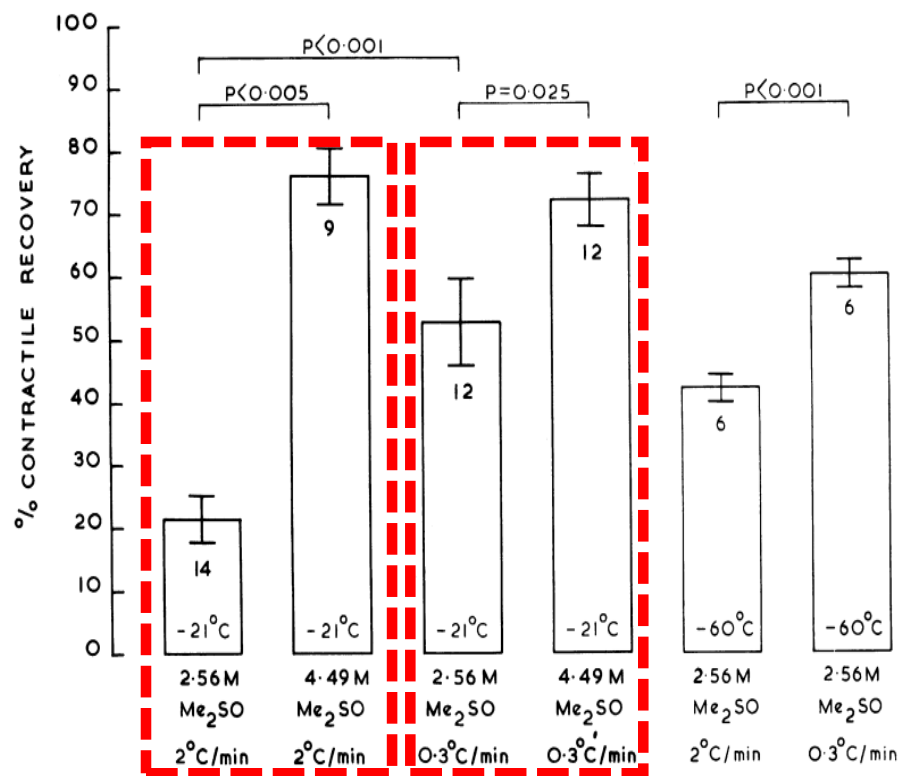
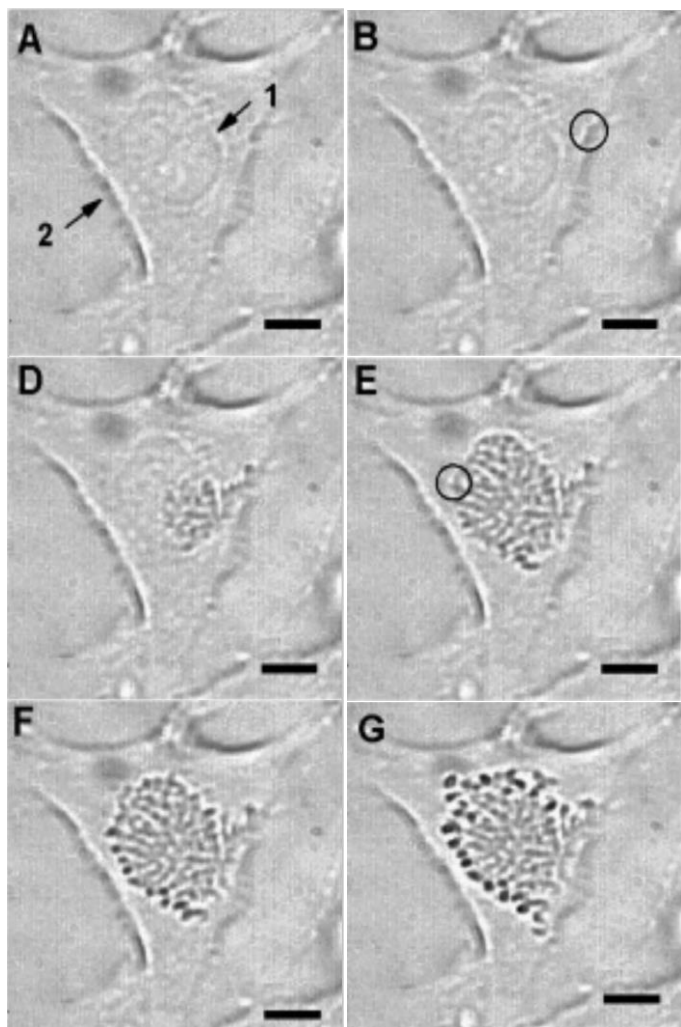
挑战：细胞损伤

冰晶生成 细胞缩水 重结晶



挑战：细胞损伤

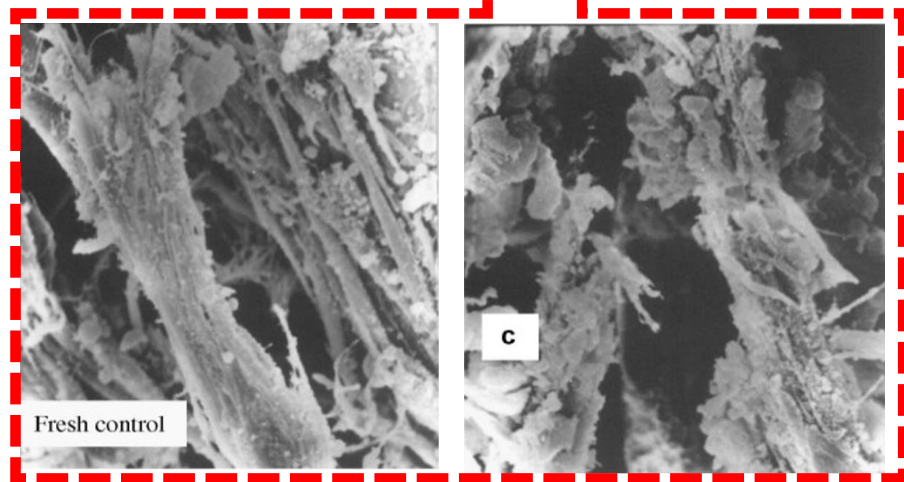
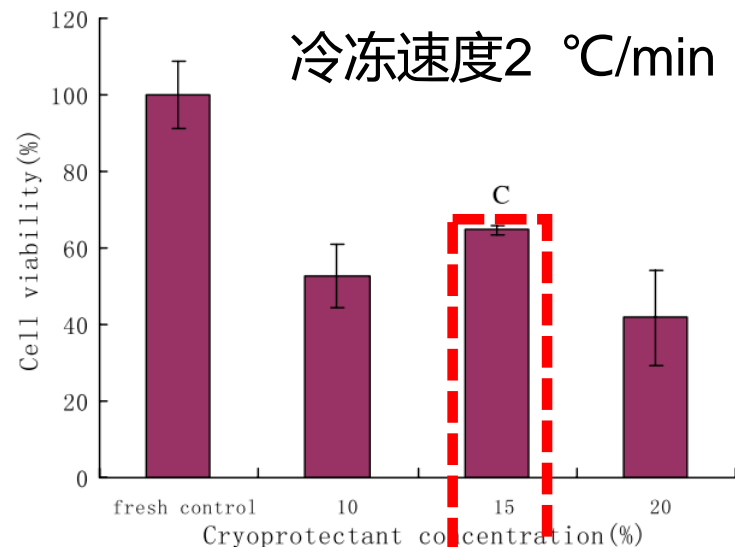
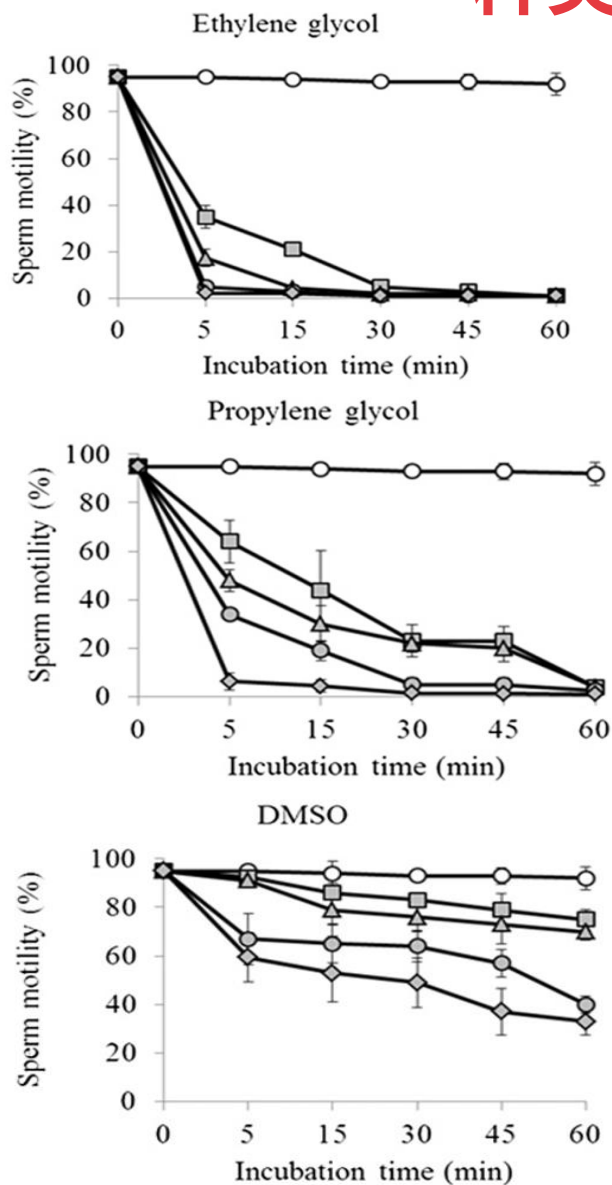
冰晶损伤



冷冻保护剂浓度低，产生更多冰晶，
细胞存活率下降

挑战：冷冻保护剂

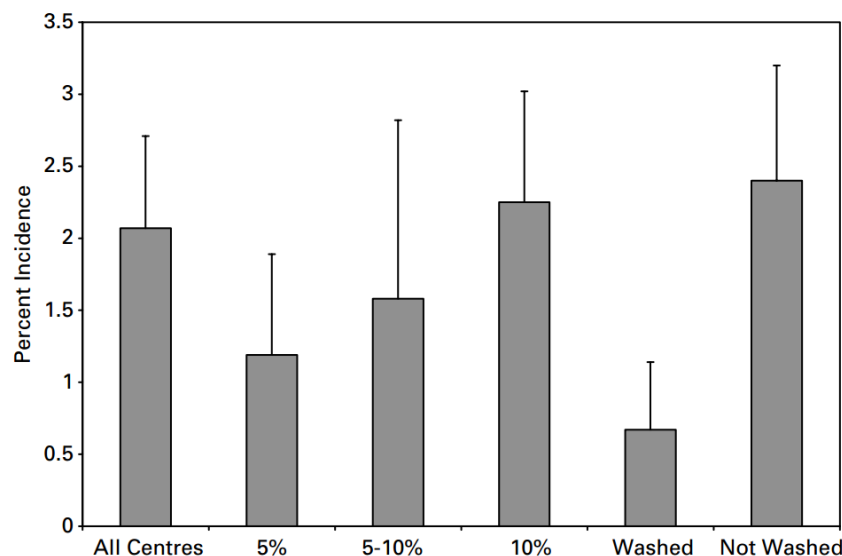
种类与浓度影响存活率



挑战：冷冻保护剂



残留毒性

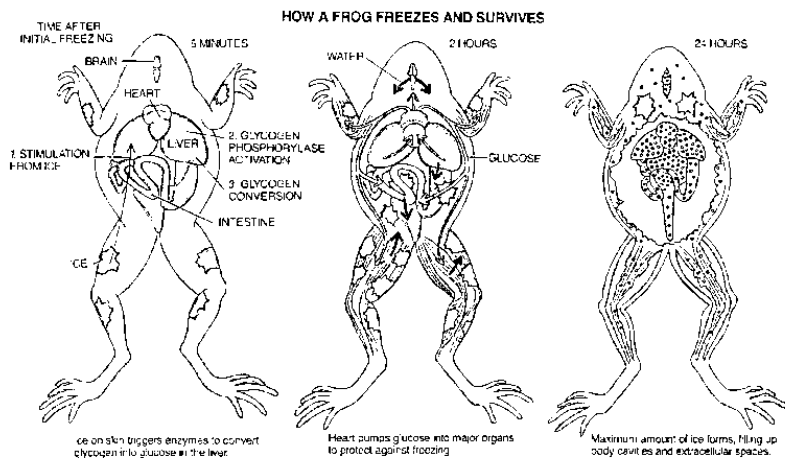
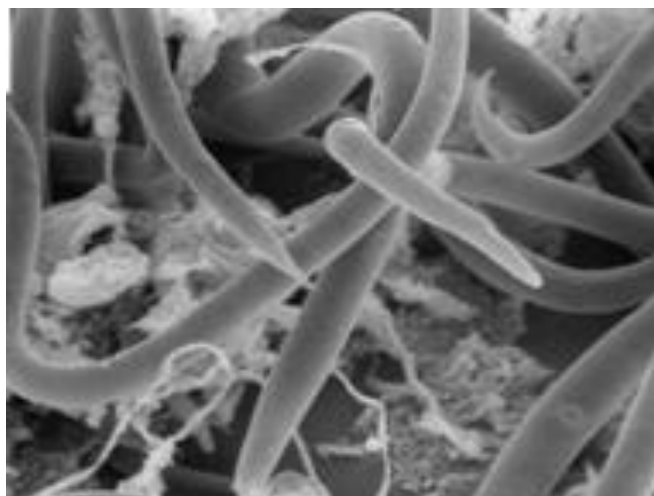


细胞冷冻中心数据显示，残留DMSO在细胞移植之后显出毒性，致使发病

<i>Toxicity</i>	<i>Probable (no. of centres)</i>	<i>Possible (no. of centres)</i>
Cardiovascular	26	14
Respiratory	14	15
CNS	4	8
Renal	4	8
Others	9	4
Total	57	49

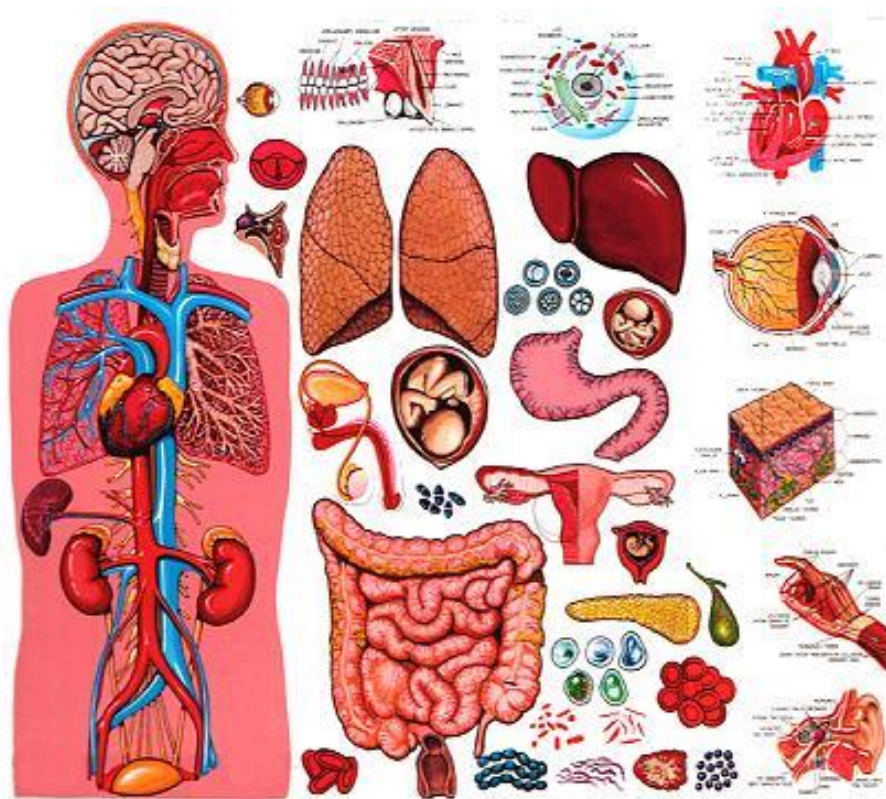
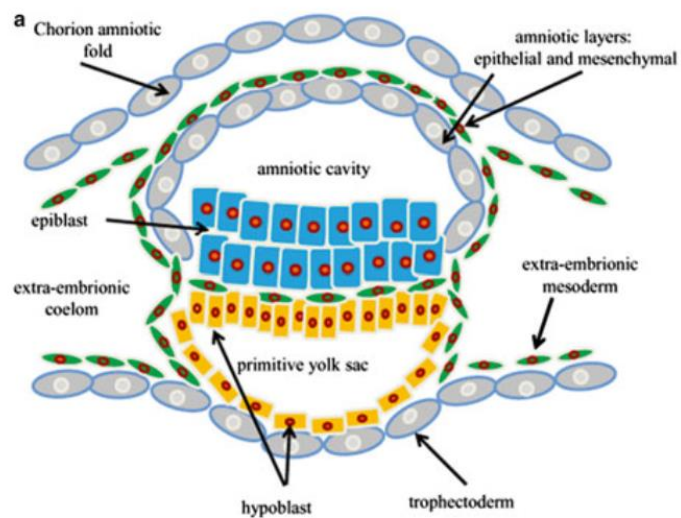
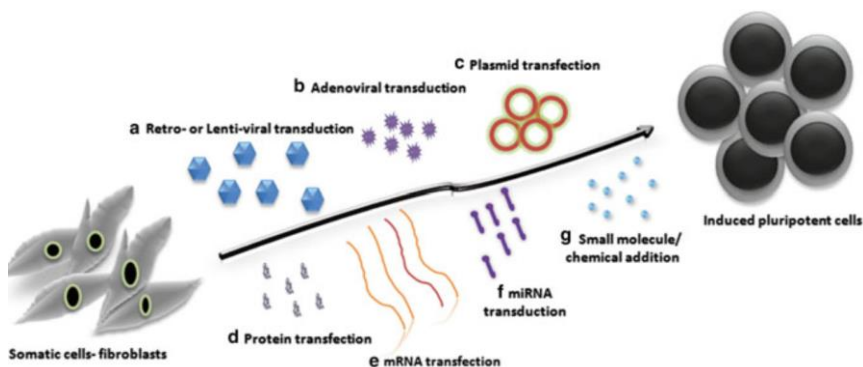
挑战：传热瓶颈

