REPRODUCTION
REVIEW

Focus on Stem Cells

The road to providing human embryo stem cells for therapeutic use: the UK experience

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Abstract

Harnessing the unparalleled properties of human embryo stem cells (hESCs) for the therapeutic treatment of disease and injury will require a convergence of scientific developments with regulatory standards. In the case of the latter, it is especially critical that standards for clinically assisted reproduction be harmonized with those governing human cell and tissue transplantation, most notably with respect to procurement, donation, testing, processing, preservation, storage and distribution of cells. In the UK, existing infrastructure to address these considerations is undergoing extensive reorganization to keep pace with evolving European Union standards. The present best paradigm for defining standards for the therapeutic use of embryo-derived stem cells is experience with adult haematopoietic stem cells (HSC). However, compared with adult-derived stem cell, the origin of embryo-derived stem cells from limiting quantities of tissue and their absolute dependence on *in vitro* culture to realise their therapeutic potential, makes optimization of their isolation and cultivation of even greater importance. Most notable is the requirement to create animal cell product-free culture environments to reduce the risk of cross-specific disease transmission. In the present paper, we review present and emerging standards in the isolation and banking of human embryo-derived stem cells for therapeutic use in the UK and international progress in the development of defined culture systems for this purpose.
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Introduction

Stem cells (SCs) are primitive cells with a regulated capacity for self-renewal and multi-lineage proliferation and differentiation (Loeffler & Potten 1997), precise control of which *in vivo* is essential for development and tissue renewal. In this context, the manipulation, isolation and transplantation of these cells or their derivatives is widely regarded as the next frontier in curative medicine. While stem cells can be sourced from a range of embryonic to adult tissues, it remains true that the proliferative and differentiative properties of this type of cell becomes increasingly restricted with tissue development, with practical consequences on their therapeutic utility in autologous (i.e. using one's own cells) or allogeneic (i.e. using donated cells) transplantation. Thus, although there will always likely be contexts where adult-derived cells provide the greatest therapeutic advantage for the treatment of a specific disease or injury, embryo-derived stem cells offer excellent long-term prospects for the development of curative therapies for a range of conditions, which presently cannot or are struggling to be met by transplantation of adult or fetal-derived tissues or alternative therapies. This includes nerve replacement or repair in spinal cord injury, provision of dopaminergic neurons for the treatment of Parkinson's disease and insulin-producing cells for the treatment of diabetes. These have been exemplified in animal models using mouse embryo stem cells or their derivatives (McDonald *et al.* 1999, Lie *et al.* 2000, Soria *et al.* 2000, Bjorklund *et al.* 2002, Kim *et al.* 2002). Similarly, there is now emerging evidence of benefit following transplantation of human embryonic stem cell derived neural progenitors and cardiomyocytes into animal models of Parkinson's disease and myocardial injury respectively (Ben-Hur *et al.* 2004, Kofidis *et al.* 2006).

Realizing therapeutic aspirations for human embryo stem cells (hESCs) requires convergence of the capacity to comply with existing and emerging standards for the

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transplantation of cells and tissues with the evolution of scientific and technical capability to preserve the properties of these cells whilst minimizing the risk of introducing unknown pathogens. Since the unique capacity of hESC for renewal and expansion offers realistic prospects for their broad usage, standards established for the procurement and processing of these cells today within individual nations should ideally be sufficiently comprehensive to accommodate their global acceptance and utility in the future. In this paper, our focus is on the progress of the UK to achieve the safe and quality-assured evaluation of hESCs in the clinic. Within this context, we discuss underpinning fundamental principles governing donation, procurement, testing, processing, preservation, storage and distribution of embryos and cells based on experience in adult cell and tissue transplantation and European Union (EU) Directives. Given the absolute dependence of hESCs on cell culture, we also address international progress in the creation of defined and animal cell product-free culture environments to isolate hESCs for cell banking. While definition and evolution of high-throughput systems for hESC expansion and differentiation are equally important to their ultimate therapeutic use, they are beyond the scope of this paper.

Existing and emerging EU/UK standards governing human cells and tissues

Globally there is widespread recognition of a need for an internationally unified framework to ensure high standards of quality and safety with respect to cell and tissue transplantation. Accordingly, in 2003, the World Health Organisation (WHO) acknowledged that the volume and complexity of activity in this arena needed to be taken into account by WHO guidelines (see report on access and safety in tissue and organ transplantation: WHO/HTP/EHT/T-2003.1; online at http://www.who.int/ethics/topics/en/madrid_report_final.pdf). Specifically, cited were:

- Poor levels of education, training and research in tissue banking globally.
- Inconsistent approaches to donor consent.
- Limited or non-existent evidence for efficacy of transplantation of some tissues.
- Unregulated commercialization.
- Inability to provide 'origin to destiny' traceability of tissues.
- Lack of harmonization of regulatory standards delivering high costs for tissue banks.
- Concern about self-sustainability of 'not-for-profit banks', and excessive income of 'for profit banks' in the context of altruistically donated human material.

