Nanobridges for 3D hESC Culture

KEY FEATURES

- **Enzyme and inhibitor free, 3D expansion of hESCs**
- **Thermoresponsive polymers for temperature-mediated cell clustering and dissociation.**
- **Polymers decorated with vitronectin to aid in natural hESC embryoid body formation, cell-cell and cell-extracellular matrix contact.**
- **Enables reproducible growth and >30-fold expansion in hESC number while maintaining pluripotency.**

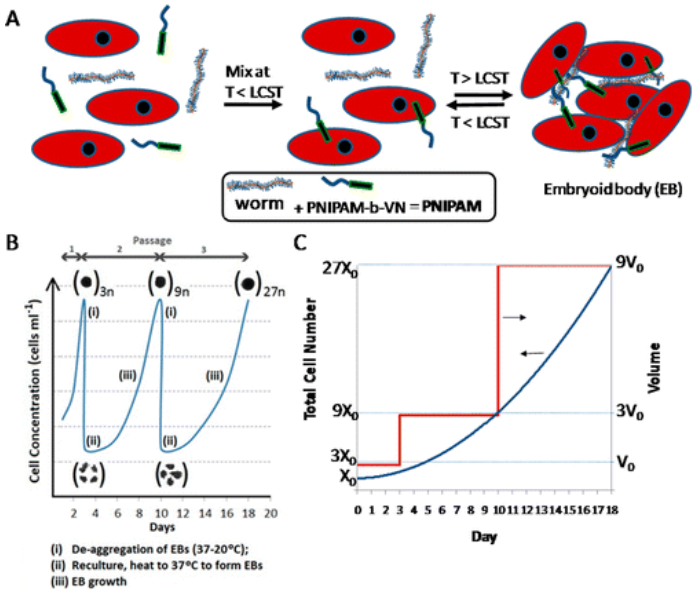

Figure 1: 3D expansion of hESC using thermoresponsive polymers. (A) Proposed mechanism for temperature reversible hESC 3D clustering (EBs) and release using PNIPAM-b-VN and polymer worms (the combination of which is denoted as PNIPAM). (B) Schematic representation for expansion and aggregation breakdown of hESC EBs using PNIPAM-b-VN and polymer worms. (C) Representation for hESC expansion with de-aggregation based on the process in graph B: total volume increases 3-fold on each passage.

The development of robust suspension cultures of human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) without the use of cell membrane disrupting enzymes or inhibitors remains a significant challenge for the production of cell sources for regenerative therapies, drug screening, tissue engineering, and the study of disease progression.

Currently, subculturing techniques generally rely on cell detachment and dissociation from other cells and from 2D or 3D surfaces with enzymatic (eg. Trypsin or acutase) or chemical treatment (eg. EDTA). Additionally, ROCK inhibitors or other small molecules are often used to significantly reduce cell apoptosis and improve self-renewal after each passage. These added enzymes or inhibitors concomitantly modify the cell surface structure and signalling pathways, thereby impacting future cell behaviour and fate.

To overcome this problem, researchers from the University of Queensland have developed a two-component polymer system for the enzyme free propagation of hESC in suspension culture.

1. The first component consists of long and flexible worms with a glassy polystyrene core coated with thermoresponsive polymer (poly(*N*-isopropylacrylamide (PNIPAM)) chains.
2. The second component consists of a block polymer of PNIPAM and a recombinant vitronectin subdomain (PNIPAM-b-VN).

The thermoresponsive polymer worms bridge hESCs, aggregating them into embryoid bodies (EBs) with good cell-cell contact and excellent nutrient penetration (see Fig1A). Whilst the vitronectin subdomain aids in hESC's natural ability to form EBs and satisfying inherent requirement for cell-cell and cell-extracellular matrix contact.

Once the cells grow and reach EB diameter where nutrient penetration becomes restricted, the EBs can be broken down into smaller aggregates by simply decreasing the temperature from 37°C to below the lower critical solution temperature (LCST) of PNIPAM (see Fig1A). These small aggregates can then be recultured and propagated at 37°C with the aid of the polymer worms (see Fig 1B and C).

In proof-of-principle studies the thermoresponsive nature of the polymer worms enabled a cyclical

dissociation / propagation of the cells. After only three cycles (over 18 days), there was a greater than 30-fold expansion in cell number while maintaining pluripotency.

Our technology provides a number of key advantages over the current gold-standard ECM mixtures:

1. **Reproducible results:** release media provides an animal-free, defined surface for hESC culture;
2. **Enzyme free:** cells can be detached using only a change in temperature and without the need for membrane-damaging enzymes;
3. **Tuneable**: easily modified to control the amount, combination and type of ECM bound to polymer bridges enabling a customisable platform for culturing specific cell lines;
4. **Mimics biological conditions:** utilises natural tendency of hESCs to form EBs but can be dissociated once nutrient penetration becomes limiting; and
5. **Low cost**: the system is low-cost and ideal for scale-up

Intellectual Property

The thermoresponsive nanobridge technology is at PCT stage (priority date of the 7th of June 2012).

Commercialisation Opportunities

We are seeking licensing or research collaboration partners to further develop this technology for 3D cell culturing applications.

Publication

Chen, X., Prowse, A. B., Jia, Z., Tellier, H., Munro, T. P., Gray, P. P., Monteiro, M. J. (2014). Thermoresponsive Worms for Expansion and Release of Human Embryonic Stem Cells. *Biomacromolecules*. **15**, 844-855.

RESEARCH LEADERS

The University of Queensland's
Professor Peter Gray
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Professor Gray's main research interests are in production of biopharmaceuticals by mammalian cell cultures. His research group works with an extensive network of international research groups and corporations. He is a Fellow of the Australian Academy of Technological Sciences and Engineering, the Australian Institute of Company Directors, the Institution of Engineers Australia, and was awarded the Centenary Medal by the Australian Government (2003). Professor Gray serves on the boards of several companies and research organisations both in Australia and overseas, and is active on a number of government committees in the areas of pharmaceuticals, education and training.

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Professor Michael Monteiro
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Professor Monteiro has established an international reputation in the field of 'living' radical polymerization to create complex polymer architectures. He is now building designer polymers for various biomedical applications, including vaccines, drug delivery and stem cells. He is dedicated to translating research into commercial outcomes, with 7 PCT and provisional patents since 2005.

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