生物体体积增大，传热速度不均匀性



挑战：传热瓶颈

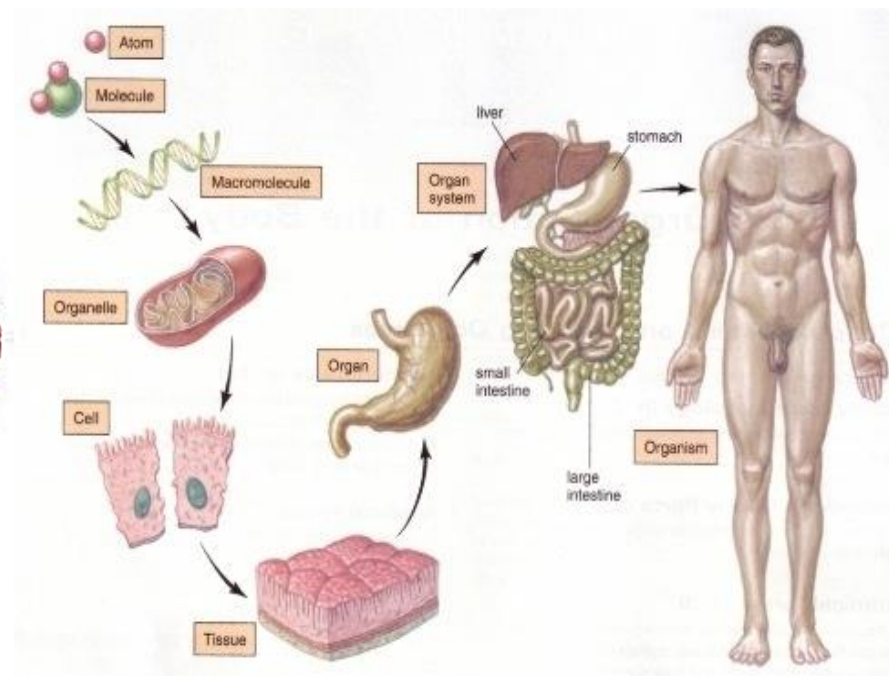
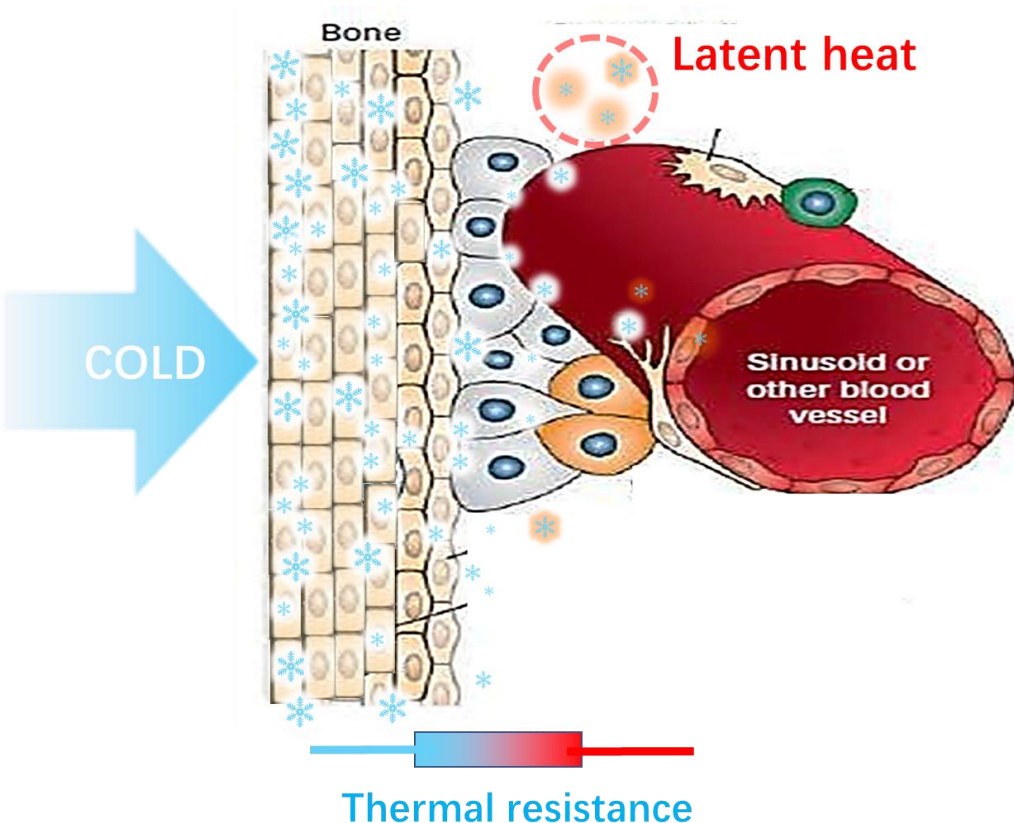
组织和器官层次的复杂性



细胞形状的多样性，组织和器官结构的复杂性，造成传热的复杂性

挑战：传热瓶颈

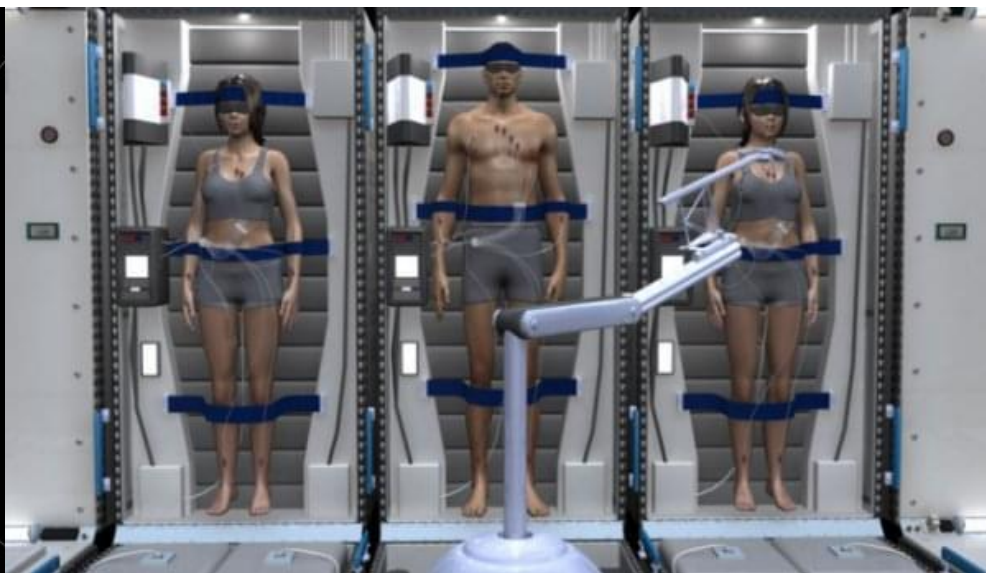
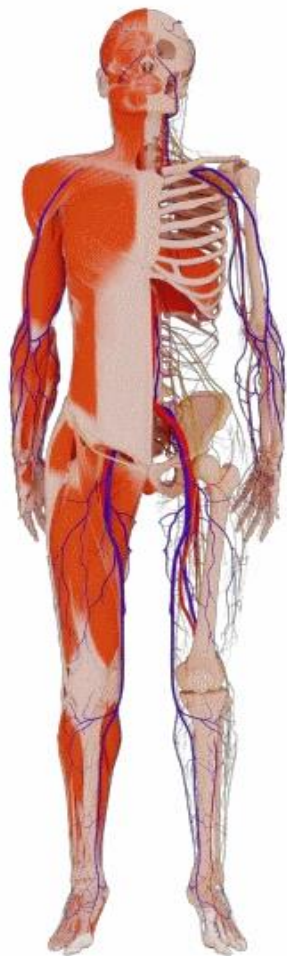
热阻与潜热



