Biochemically Equivalent Substitutive Technology for Stem Cells

(BEST-SC) - an EU FP7 project (2009 – 2012)

Workpackage 1.5: OPTIMIZATION OF HUMAN ES CELLS (hESCs) CRYOPRESERVATION.

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Backgrounds & Aims

Efficient recovery of biologically safe and undifferentiated hESCs is a major challenge after cryobanking, especially when their use for human therapy is foreseen. One drawback of actual cryopreservation protocols is the unavoidable direct contact of the cells with liquid nitrogen (LN2), due to cooling-induced vacuum. Therefore cells can be exposed to heavy metals, bacteria, viruses or fungi during cooling and storage. We describe a novel cryopreservation method based on aseptic vitrification[1] (no direct contact with LN2) using only chemically defined materials and media, and amenable to automation.

[1:] Vitrification is a cryopreservation method based on the conversion of a liquid into a glass-like state by an infinite enhancement of its viscosity and without formation of ice crystals.

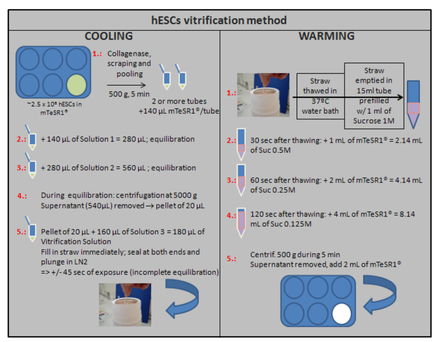
Experimental Design

A
- Replacement of undefined by chemically defined compounds in the vitrification medium:
  - hESC line used: RCM1 (Roslin Cells)
  - medium used: mTeSR1® from StemCell Technologies®
- Adaptation of procedures and materials to address requirements for automation and aseptic vitrification.
- Comparison between conventional slow freezing (SF) (non aseptic) in cryotubes and vitrification (V) in sealed straws.
  - morphometric studies, karyotyping, IF, teratomas

B Test of polysaccharides extracted from marine microorganisms as viscosity enhancers (=vitrification helper)

Results (1)

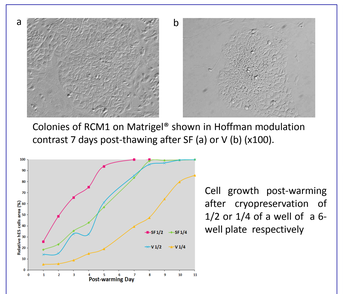
A
- Definition of an efficient vitrification protocol fitting to the constraints, and not affecting hESCs biology :



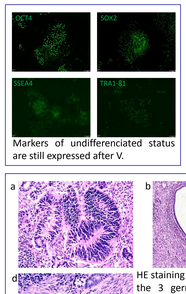
Aseptic vitrification in sealed straw is in accordance with EC and FDA directives on cell and tissue storage: requiring that cells are protected from any possible contamination during cooling and storage.

Results (2)

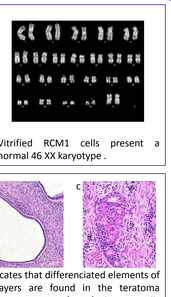
A



Colonies of RCM1 on Matrigel® shown in Hoffman modulation contrast 7 days post-thawing after SF (a) or V (b) (x100).



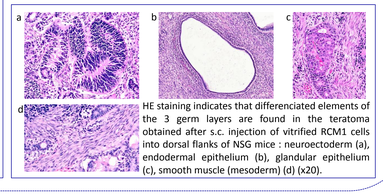
Markers of undifferenciated status are still expressed after V.



Vitrified RCM1 cells present a normal 46 XX karyotype .

Cell growth post-warming after cryopreservation of 1/2 or 1/4 of a well of a 6-well plate respectively

Post-warming morphometric analysis indicate that cell morphology is not affected by both cryopreservation methods. Even if cell number is affected by V, growth curves evolue parallely.



HE staining indicates that differenciated elements of the 3 germ layers are found in the teratoma obtained after s.c. injection of vitrified RCM1 cells into dorsal flanks of NSG mice : neuroectoderm (a), endodermal epithelium (b), glandular epithelium (c), smooth muscle (mesoderm) (d) (x20).

B Selection of two marine polysaccharides as candidate cryoprotectants

Discussion

- Aseptic vitrification of RCM1 hESCs is now possible in completely defined media. Because straws are directly immersed in LN2, this method does not require any specific and expensive material. To limit cell manipulations, our protocol implies stepwise addition and dilution of cryoprotectants before cooling and after warming, respectively.
- Vitrified RCM1 cells maintained their stem state after cooling & warming.
- Cell number reduction after vitrification can be explained by the conditions allowing aseptic and chemically defined conditions in the process of cryopreservation.
- Some marine polysaccharides have a positive effect on vitrification by reducing ice crystal formation.

Future

- Procedures and media for efficient, biochemically defined, aseptic and automatable vitrification have been set up, but can still be enhanced.
- The whole procedure should be tested by partners on various hES cell lines.
- Use of marine polysaccharides is still to be evaluated as substitutes for cryoprotectants or additives as vitrification helpers.