technology opportunity

Use of connexin inhibitors for improved cryopreservation of cells and tissues

Ghent University is seeking partners interested in commercializing improved solutions for cryopreservation of cells and tissues.

Introduction

Cryopreservation, freezing, vitrification and cold storage are techniques often used for storage of tissues or cells. These procedures, however, are intrinsically associated with various degrees of cell stress that may ultimately result in cell damage and cell death. Ice crystal formation and subsequent local hyperosmolarity are crucial factors in cell damage. Cryoprotectants are often used to lower the freezing point of water and diminish cell damage but at the same time these substances have intrinsic toxicity.

Connexin proteins are expressed in cells and form hemichannels and gap junction channels. Hemichannels are precursors of gap junction channels and these channels are normally closed. When two hemichannels interact, they open and form a gap junction channel that connects two adjacent cells. Gap junctions are crucial for the physiological function of cells and tissues but they also form a conduit that may communicate cell death between cells (bystander cell death). Hemichannels not incorporated in gap junctions are normally closed but open in response to various stressor conditions and may promote cell death when open. Connexins are named according to their predicted molecular weight and Cx26, Cx32, Cx37, Cx40 and Cx43 are examples of widely distributed isoforms.

It has been investigated whether blocking gap junctions and hemichannels, using connexin channel inhibitory peptides like Gap26 and Gap 27 that target a range of different connexin species, could prevent cell death after cryopreservation.

Technology

Researchers at Ghent University have demonstrated that connexin channel inhibitors (such as Gap26 and Gap27 peptides, consisting of 13 and 11 amino acids respectively), when added to the preservation solutions, strongly reduce cell death in blood vessel grafts rescuing ~12 % of the endothelial cells that would otherwise die.

This technology was also applied to hepatocytes exposed to freezing/thawing resulting in a strong reduction of the dead cell mass.

Similarly, application of this approach to cumulus-oocyte complexes (COCs) exposed to vitrification/warming demonstrated reduced cell death of cumulus cells, diminished shrinkage and plasma membrane abnormalities of oocytes, increased fertilization success and even speed-up the cell cleavage to 2-4 cell stage. Recent evidence furthermore indicates that the approach significantly improves embryo development following vitrification of blastocysts.

Experiments are now running to explore gap junctions/hemichannel inhibition to improve heart and uterus function exposed to cold ischemia during transfer/transportation from donor to recipient.

technology opportunity

Applications

Improved solutions and methods for cryopreservation of different cell types (o.a. hepatocytes, COCs, stemcells,…), blood vessel grafts and whole organs during transportation from donor to recipient.

Advantages

- Gap26/27 are short (11-13 amino acid) peptides, i.e. chemically well-defined products which can be manufactured cost-effectively
- State of the art in cryopreservation is adding DMSO to the medium, a compound which is by itself cytotoxic for many tissues and cells. Using the new technology, DMSO may be diminished out omitted.
- Inclusion of Gap26/27 or related peptides may prevent part of the cell death and functional disturbances related to cryo-injury and/or DMSO

Status of development

1. tissues – blood vessels: Immuno-staining demonstrated the presence of the major vascular connexins, Cx37, Cx40 and Cx43 in the intimal and medial layers of human cryopreserved blood vessels using TUNEL stainings. We demonstrated that the connexin inhibitor peptide Gap27 reduced cell death of smooth muscle cells by 70% in saphenous veins and femoral arteries and reduced endothelial cell death by 50% in saphenous veins and by 30% in femoral arteries **(Figure 1)**

2. various cell types:
   Hepatocytes: hepatocyte adhesion on collagen type I is 67% better with Gap27 during freezing/thawing process, compared to controls
   Oocyte vitrification (work in progress) in cumulus-oocyte complexes (COCs): strong improvements were seen on cell death in the cumulus cell mass and fertilization success was promoted **(Figure 2)**
   Embryo development: Gap26 inclusion in the medium used for vitrification of blastocysts (an early embryonic stage after fertilization) strongly improves 'hatching'. Hatching is the process of zona pellucida disruption and decomposition such that the embryo can attach to the uterus wall for further development **(Figure 3)**.

Partnership

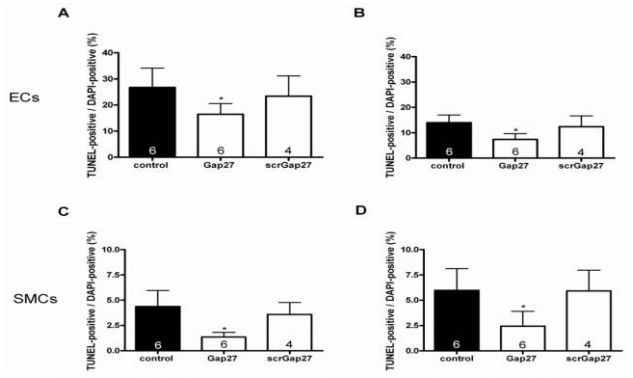
Ghent University is looking for a commercial partner who is interested to develop cryopreservation solutions containing connexin channel inhibitors. We are also interested in research collaborations with this partner.