人体自身热阻和冰晶形成所释放的潜热造成均温难题

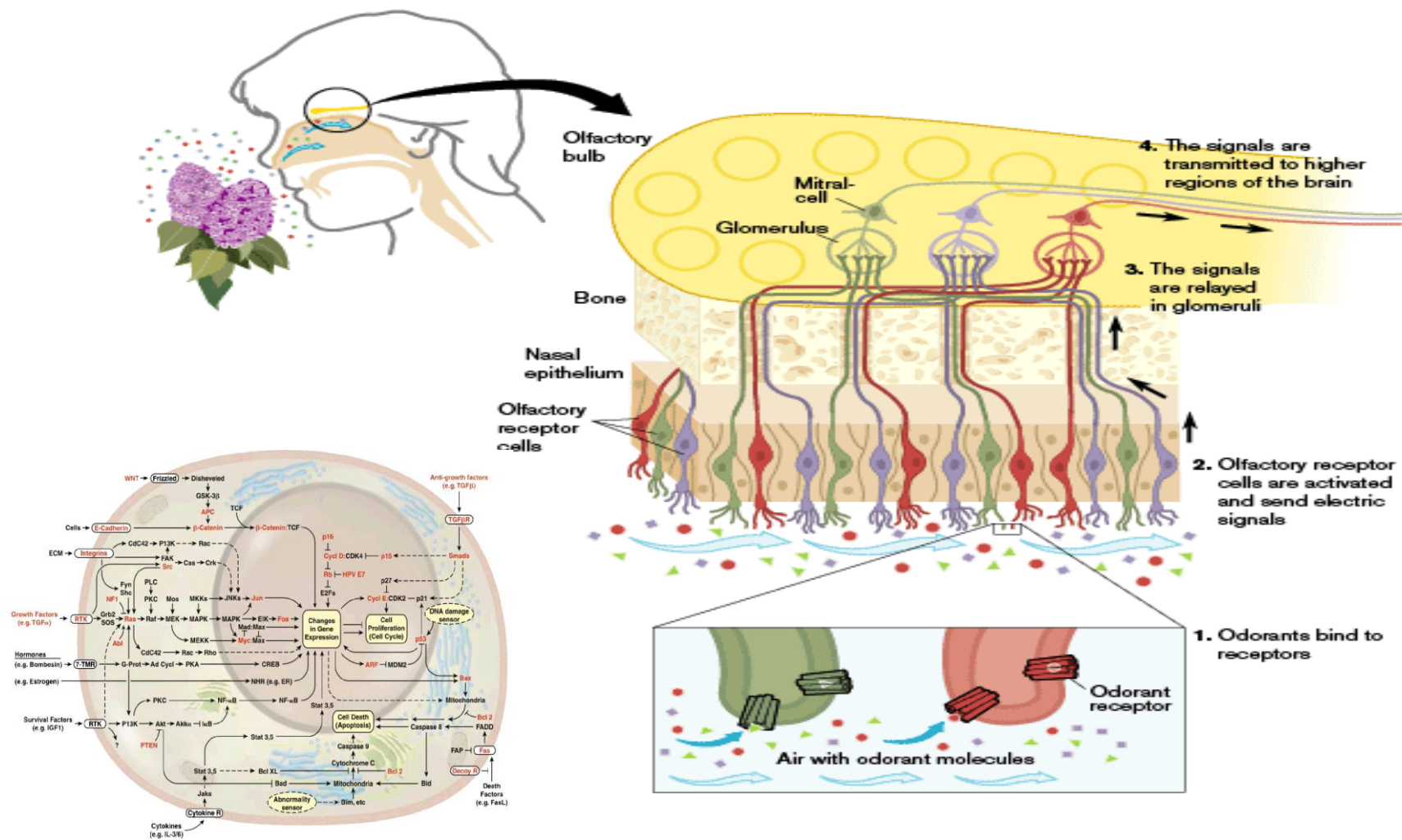
挑战：传热瓶颈

降低人体局部或全身体温面临大量理论与技术挑战



挑战：记忆的保存

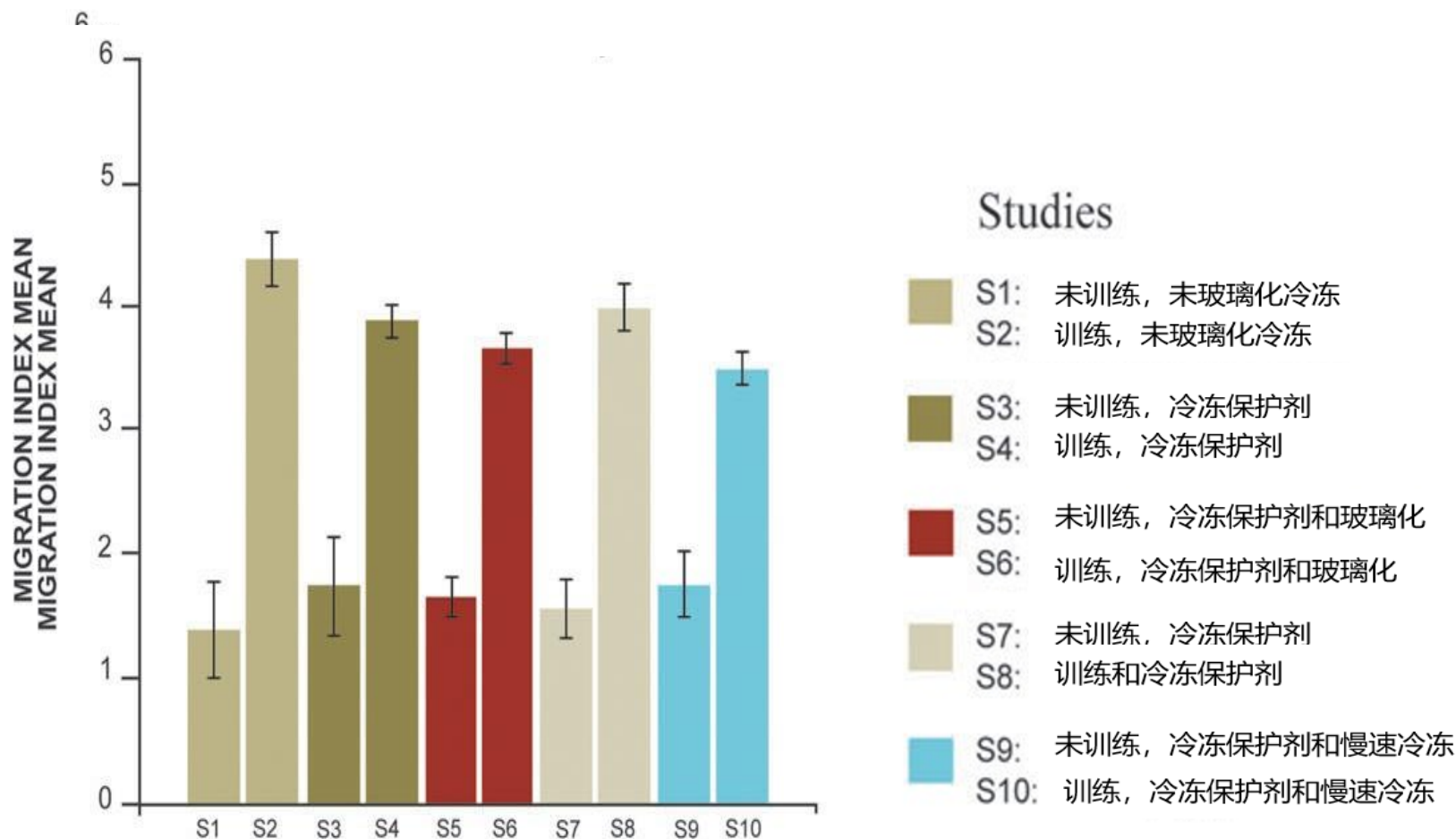
以味觉为例



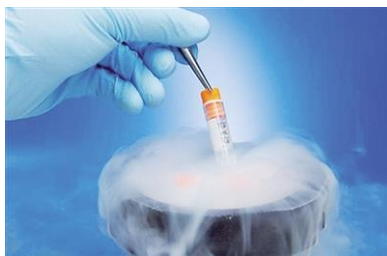
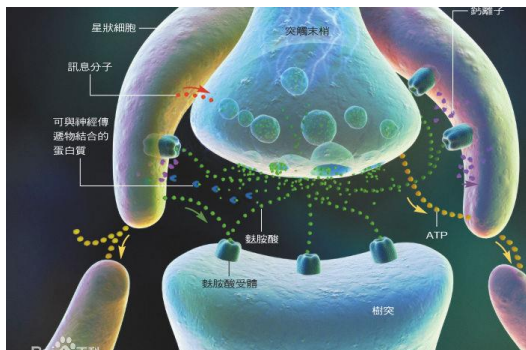
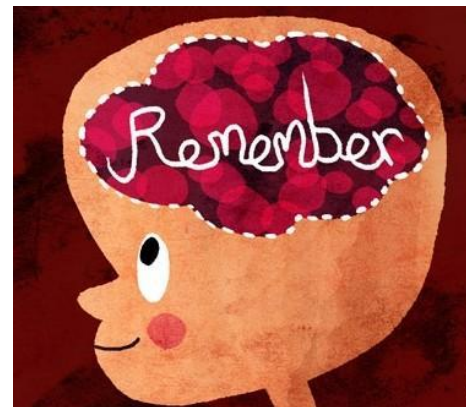
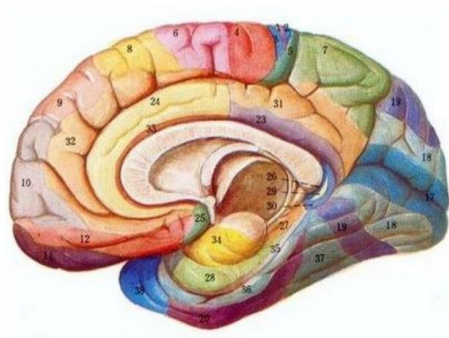
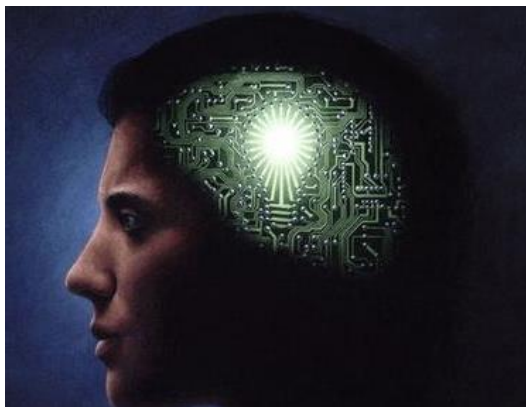
挑战：记忆的保存



线虫在冷冻复活之后仍旧存有味觉记忆

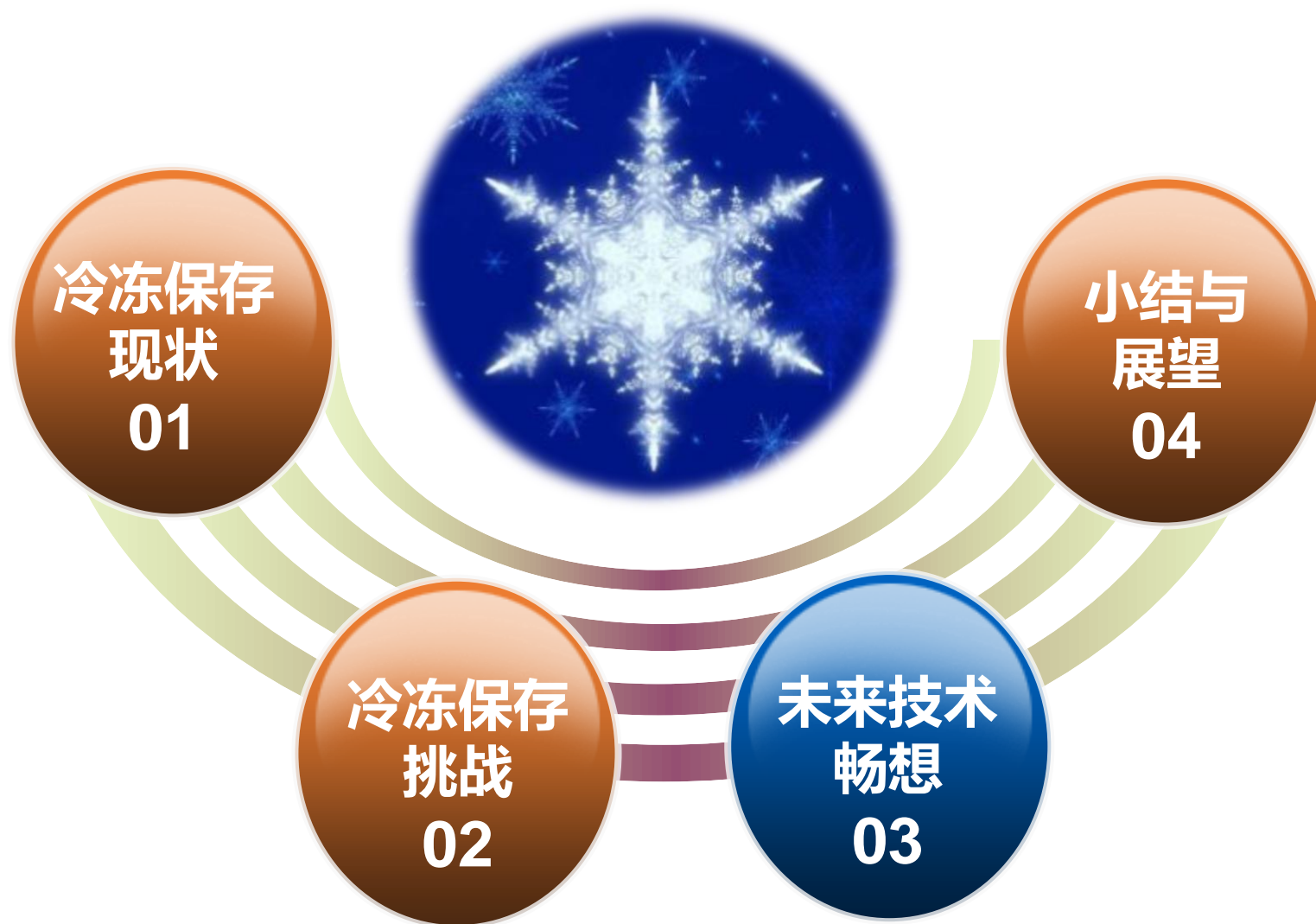


挑战：记忆的保存



解决复杂生命个体特别是高等动物的记忆在冷冻及复温过程中不被破坏

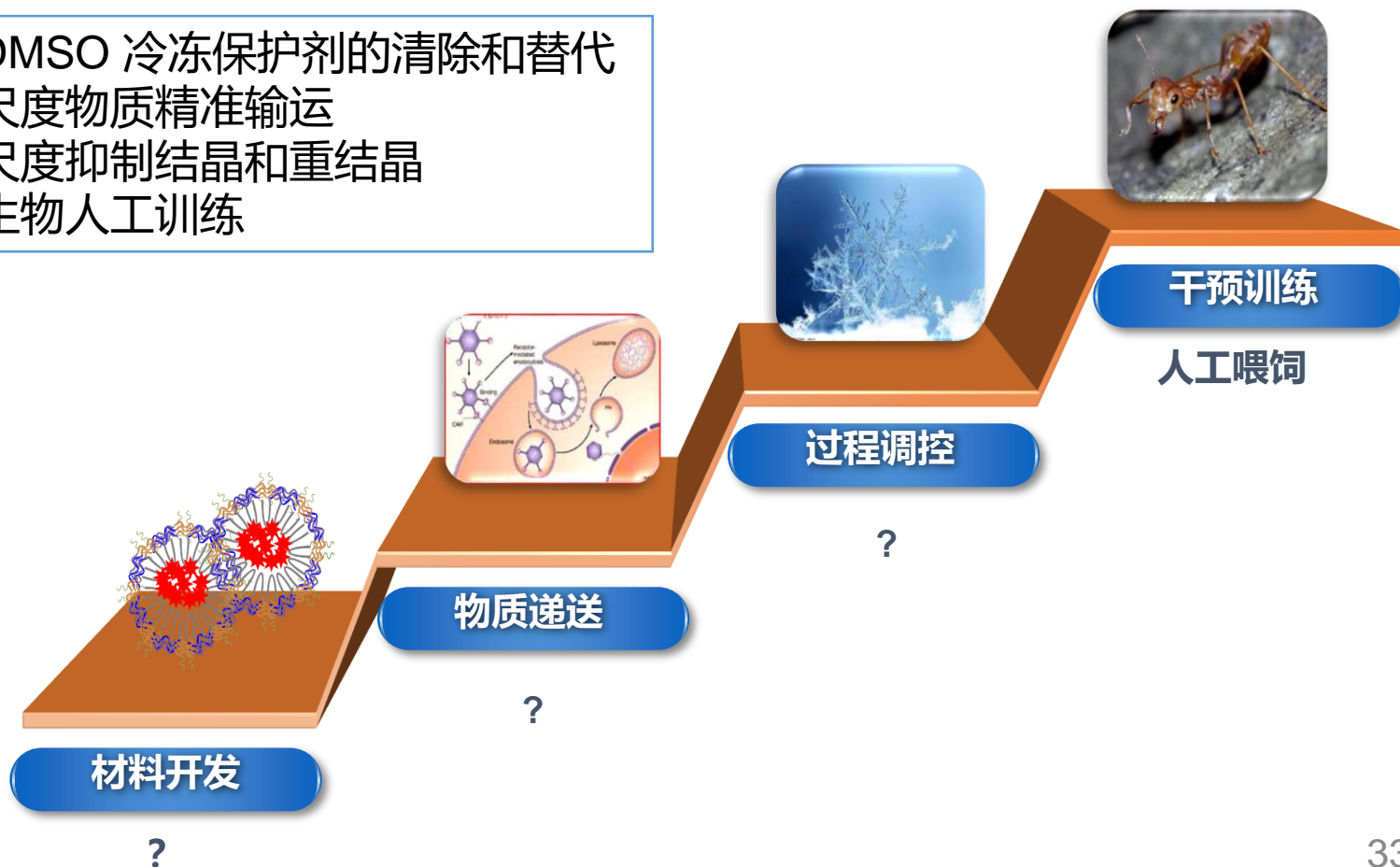
报告内容



物质 / 能量的精准控制对器官/活体的冷冻保存至关重要

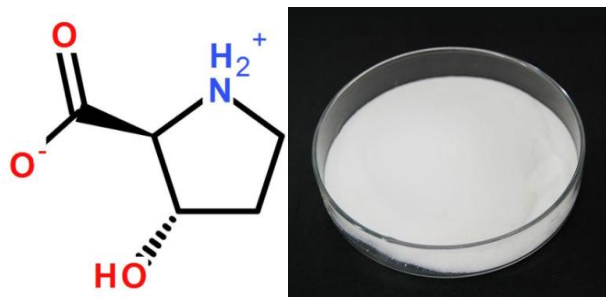
关键因素

- 材料：毒性DMSO 冷冻保护剂的清除和替代
- 递送：细胞尺度物质精准运输
- 过程：分子尺度抑制结晶和重结晶
- 干预：活体生物人工训练



绿色纳米冷冻保护剂

耐寒动物自身合成天然抗冻剂



脯氨酸

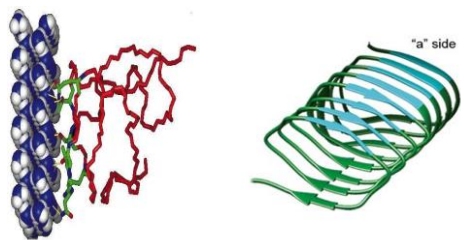
天然高分子纤维蛋白



蚕茧

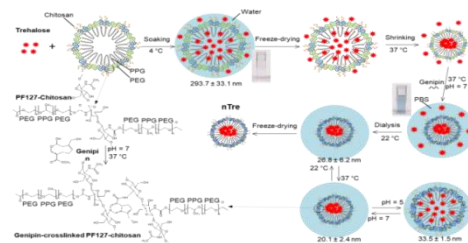
丝素

冰活性蛋白质



抗冻蛋白(AFP)&重结晶抑制蛋白(RIP)

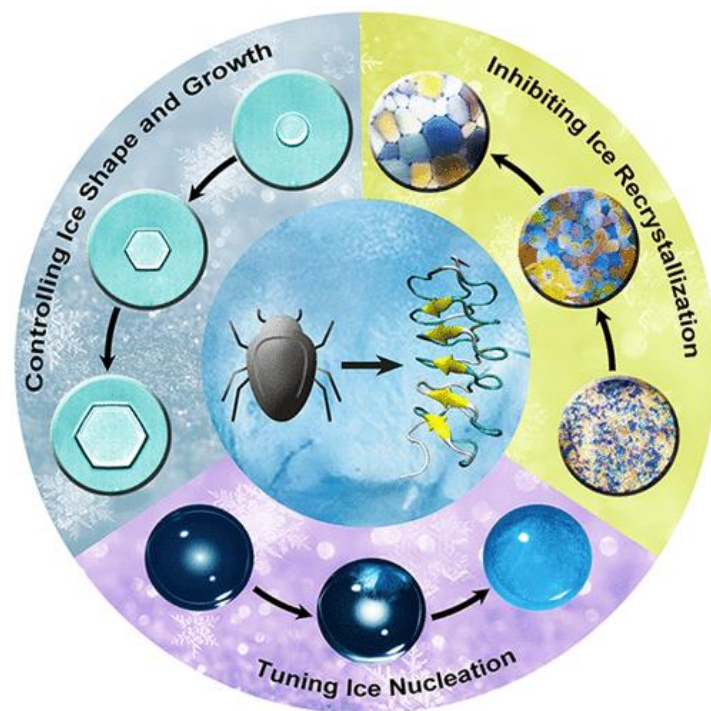
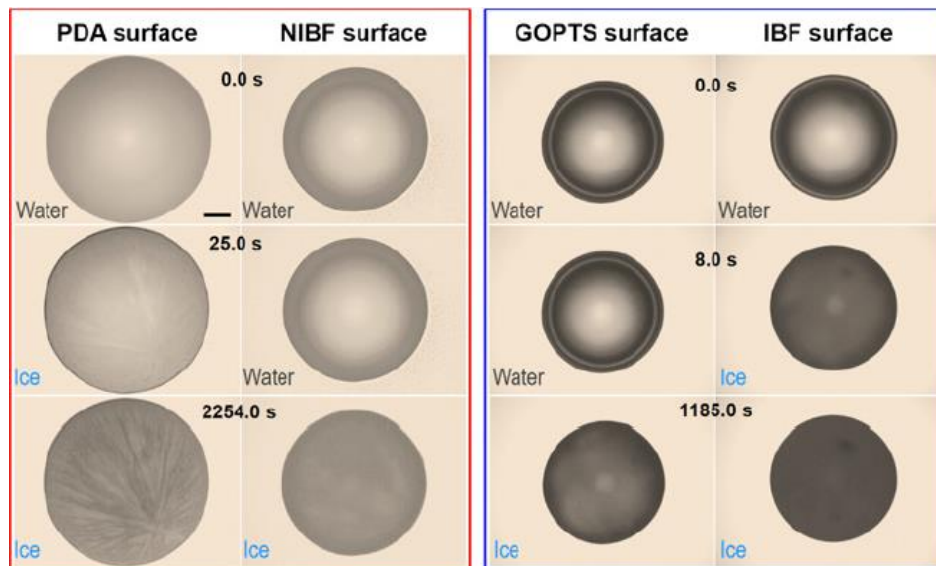
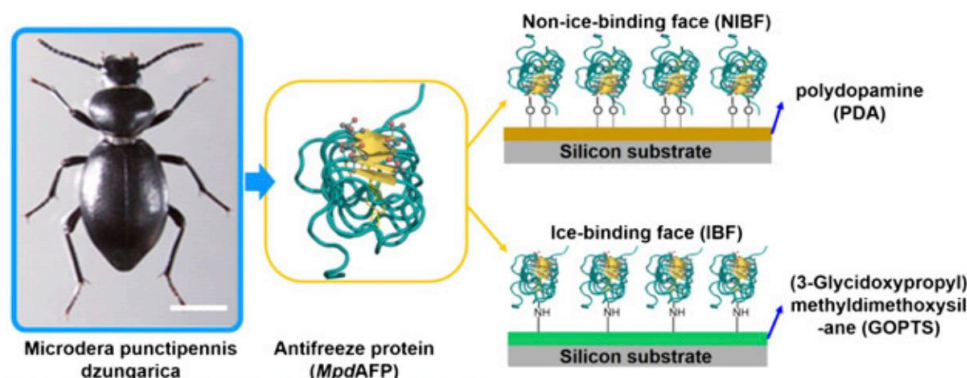
基于非渗透冷冻保护剂的纳米胶囊