While WHO recommendations to National Health Authorities are anticipated in late 2006, the intervening period has witnessed significant progress in defining and legislating improved standards by the Councils and Parliament of European Union, which in turn have been ratified or are in the process of being ratified by member states, including the UK. Specifically, in March 2004, EU directive 2004/23/EC was issued, followed in November by Royal assent of the Human Tissues Act (2004) in the UK. These have set new standards of quality and safety for the donation, procurement, testing and distribution of human tissues and cells. The first technical annex providing detailed requirements for donation, procurement and testing was published in February 2006 (directive 2006/17/EC) with the technical annex for coding, processing, preservation, storage and distribution presently in consultation in draft form (see Draft 29/03/2005). Copies of issued and draft EU directives are available online (http://europa.eu.int/). The scope of these directives encompasses blood, bone marrow, reproductive cells (i.e. eggs, sperm and embryos), cells derived from fetal and adult tissues, and embryo stem cells. The inclusion of reproductive (i.e. eggs, sperm and embryos) in this directive has generated the greatest concern amongst practitioners of assisted reproduction, who have questioned the relevance of standards normally associated with transplantation medicine. It is worth noting that autologous grafts of tissues or cells within the same surgical procedure, allogeneic transplantation of blood, blood products, and tissues or cells or animal origin are covered by other directives and recommendations (i.e. Directives 2001/83/EC & 2000/70/EC, 2002/98/EC and Recommendation 98/463/EC). Also excluded are the uses of human tissues for research, unless those tissues are used in clinical trials applied to the human body. In this context, it is possible that the non-reproductive use of embryonic cells was a contributing factor to their grouping with other cells in a single legislative document, namely EU directive 2004/23/EC.

To the credit of EU legislators and contributors involved in its drafting and consultation, Directive 2004/23/EC comprehensively identifies the multitude of factors that must be considered for quality-assured and safe therapies involving human cells. As summarized in Tables 1 and 2, it specifies provisions to be addressed by multiple parties within member states from designated authorities to tissue facilities, third parties and individuals. It not only allows for member states to impose more restrictive measures but also promotes unification of standards that ultimately benefit all participants. The implications of these provisions are significant with increased burdens of responsibility and costs to the public and private sectors and end beneficiary awaiting new treatments. While it is easy to envisage that the therapeutic use of cells in general or with hESC derivatives in specific could be achieved more expediently without these standards, the consequences associated with inconclusive or adverse results that could occur without having them cannot be overlooked. This is borne out by recent experience with gene therapy trials, or transmission of infectious

Table 1 Summary of Directive 2004/23/EC of the European Parliament and Commission on setting standards of quality and safety for the therapeutic use of human cells.

