EUFP7 BEST Stem Cells project

The BEST-Stem Cells consortium's goal was to discover and validate knowledge and reagents leading to the development of Biochemically Equivalent Substitutive Technology to improve the safety and efficacy of human pluripotent and multipotent stem cell culture by reducing or eliminating current dependence on animal cell and tissue derived biological reagents (i.e. extracellular matrix, blood serum/serum fractions). It involved 8 academic and commercial partners across 5 EU member states at the: University of Edinburgh (UEDIN), Roslin Cells (RC) and Glycomar (GLYCO) in the UK; INSERM and CNRS in France; University of Bonn (UBONN) in Germany; Bioneer in Denmark, and University of Liege (ULg) in Belgium. Its principle objectives were:
1) Discovery and validation of chemical polymer substrates for support of pluripotent human embryonic stem cells (hESC) and multipotent mesenchymal stem cell (hMSC) growth (WP1.1 - UEDIN)

2) Discovery and validation of chemical small molecules to substitute or reduce current requirements for protein growth factors and serum supplements for growth of hESC and hMSC, respectively (WP1.2 – UEDIN).

3) Develop recombinant high-affinity and/or membrane permeant proteins affecting hESC survival and growth and validate the significance of the pathways being targeted (WP 1.3 & 1.4 – CNRS, UBONN, INSERM, Bioneer).

4) Develop an aseptic serum free cryopreservation protocol for hESC and validate non-vertebrate of origin cryoprotective agents (WP1.5- ULg, Glycomar).

5) Provision of training in research quality assurance to support future translation to Good Manufacturing Practice (WP0.2 – Roslin Cells).

6) Public dissemination of BEST-Stem Cell consortium principles and outputs (WP0.1 – All).

Polymer substrates
UEDIN discovered thermomodulatable chemically defined polymer hydrogels supporting hESC and hMSC growth as high-affinity alternatives to complex extracellular matrices or uncoated plastic, respectively. These provide the added advantage of gentle enzyme free cell dissociation for passaging cells between vessels. The former was validated in a biologically defined serum-free medium using multiple hESC lines approved for use in this project (Zhang et al., 2013; Nat Commun 4:1335) and has been commercially licenced. UEDIN also developed a high density polymer array for cell screening providing a ten-fold improvement in screening capacity.

Small molecules
UEDIN discovered multiple small chemical molecules that substitute for the absence of either or both basic Fibroblast Growth Factor and Transforming Growth Factor-beta in short term growth of hESC. The best characterized of these affected calcium signaling (Ermakov et al., 2012, Stem Cell Research 9: 171). The project also identified promoting growth of hMSC or increased expression of a traditional hMSC marker, STRO-1. Additionally, a side project supported by matching resources validated an affinity targetable controlled release approach to delivery of growth factors using a biodegradable nanoparticle platform also validated for delivery of small molecules (Corradetti et al., 2012, Biomaterials 33:6634). This reduces the requirement for costly growth factors by a factor of over 10,000.

Recombinant proteins and target significance
CNRS and *Bioneer* discovered toxic aspects and poor affinity of its recombinant protein probes respectively. However, the former provides novel tools to selective ablate undifferentiated human pluripotent stem cells. This has potential value in the context of manufacturing hESC-derived cell products for therapeutic application as it provides a means to remove cells with the potential to form a tumour should they contaminate the final product. *INSERM* experimentally validated alternative signaling pathways to mediate maintenance of a

more naïve state of pluripotency in undifferentiated hESC with important implications for enhancing the properties of these cells. *UBONN* also validated the capacity of a recombinant membrane permeant version of pluripotency associated transcription factor Nanog from the mouse to maintain hESC growth and pluripotency. They also demonstrated this protein could be used to enhance reprogramming of adult cells into an embryonic stem cell like state of pluripotency. In partnership with *Bioneer*, membrane permeant human Nanog and other pluripotency associated proteins have been produced.

Cryopreservation and non-vertebrate cryoprotectants
ULG developed an aseptic, serum-free biologically defined method for rapid freezing (ie. Vitrification) of hESC and with *GLYCO* identified two new microalgal polysachharides as cryoprotectant alternatives to serum and serum albumin in vitrification. These were demonstrated to not be toxic in established mouse embryo and embryonic stem cell assays.

Promotion of research quality assurance
RC provided questionnaire and partner site-specific assessment and training in methods and standards for ensuring the quality and reproducibility of research practices. Training included presentations and practical workshops at annual consortium meetings. As a focused case study, research underpinning the discovery of a high affinity thermomodulatable hydrogel supporting hESC growth was subject to a detailed GAP analysis of records and requirements to achieve a Good Manufacturing Practice level of research compliance.

Public dissemination
The public launch of the project coincided with the first General Assembly meeting held in Lyon, France on 29th & 30th October 2009. The launch was communicated by a press release which received regional press coverage in the UK and France. The public launch of the project also coincided with the establishment of the project website: **www.beststemcells.ed.ac.uk.** In the course of the project partners communicated ongoing research progress at international and national meetings, invited lectures and public forums. Press releases were issued in association with high profile accomplishments, most notably the communication of the hESC supportive thermomodulatable hydrogels. Lastly the consortium held a public dissemination meeting in Edinburgh on the 22nd October, 2012, attended by over 60 participants at which partners presented project outputs and expertise together with invited national and international scientists presenting complementary research in keynote addresses.

Socio-economic impact of project
The BEST-Stem Cells project has yielded new and improved technology for the cultivation of human stem cells of embryonic and adult origin. It has yielded publications in high impact academic journals and commercial products that have already been licenced or are in the process of being filed as new intellectual property. These products contribute to the strength of the biotechnology sector in Europe. Their utilisation in regenerative medical programs in the future should contribute to the safety and efficacy of human stem cell derived cell products and thus health and welfare promotion.

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