纳米海藻糖

从自然界产物筛选，开发全新绿色冷冻保护剂

抗冻蛋白(AFP)

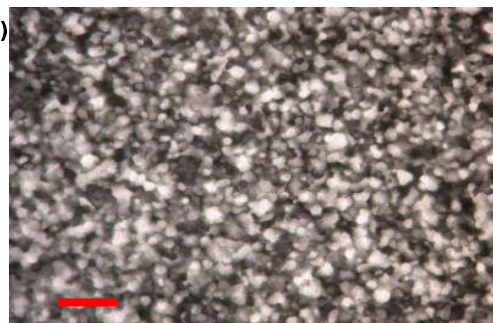
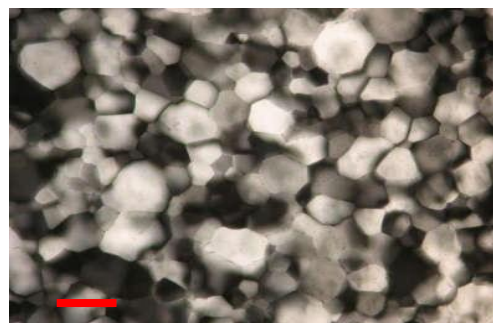
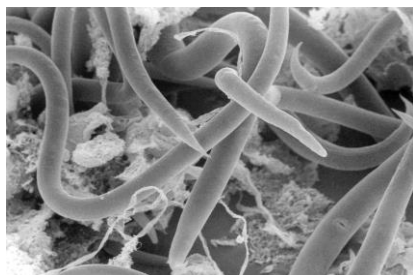


IBF促进冰晶生长
NIBF抑制冰晶生长

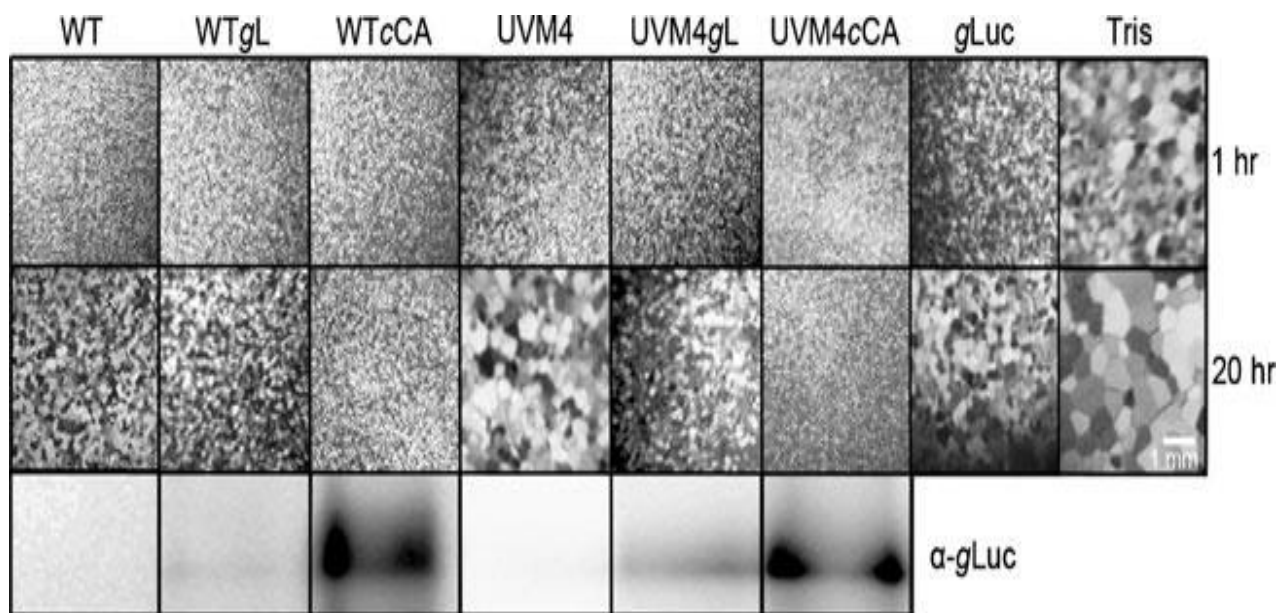
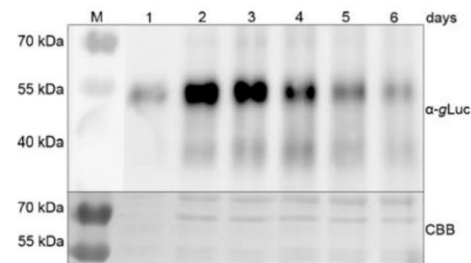
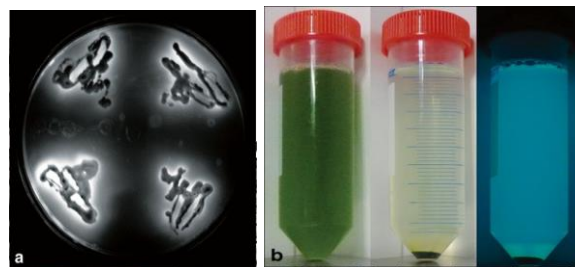
He Z., et al., Accounts of Chemical Research, 2018, 51(5).

Liu K., et al., Proc Natl Acad Sci USA, 2016, 113(51):14739-14744.

重结晶抑制蛋白(RIP)



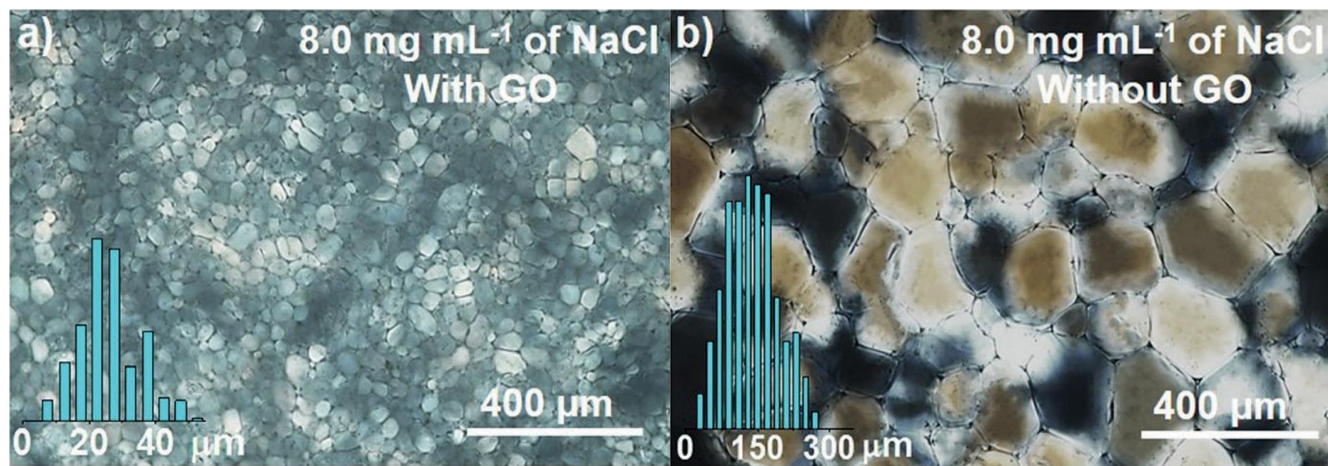
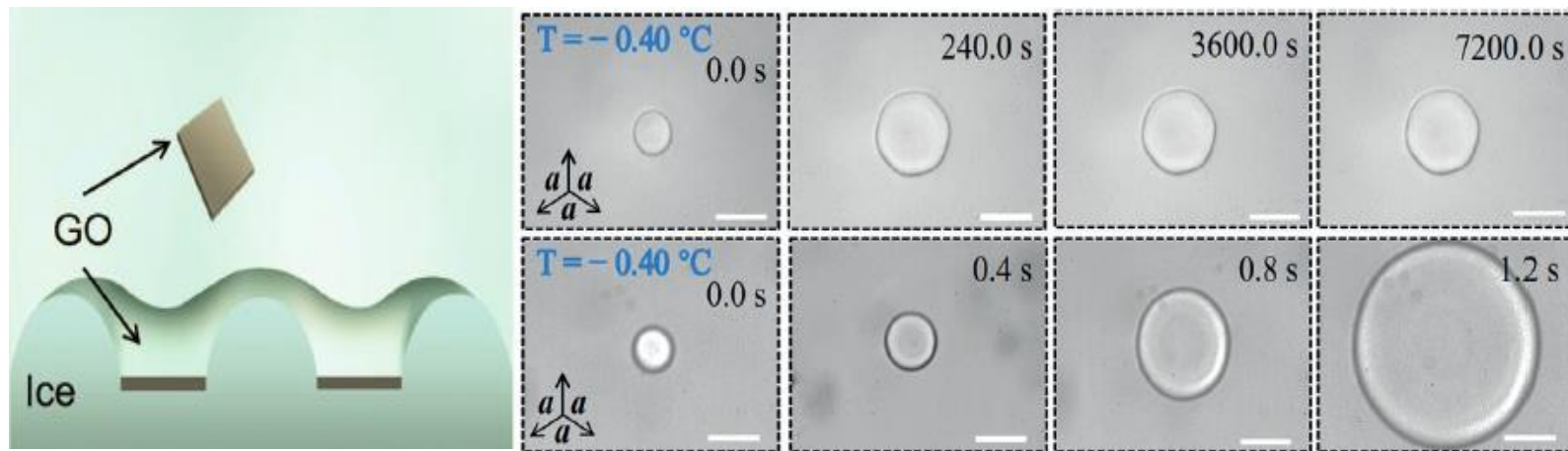
线虫的重结晶抑制蛋白



黑麦草中的重结晶抑制蛋白

Wharton D. A., Barrett J., Goodall G., et al., Cryobiology, 2005, 51(2):198-207.
Lauersen K. J., et al., Applied Microbiology and Biotechnology, 2013, 97(22): 9763-9772.

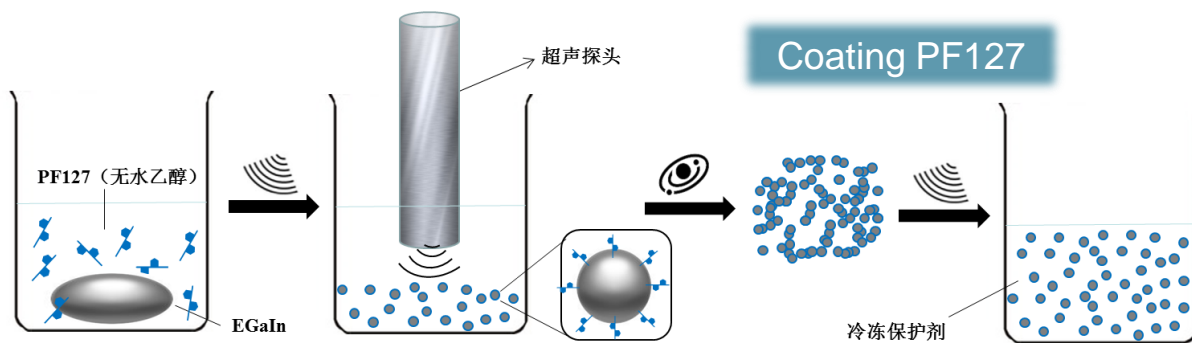
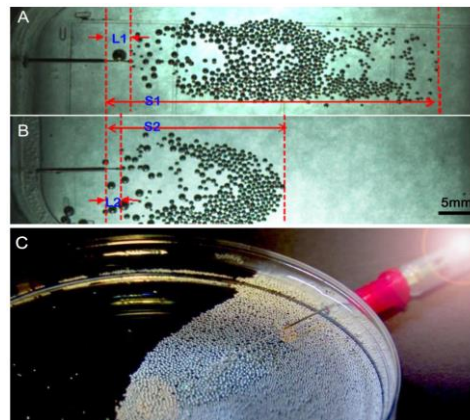
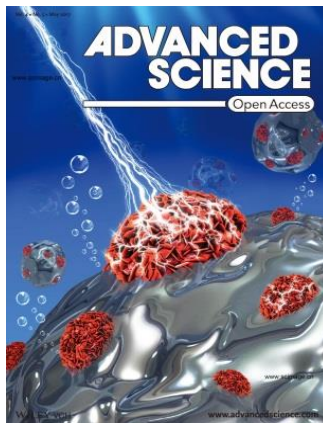
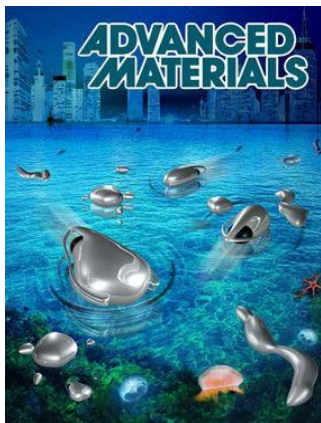
氧化石墨烯(GO)



减小冰晶尺寸

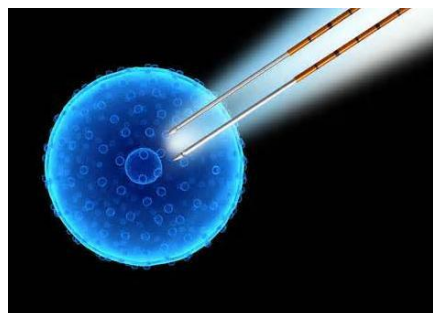
提高精子冷冻
保存的成功率

纳米液态金属低温保护剂初探

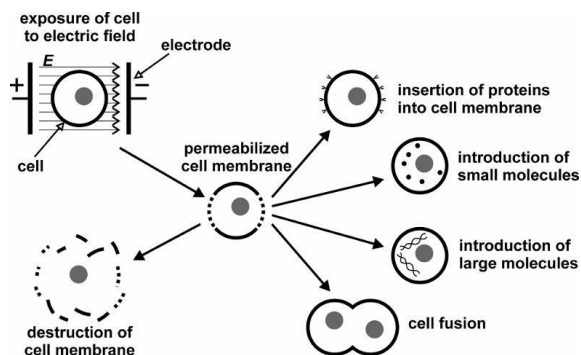


纳米材料实现非渗透冷冻保护剂的细胞递送

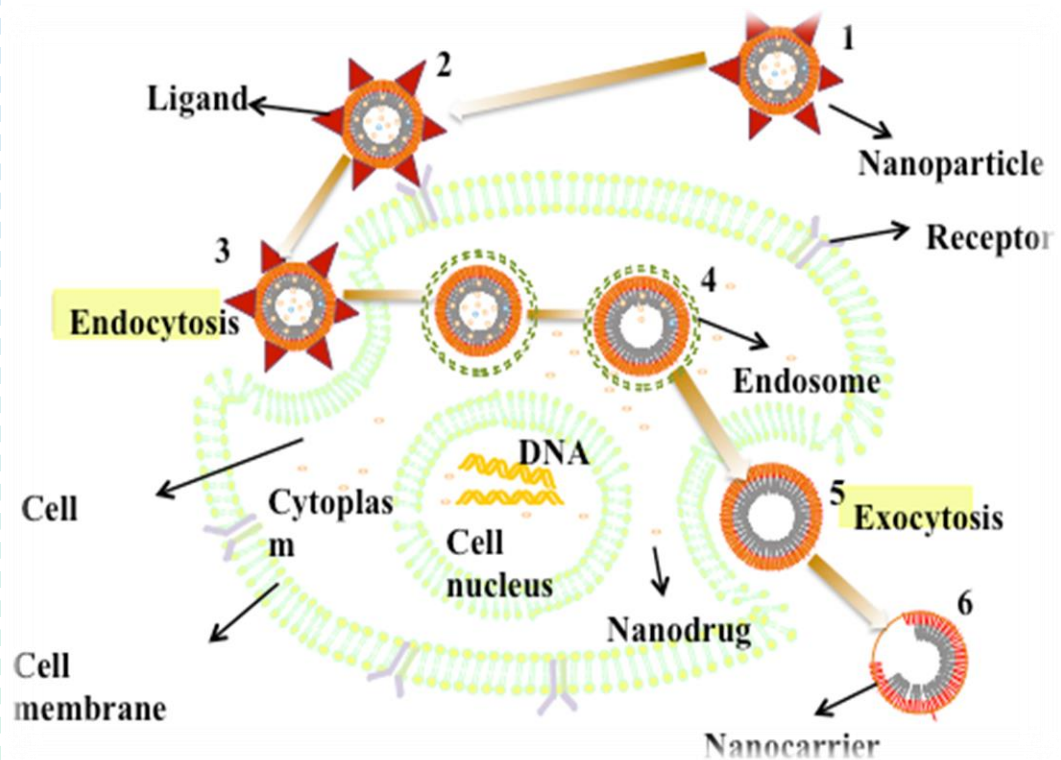
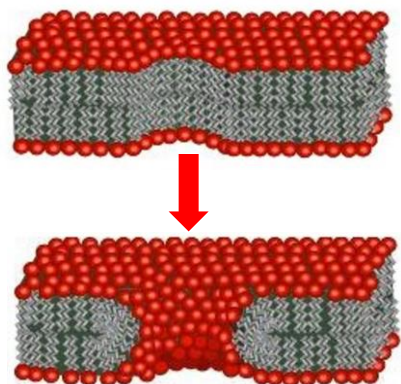
微创手段 | 无创手段