technology opportunity

Intellectual property

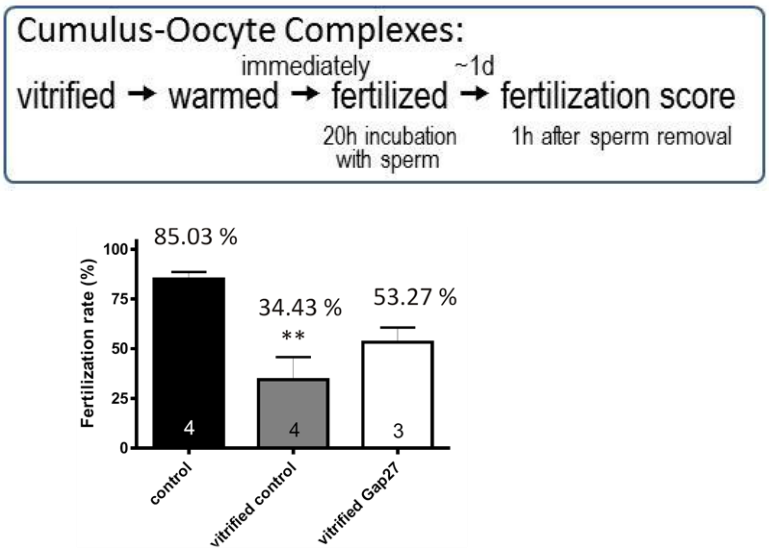
A European patent application was filed as a priority application on **19 December 2012** . The latter application has been extended into the PCT phase and has been recently nationalized in Europe, the US and South-Africa.

Figure 1



Gap27 reduces apoptotic cell death in cryopreserved human blood vessels – summary graph of average data. Apoptotic cell death in endothelial cells (EC) (A, B) and smooth muscle cells (SMC) (C, D) of femoral arteries (A, C) and saphenous veins (B, D). The number of TUNEL positive cells was significantly reduced by Gap27 in ECs and SMCs. Gap27 with a scrambled peptide sequence had no significant effects on cell death counts. The numbers of vessels are indicated in the bars. * P < 0.05

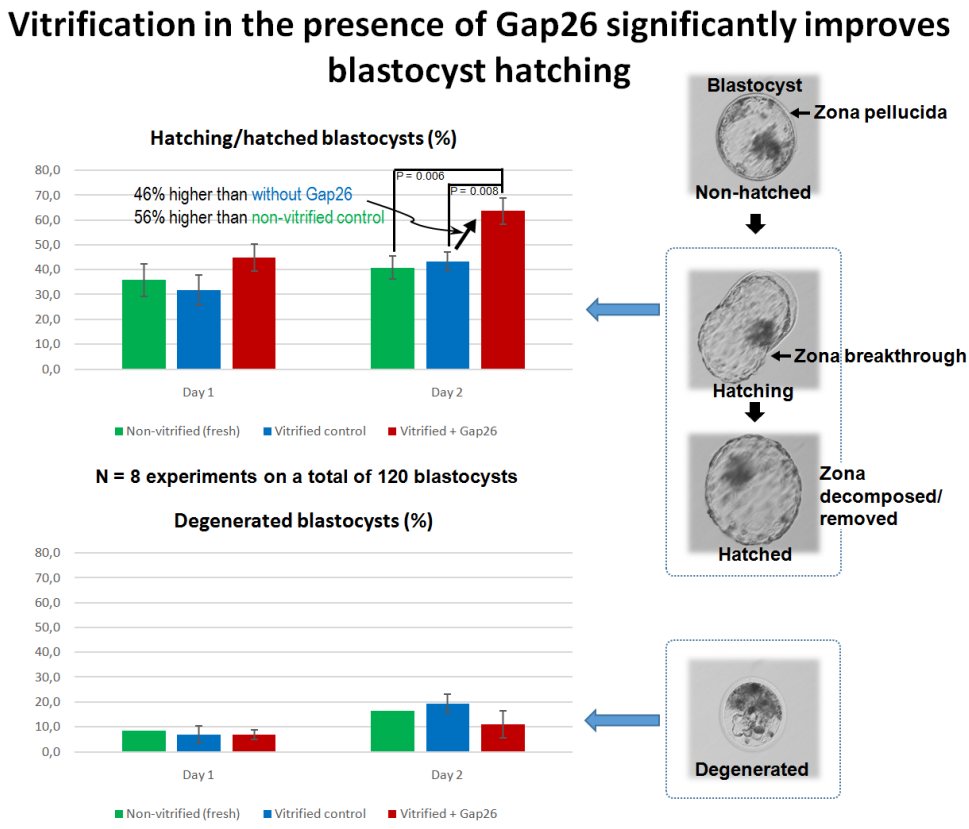
Figure 2



Gap27 tends to increase fertilization success using the process steps as shown.
As a control, COCs not exposed to vitrification/warming was used.
(Non-published results)

technology opportunity

Figure 3



References

Bol M., et al. Inhibiting Connexin Channels Protects Against Cryopreservation-induced Cell death in Human Blood Vessels. Eur. J. Vas. Endovasc. Surgery, Vol 45(4), 382-390, 2012

Decrock E, Vinken M, De Vuyst E, Krysko DV, D'Herde K, Vanhaecke T, Vandenabeele P, Rogiers V, Leybaert L. Connexin-related signaling in cell death: To live or let die? Cell Death Differ. 2009;16:524-536

Contact

Lieve Nuytinck, PhD
Valorisation Advisor
Bimetra
Ghent University Hospital, 3K3
De Pintelaan 185
9000 Gent, Belgium
tel. + 32 9 332 05 32
Lieve.Nuytinck@UGent.be

Piet De Vos, PhD
Licensing Manager
Ghent University
Technology Transfer
Bollebergen 2B
9052 Gent, Belgium
Tel. +32 9 264 7961
Piet.Devos@UGent.be

Are you interested in more technology offers from Ghent University? Check out www.techtransfer.ugent.be