微注射

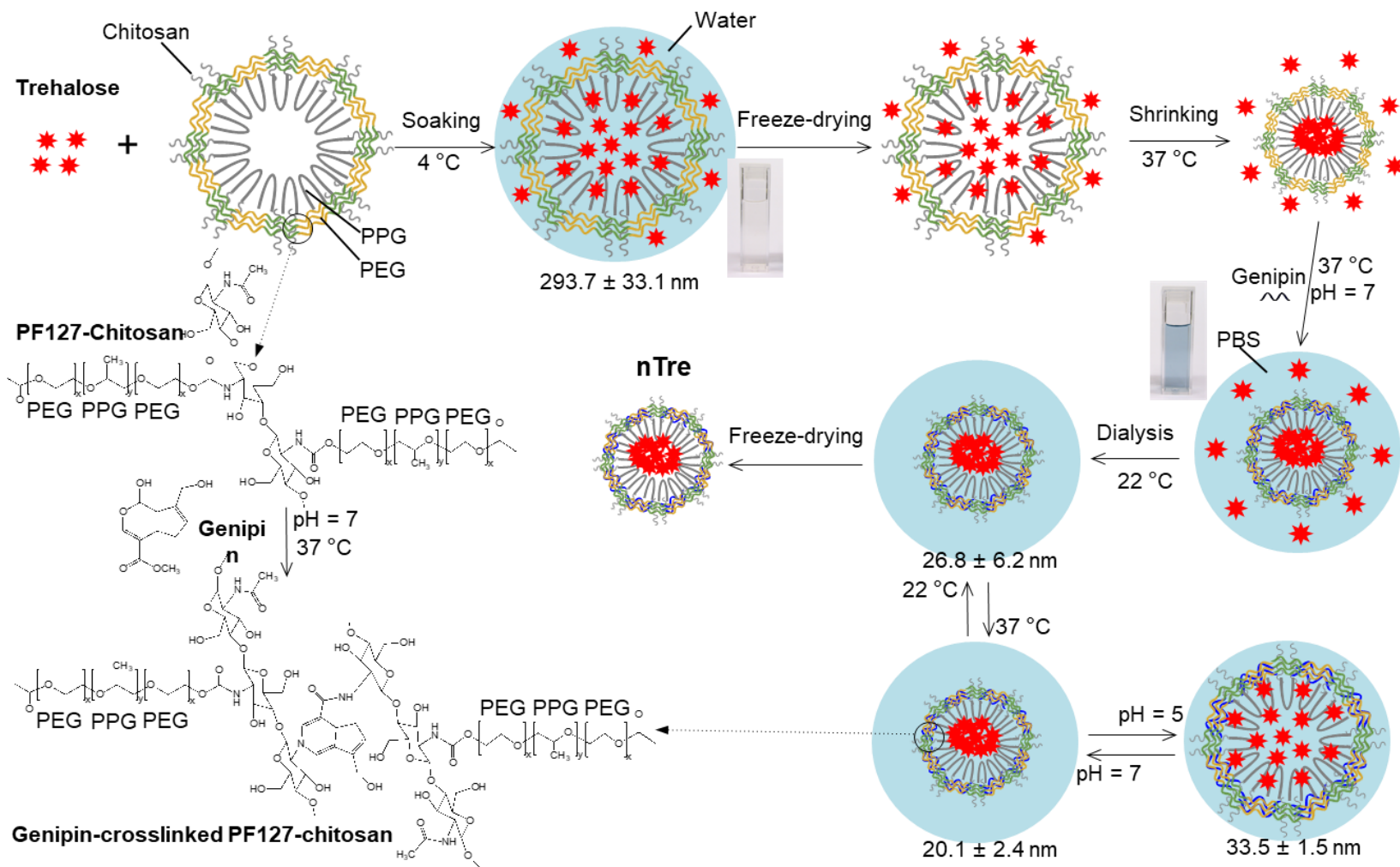


电穿孔

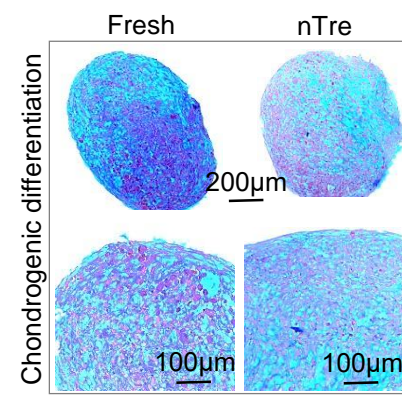
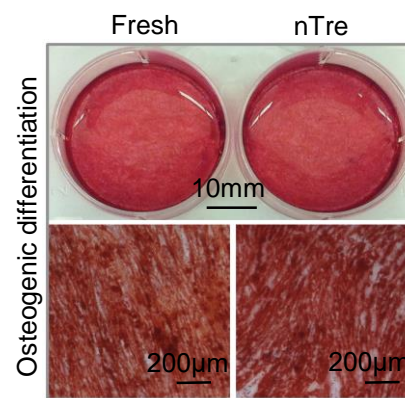
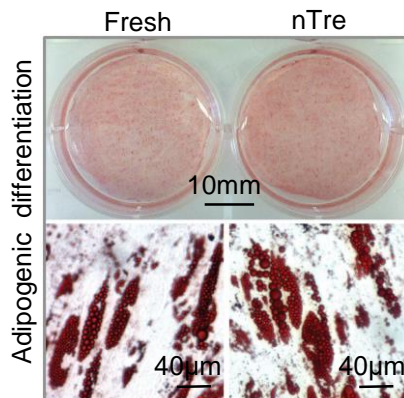
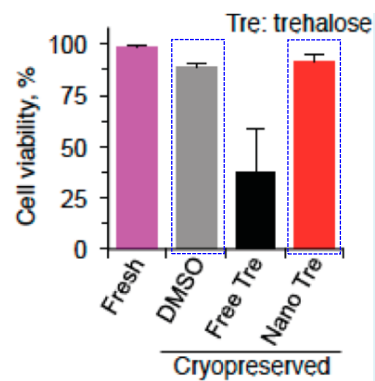
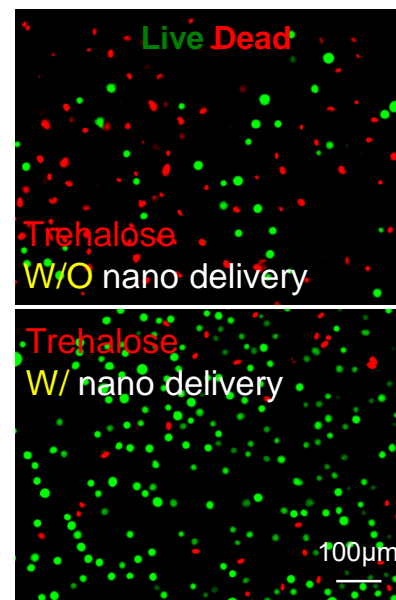
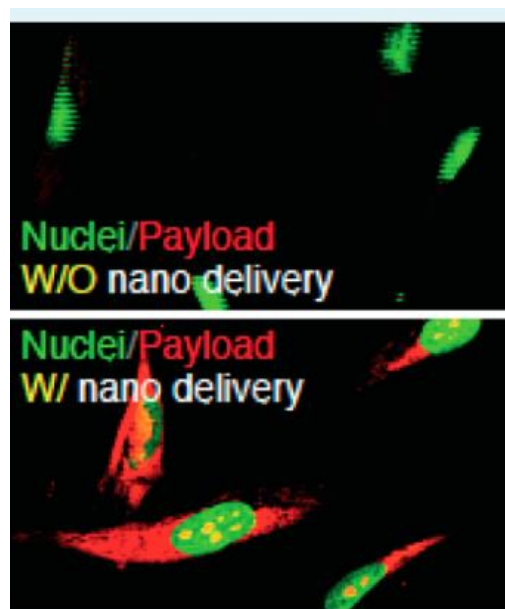
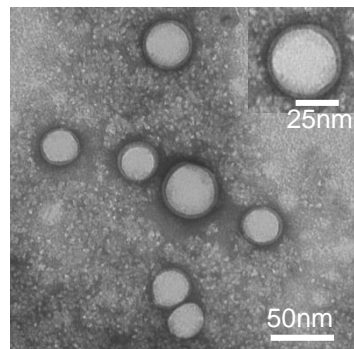
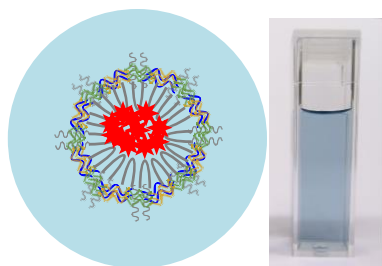


细胞内吞

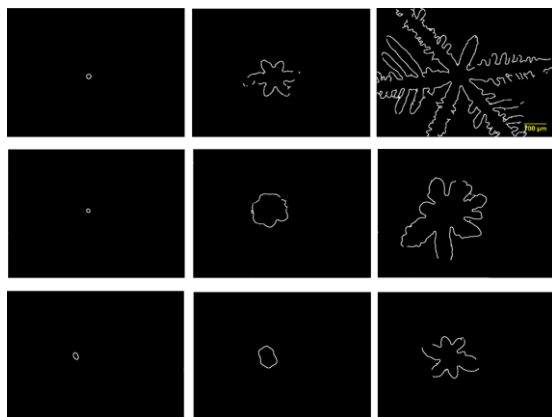
纳米材料实现非渗透冷冻保护剂的细胞递送



纳米材料实现非渗透冷冻保护剂的细胞递送



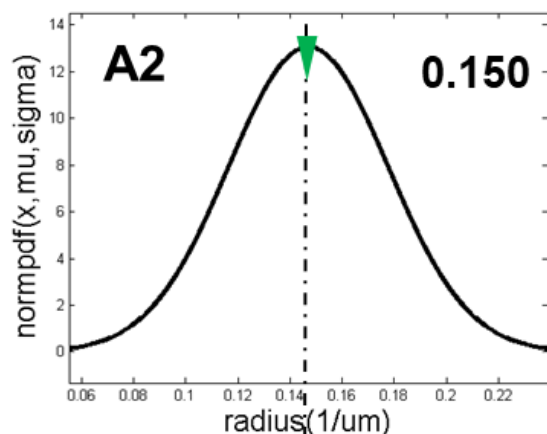
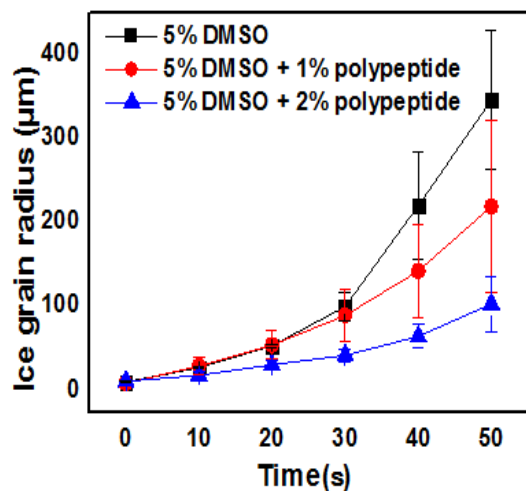
多肽可显著降低冰晶生长速率，修饰冰晶生长



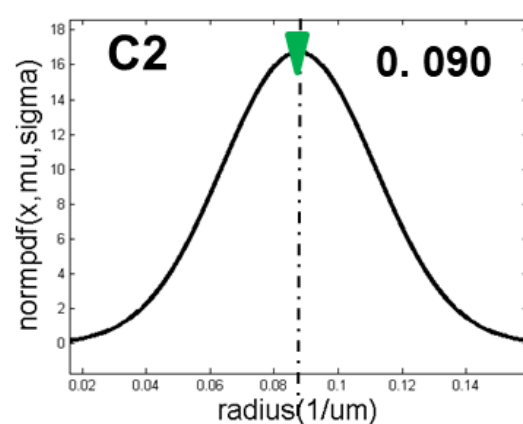
5%DMSO



5%DMSO+2%Pyepptide

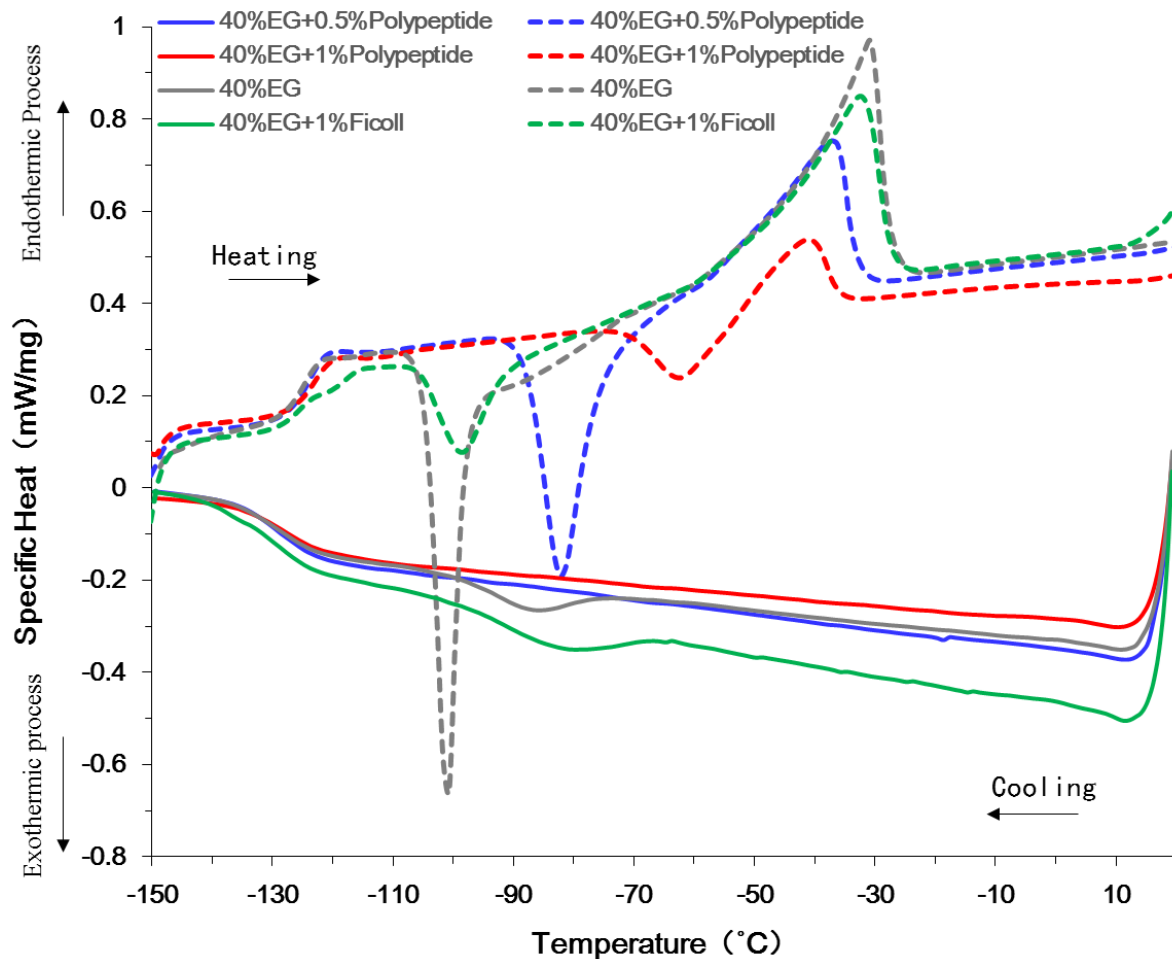


Curvature Radius: 4-7 μm



Curvature Radius : 10-12 μm

仿生纳米材料对冰晶生长的调控



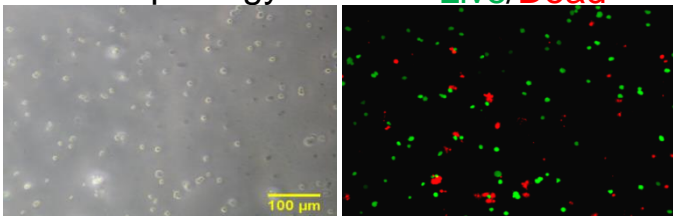
- 抑制结晶，有助于玻璃化冻存
- 抑制重结晶

仿生纳米材料对冰晶生长的调控

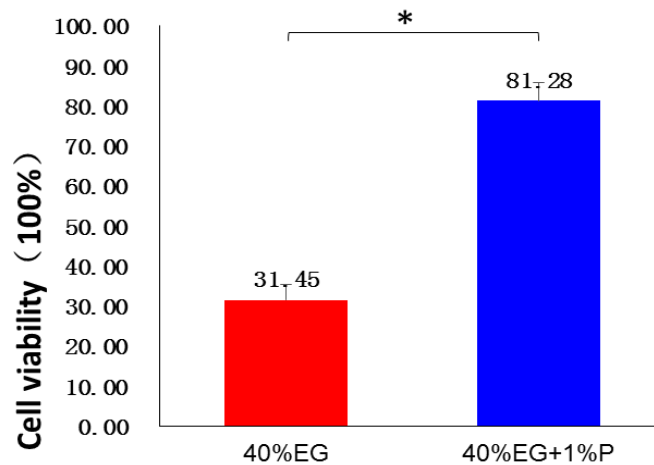
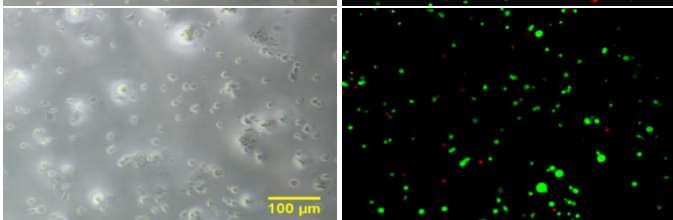
Morphology

Live/Dead

40%EG

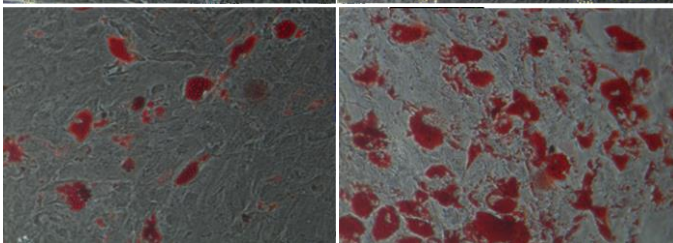
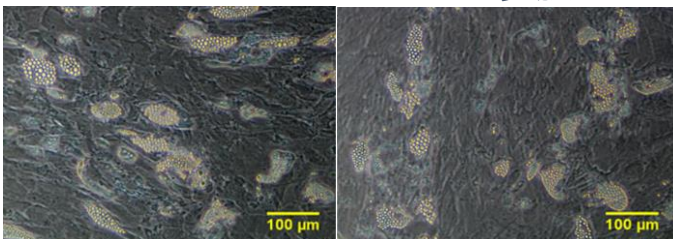


40%EG+
1%Polypeptide

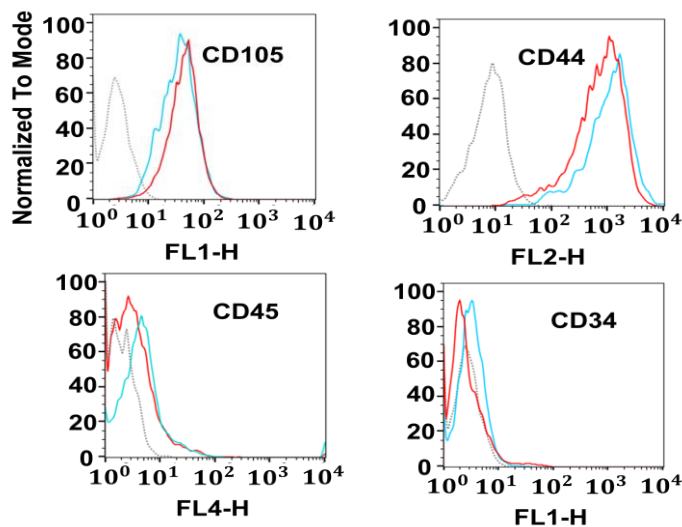


控制组

多肽



Condition



微胶囊营造保护屏

Microfluidic to produce cell microcapsules

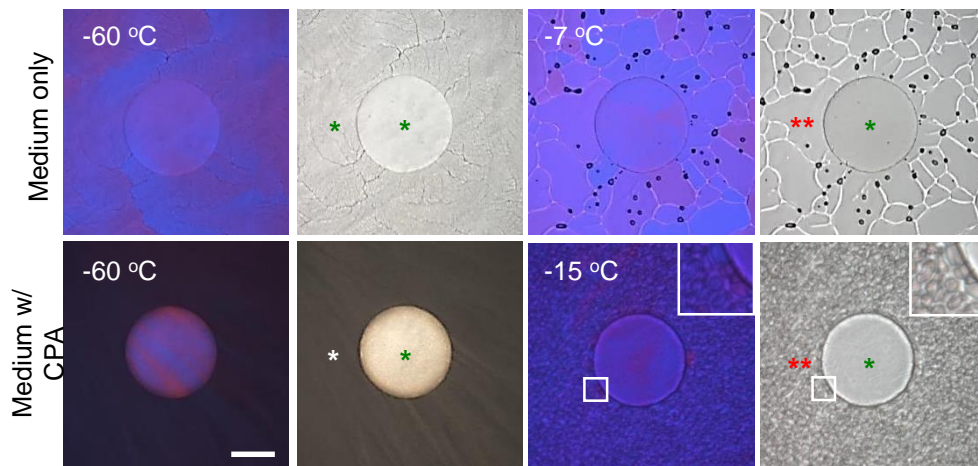
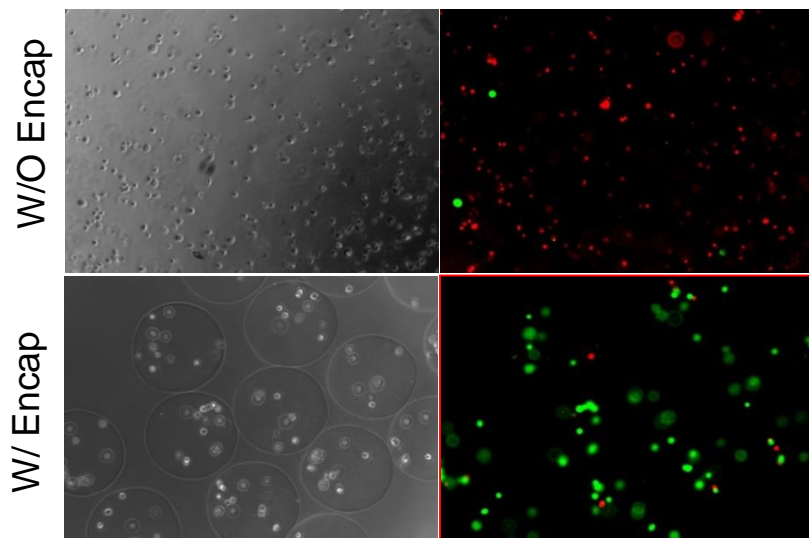


Freezing

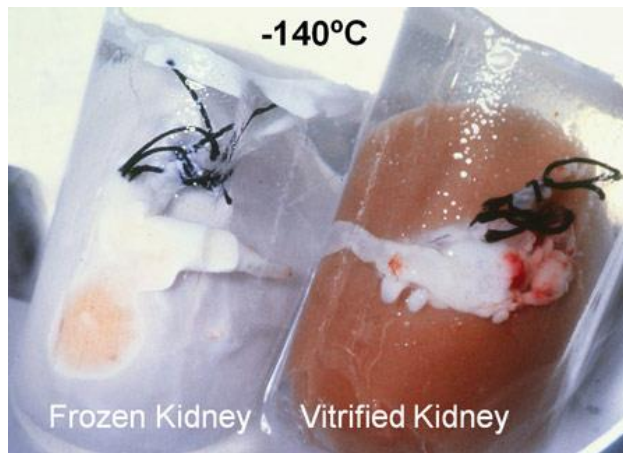
Thawing



After vitrification: Phase & Fluorescence



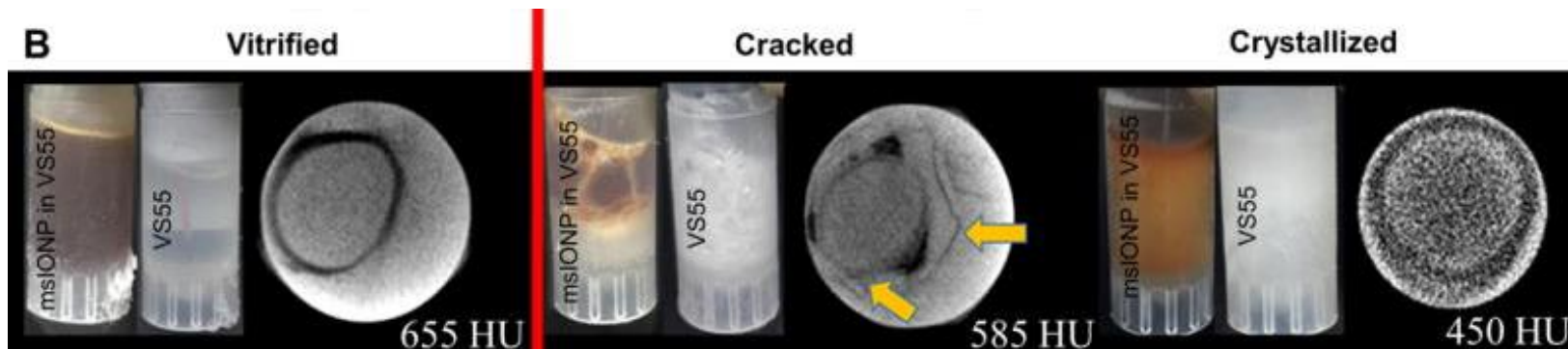
玻璃化冷冻



兔子肾脏玻璃化



卵巢组织玻璃化冷冻前（左）后（右）的切片



1ml猪颈动脉冷冻保存

Fahy G. M., et al., Cryobiology, 1984, 21(4): 407.

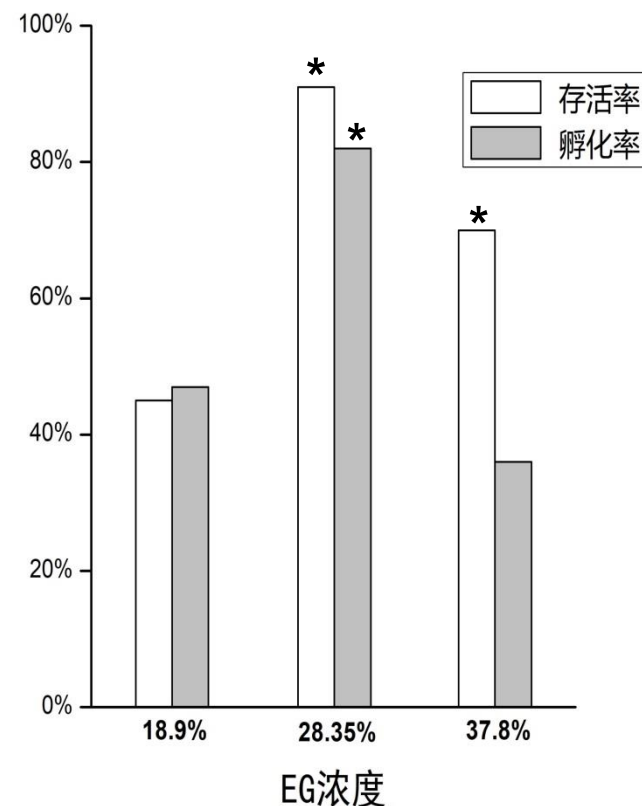
Manuchehrabadi N., et al., Science Translational Medicine, 2017, 9(379)

促进玻璃化

增加冷冻保护剂浓度

Type of embryos frozen	Postthawing survival rate definition	Type of cryoprotectant used for vitrification	Type of cryoprotectant used for slow freezing
Blastocyst	Expanding blastocyst with intact trophoctoderm and ICM	20% EG (3.6 mol/L), 20% DMSO (2.4 mol/L), and sucrose (0.5 mol/L) in HTF medium with 20% HSA	9% Glycerol with sucrose (0.2 mol/L) in G2 medium with 20% albumin
2PN, 4 cell, blastocyst	Normal response to osmotic changes during the dilution process	15% EG, 15% DMSO, and sucrose (0.5 mol/L) in TCM199 with 20% SSS	1.5 mol/L propylene glycol plus sucrose (0.1 mol/L) in HEPES-buffered P1 medium
Day 3	Embryos with >50% intact blastomeres immediately after thawing	EG (30%), sucrose (0.5 mol/L)	1.5 mol/L PROH with sucrose (0.1 mol/L)
Day 3	Not reported	EG (40%), sucrose (0.6 mol/L)	Commercial kit (Vitrolife)

冷冻保护剂毒性

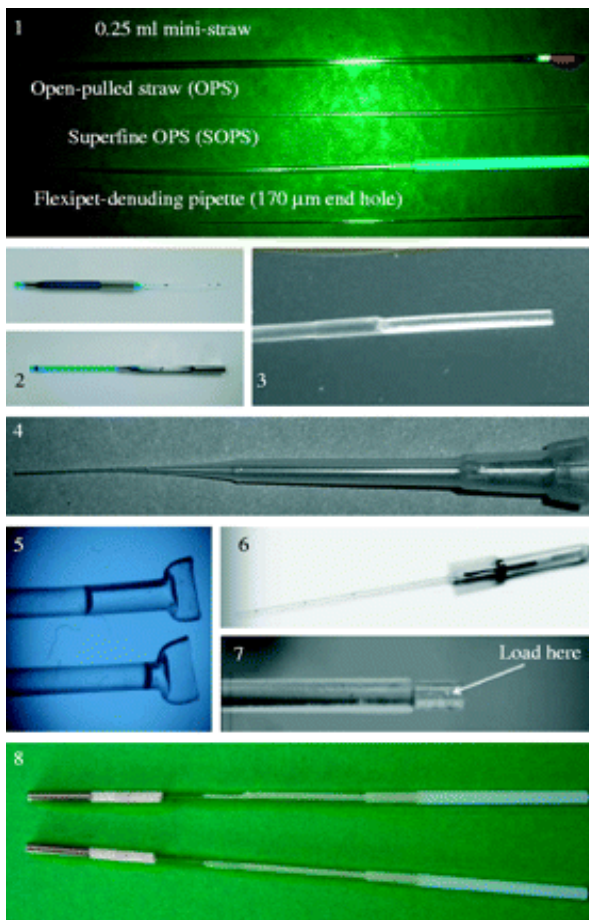


慢速冷冻和玻璃化 冻存冷冻保护剂用量对比

提高降温速率——提高导热速率的冻存管



玻璃化平面冻存管

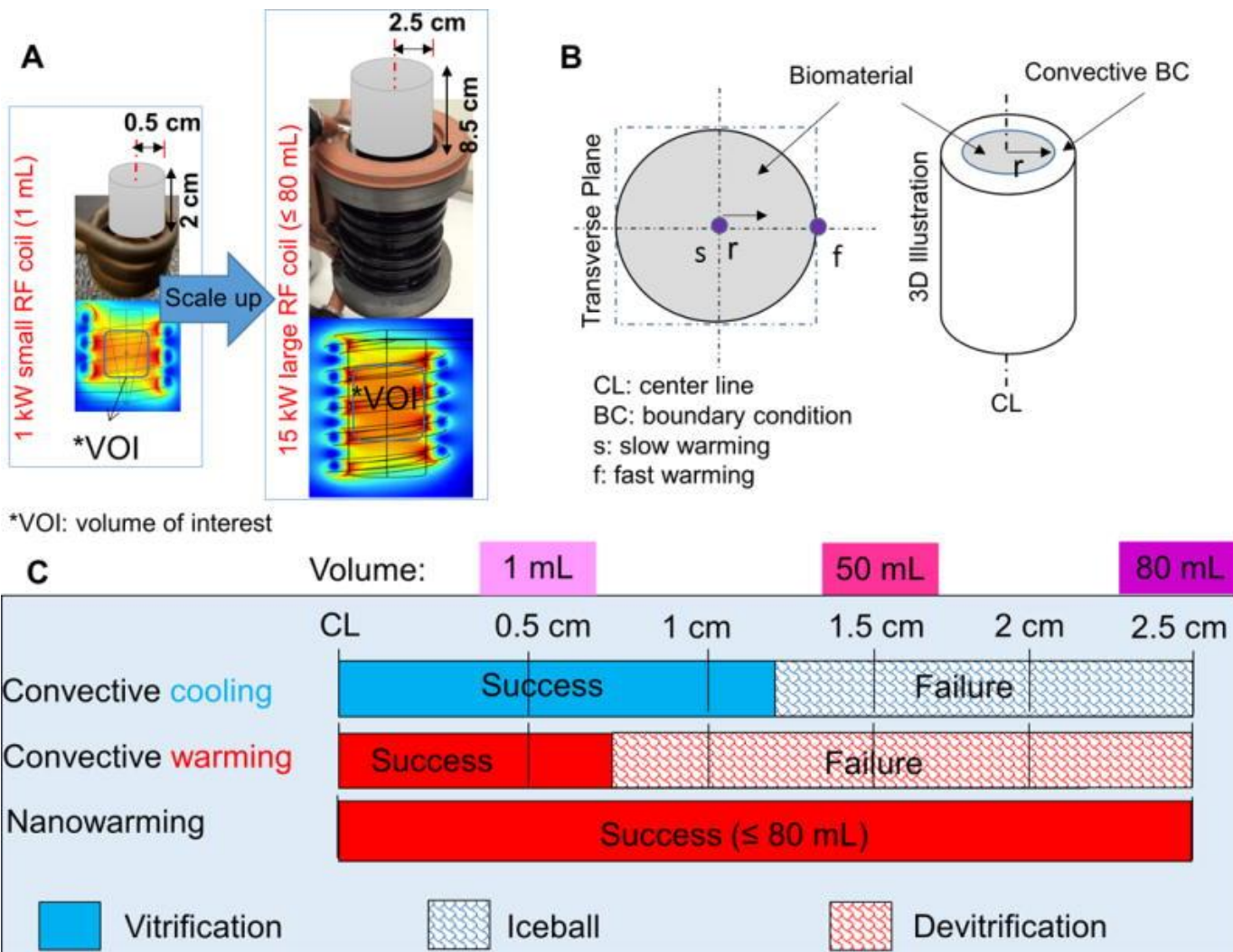


玻璃化圆管冻存管

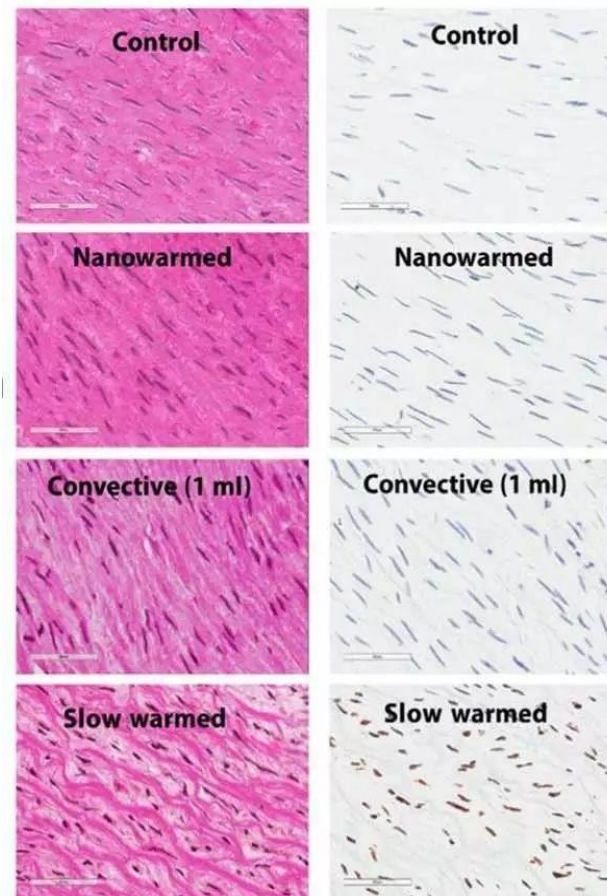
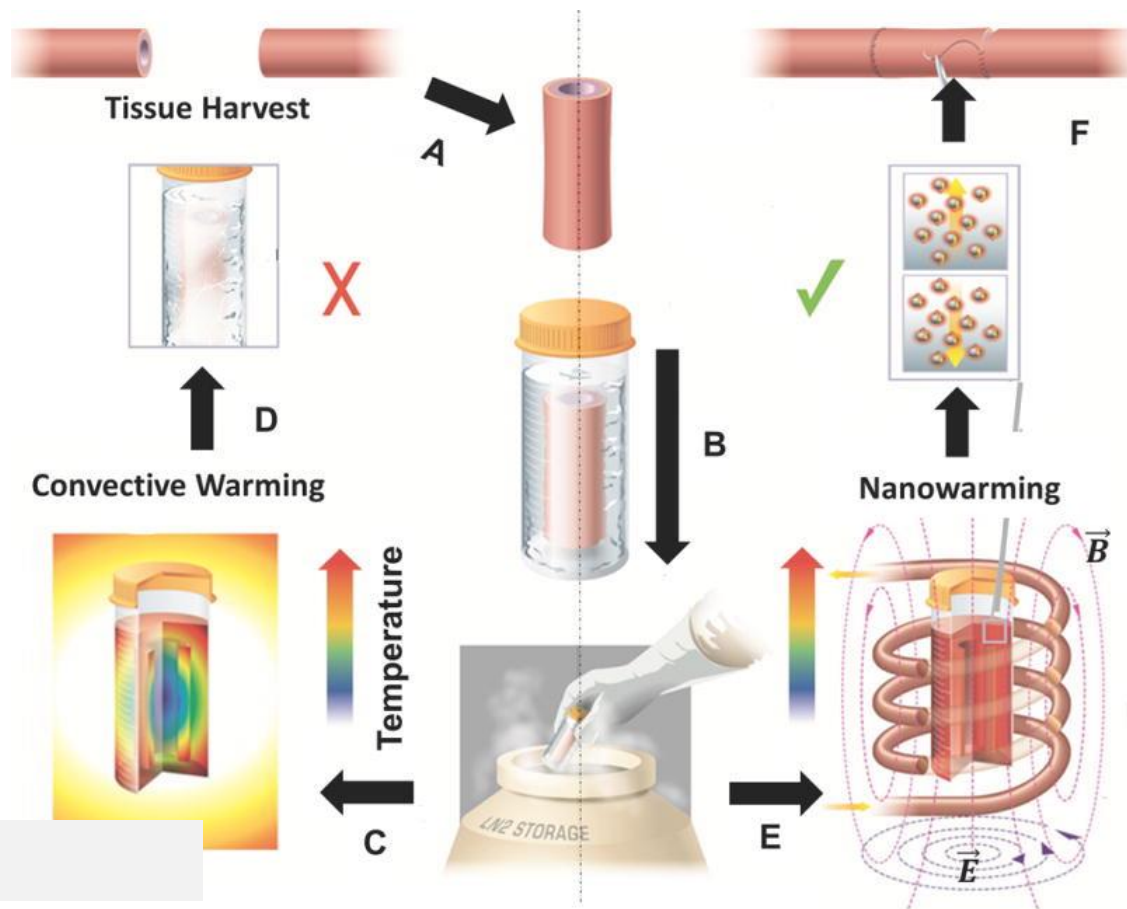


卵巢组织玻璃化冻存管

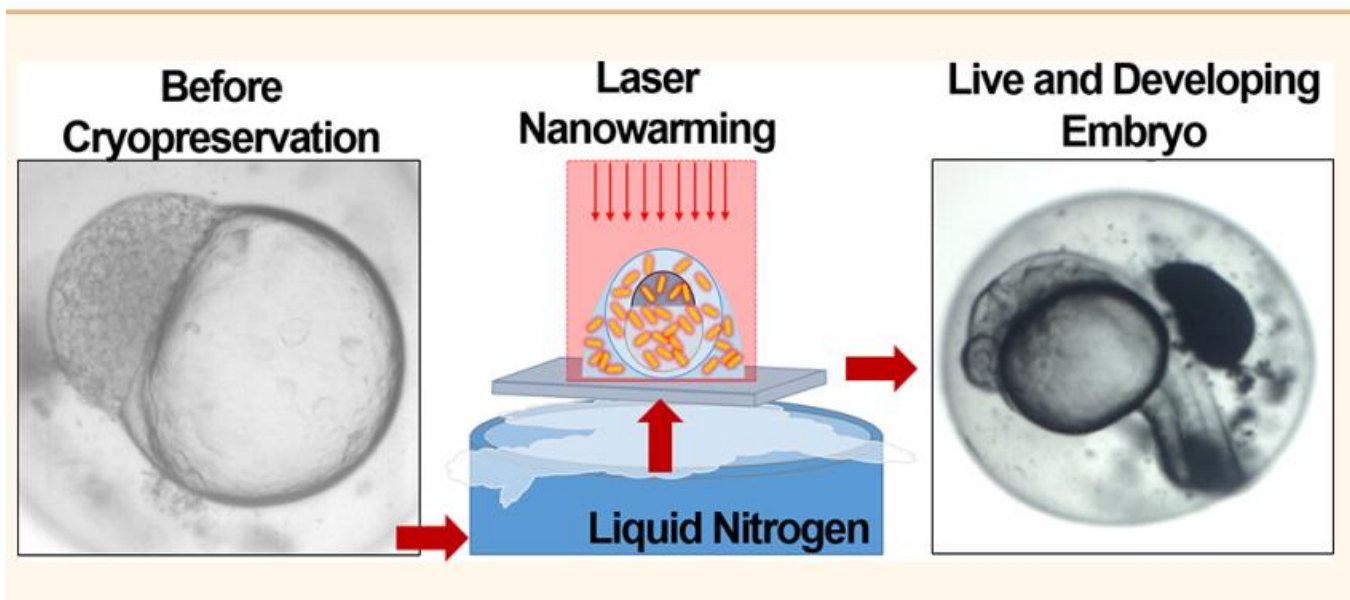
纳米加热拓宽冷冻体积



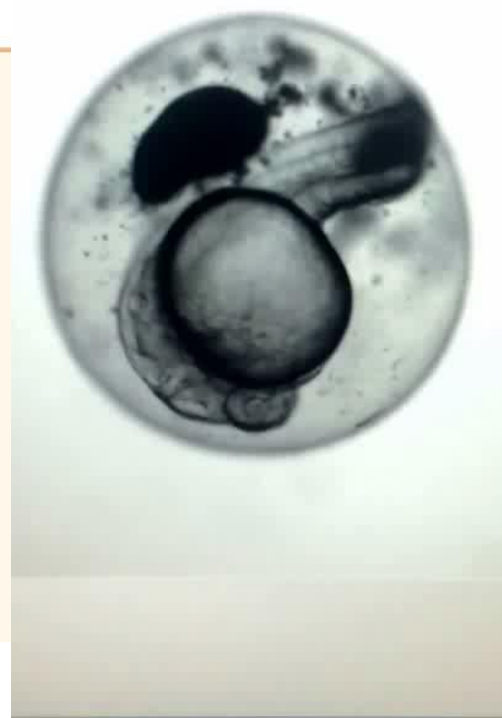
磁性纳米粒子的感应加热改善组织冷冻保存



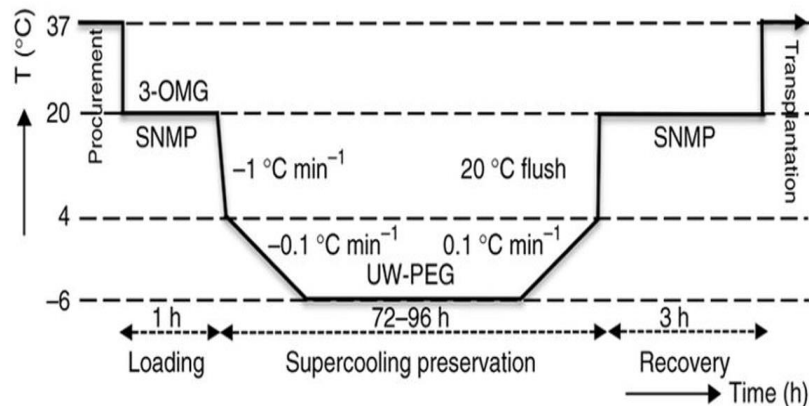
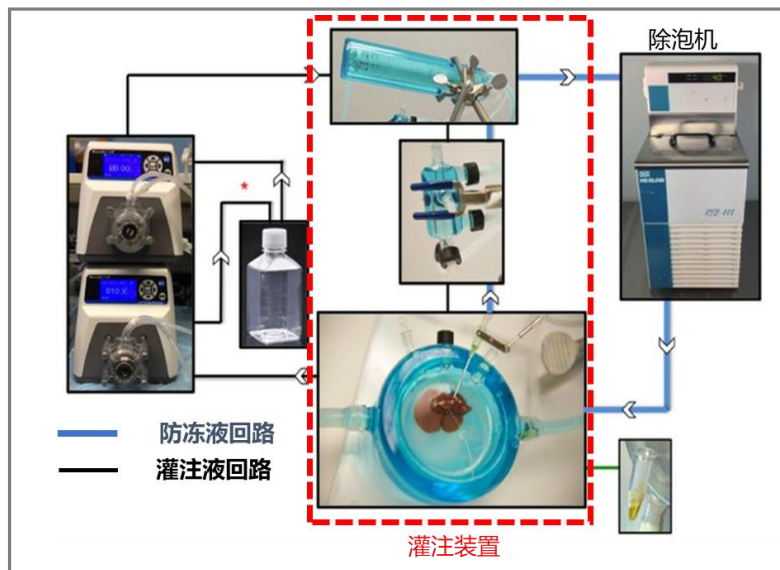
激光加热纳米复温



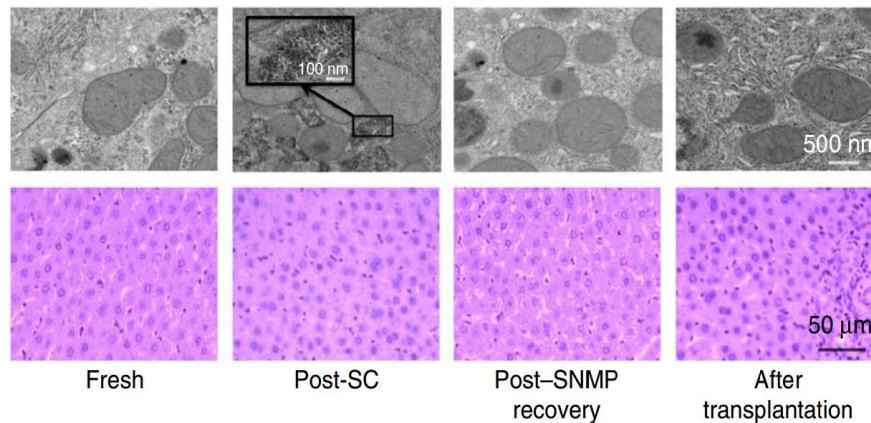
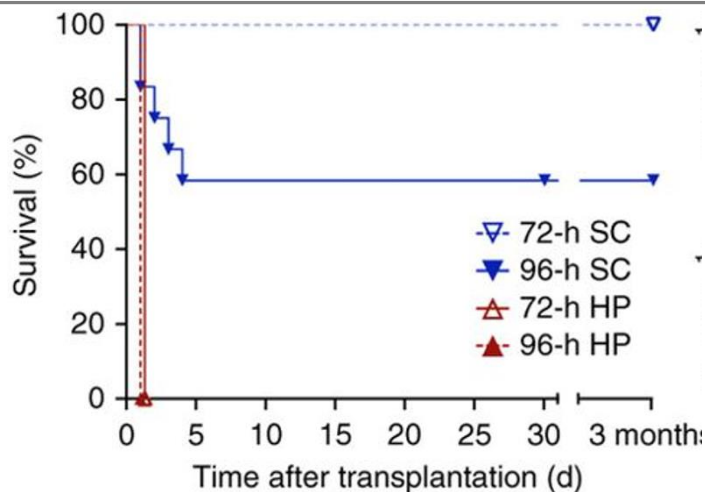
斑马鱼胚胎



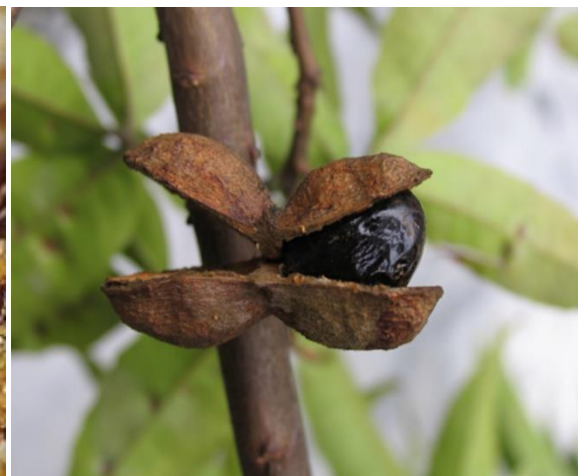
低温机械灌注



SNMP: 常温机械灌注技术 HP: 低温保存 SP: 过冷保存



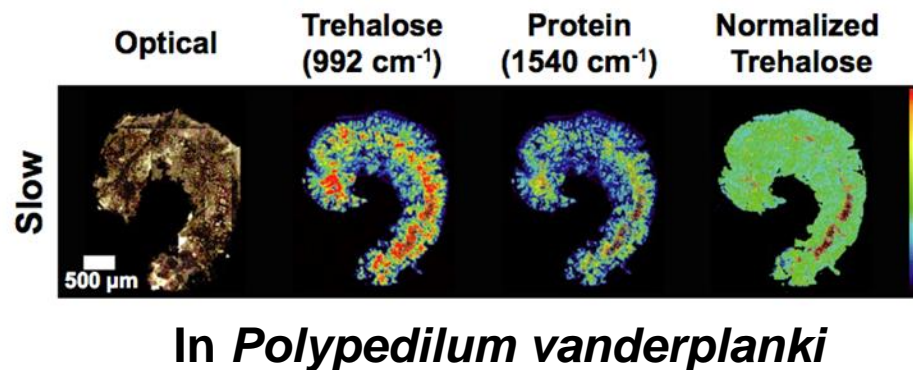
畅想：仿生



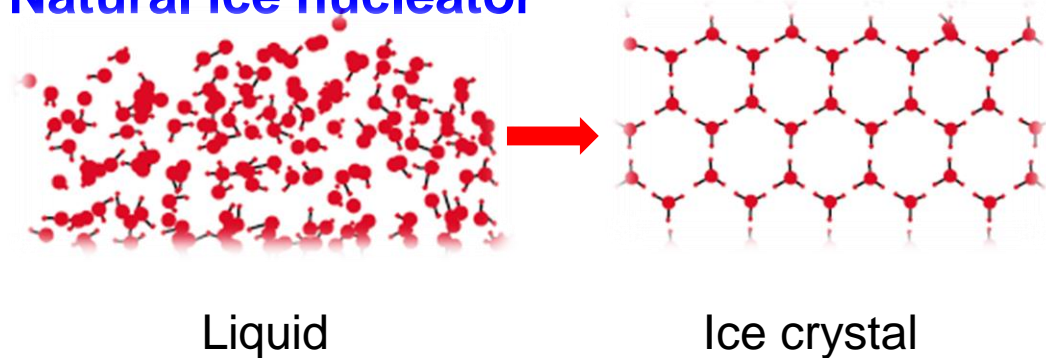
深入揭示耐寒（旱）昆虫或动物的抗寒（旱）机理，解决制约仿生型低温保存技术发展的瓶颈问题，实现复杂生命对象的低温保存，从而推动低温保存技术的跨越式发展

训练和干预

Produce antifreeze agents



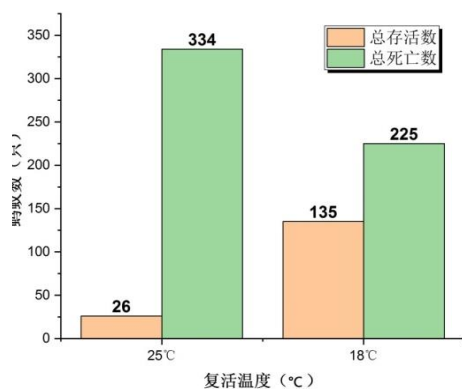
Natural ice nucleator



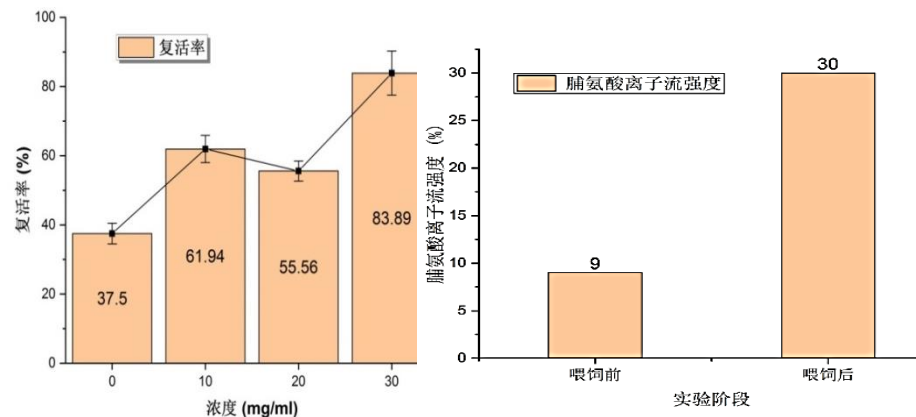
Cryoprotective dehydration



训练和干预



复温温度为18°C，冷冻存活率大幅提升



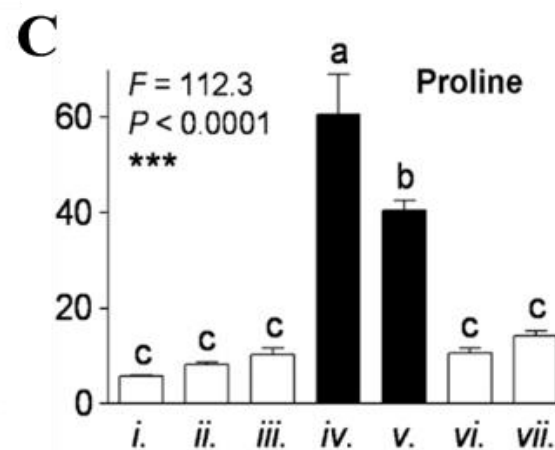
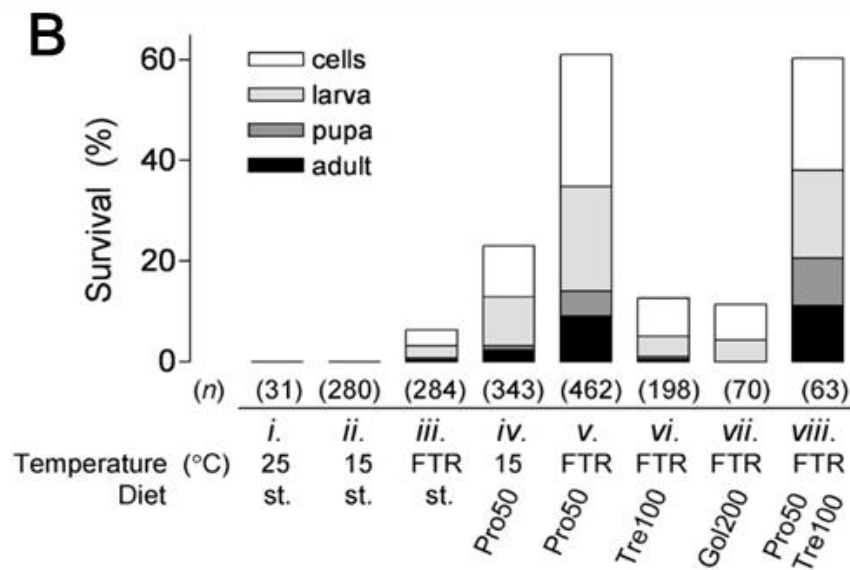
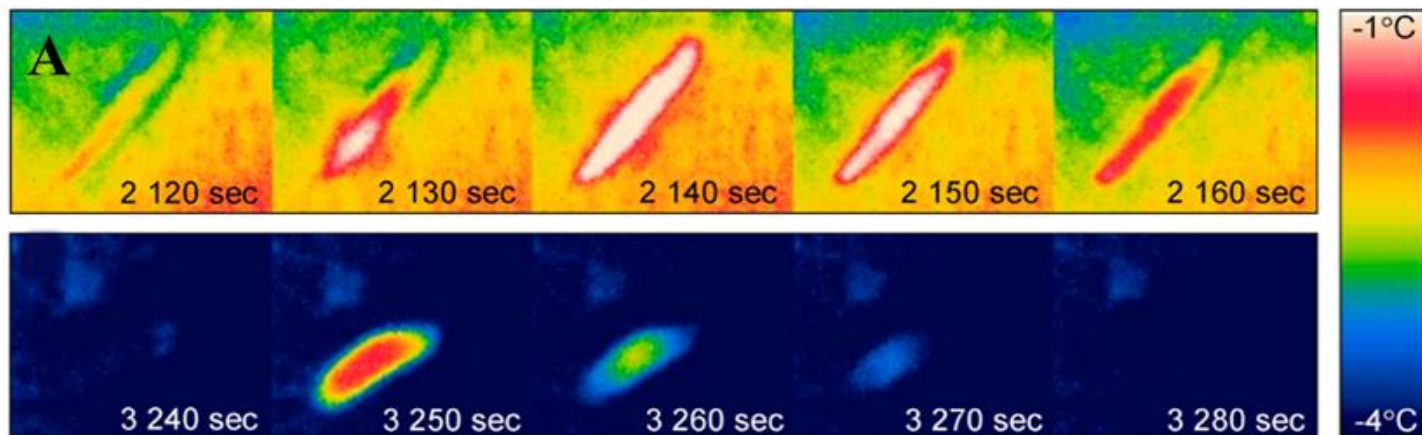
30 mg/ml 喂饲浓度可最大限度提升冷冻存活率

实验组	质量m (mg)	含水量m _h (mg)	结冰温度 (°C)	计算冻结焓t _f (J/g)	放热焓H _f (J/g)	OA质量 (mg)	OI质量 (mg)	OA/OI
对照组	16.01	11.29	-18.3	263.47	130.53	7.93	8.08	0.98
DI10组	13.40	6.30	-25.50	260.81	111.25	3.59	5.35	0.67
DI20组	17.14	12.08	-25.51	278.23	102.10	6.29	10.85	0.58
DI30组	25.86	18.23	-26.30	279.87	96.41	8.91	16.95	0.53

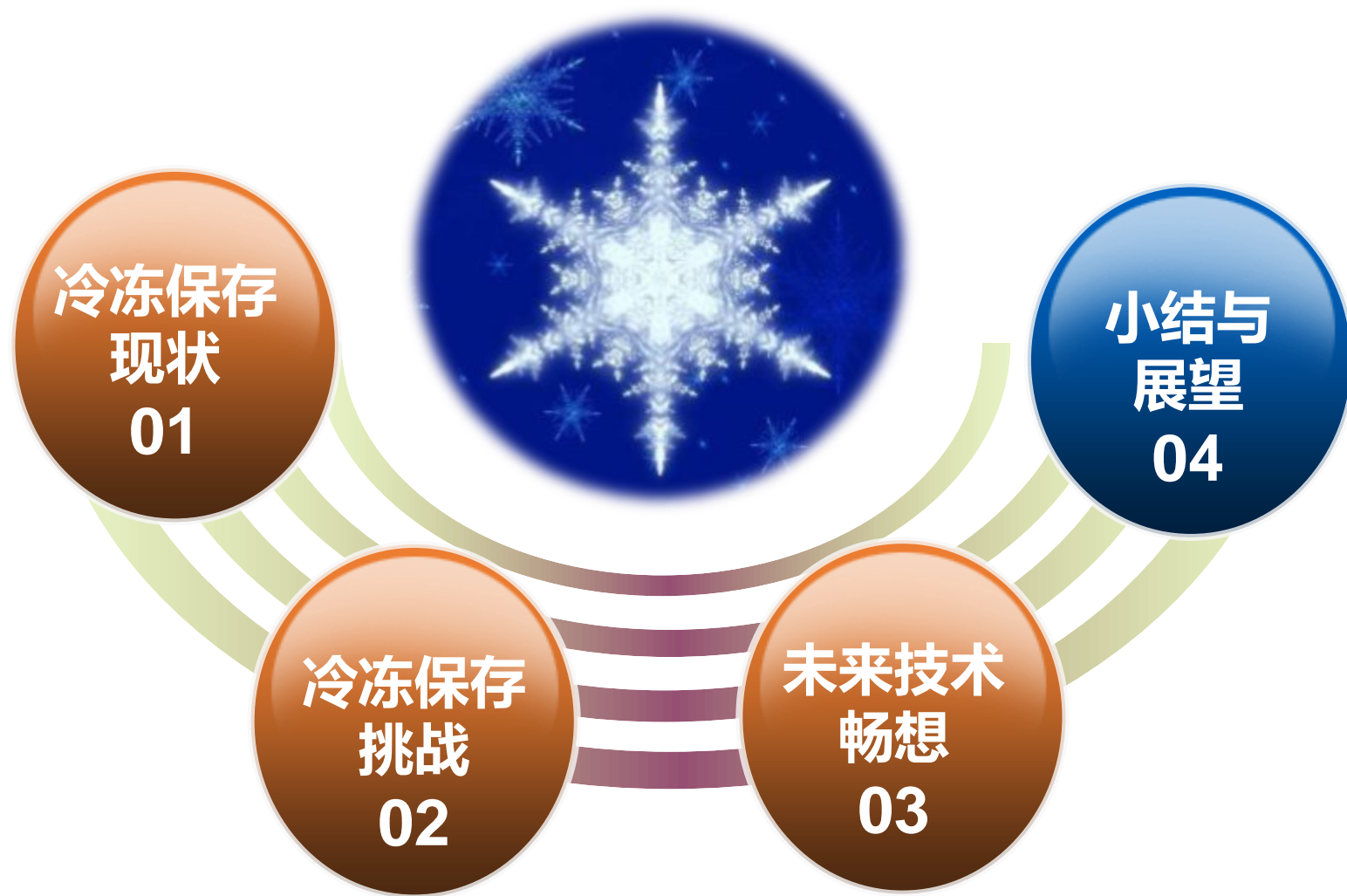
30 mg/ml 喂饲组渗透活性水量（结晶水量）/非渗透活性水量值最低

喂饲天然氨基酸可显著提升非耐寒蚂蚁的冷冻存活率

训练和干预



报告内容

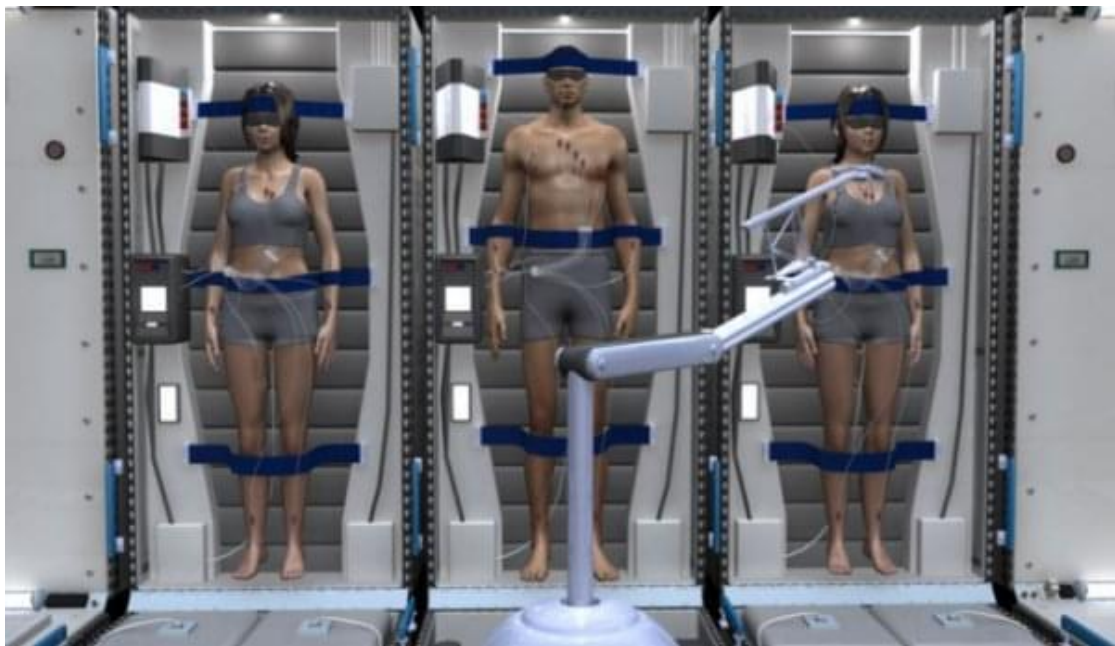


选择性快速低温脑保护



解决大脑在体选择性快速、均匀降温等问题，
满足脑卒中、脑血栓、心血管病、高热、交通伤、战伤等重大需求

人工冬眠



深入揭示冬眠机理，
解决哺乳动物或人体冷休克问题，实现高等动物甚至人类的人工可控冬眠技术

通向未来的技术路线图



**智能机器操纵的诊疗
一体化冷冻治疗**



记忆冷冻保存与恢复



人体冷冻复活



冬眠疗法



人工冬眠



人类星际旅行



谢谢大家！ 敬请批评指正！