Consideration	Provisions to be met by member state, designated competent authorities, tissue facilities, third parties and persons
Implementation	Member state designation of responsible competent authority/authorities to regulate all forms of tissue banking activities
	Member state freedom to introduce more stringent provisions/prohibitions. (i.e. restrictions on tissue imports)
Procurement and testing[a]	Adherence to principle of voluntary unpaid donations
	Performed by appropriately trained and experienced personnel in accredited, designated, authorized or licensed premises
Facilities and processes	Accredited for purpose
	Operation within boundaries issued by competent authority
Inspections and controls	By competent authority of tissue establishments and by latter of third parties to confirm compliance, on regular basis not exceeding 2 years
	Institutional response capacity for serious adverse reaction or event
Traceability	From donor to recipient of all tissues and cells procured, processed, stored or distributed within member state
	Of all products and materials coming into contact with cells and tissues
	Implementation of a donor identification system assigning unique code to donation and associated products
	Fully traceable data storage for 30 years after clinical use
Import/export	By tissue establishments accredited for purpose by competent authorities
	Compliance of imported tissue with directive requirements
	Option of direct imports to the end clinical user stopped
Registry and reporting	Recording by tissue establishment of activities, types and quantities of tissues and/or cells procured, tested, preserved, processed, stored, distributed, or disposed and origin and destination of intended applications
	Annual public reporting of tissue establishment to competent authority
	Establishment of publicly accessible and EU linked register of tissue establishments
Adverse events and reactions	Member state system to report, investigate, register and transmit information pertinent to quality and safety of tissue and cells
	Responsibility of users and providers of human cells or tissue to report adverse events/reactions across chain
	Designation of responsibility for notification of competent authorities
	Delineation by tissue establishments of recall-procedures
Donor procurement, selection, consent, evaluation and confidentiality[a]	Non-profit procurement
	Voluntary and unpaid donations excluding reimbursement for expense and inconvenience
	Informed authorization by donor or family and documentation of donor testing
	Anonymity, security and quality control of donor and recipient identity and data collated within scope of directive, without prejudice to legislation in member state
Quality management (i.e. tissue establishments)	Implementation of quality systems of documentation covering Standard Operating Procedures, Guidelines, Training and Reference Manuals, Reporting Forms, Donor Records, Information on the Final Destination of Tissues or Cells
	Inspection by competent authorities
	Designation, qualification and duties of responsible person and personnel
	All operations covered by the principles of GMP
Tissue and cell reception[a]	Testing, selection, acceptance and identification of tissues or cells received by tissue establishment
	Verification of packaging
	Capacity to quarantine tissues until donor testing requirements fulfilled
Tissue and cell processing	Validation by tissue establishment of working environment, equipment, and process design
	Disposal and decontamination procedures
Tissue and cell storage, labelling and distribution	Documented and controlled packaging and storage
	Agreements/procedures for tissue distribution and transfer in the event of facility closure
Third party relationships	Written agreements with third party suppliers of goods or services
	Evaluation and selection by tissue of third party suppliers of goods or services
Information coding	Establishment of national and EU systems for tissue identification
Penalties	Establishment by member state of penalties of infringements of national provisions that are effective, proportionate and dissuasive
Technical consultations	Requirements for licensing of tissue establishments, procurement processing, storage and distribution of tissues and cells, quality system including training, donor selection criteria and testing

[a]Elaborated further in Commission Directive 2006/17/EC, summarized in Table 2.

pathogens via blood products or tissues (Gode & Bhide 1988, Simonds *et al.* 1992, MacLaren *et al.* 2005, Ludlam *et al.* 2006). While some of the complications that have been experienced could arguably be described as unforeseen on the basis of available knowledge, this in itself does not substantiate less rigorous standards. Rather, it emphasizes the need for the forethought that such standards invoke.

Table 2 Summary of Directive 2006/17/EC selection criteria and laboratory tests for donors of reproductive cells for non-direct use (i.e. gamete donation or stem cell derivation).

Consideration	Provisions to be met by member state, designated competent authorities, tissue facilities, third parties and persons
Donation	Clinical documentation of donation justification and safety to recipients Based on age, health and medical history provided by questionnaire and interview with qualified and trained healthcare professional, to screen out persons whose donation represents health risk to others or themselves
Donor testing	Testing of donor blood serum at time of donation •HIV 1 and 2 (anti-HIV-1, 2 or nucleic acid amplification) •Hepatitis B (BbsAg, anti-HBc or nucleic acid amplification) •Hepatitis C (anti-HCV-Ab or nucleic acid amplification) •Syphilis (*Treponema pallidum*) •HTLV-I (if donor is high risk) Additional testing (i.e. RH D, malaria, CMV, *T. cruzi*) depending on donor travel and exposure history Pre-testing storage of gametes or embryos when testing positive or unavailable Deference to member state rules governing donation from positive donors Genetic screening for autosomal recessive genes known to be prevalent, according to international scientific evidence, in the donor's ethnic background in accordance with member state requirements

Changes in EU and UK legislation have prompted restructuring of non-departmental regulatory bodies within the UK Department of Health, for purposes of harmonizing oversight of what previously were regarded as distinct domains encompassing assisted reproduction and transplantation. Since 1991, the Human Fertilisation and Embryology Authority (HFEA) has licensed and monitored UK clinics offering assisted conception, as well as all UK-based research into human embryos and storage of eggs, sperm and embryos. The HFEA has also had the duty to review all new developments in treatment and research, to engage in public debate and to advise ministers. By contrast, in 2001, the Medicines Control Agency acquired responsibility for accreditation of cell and tissue banks processing and storing human tissues for therapeutic use, and became the Medicines Healthcare-products Regulatory Agency (MHRA). With effect from 7 April 2006, a new Department of Health body, the Human Tissue Authority (HTA) came into force. Together with the HFEA, it will serve as the competent authority for the EU Cells and Tissues Directives and the UK Human Tissue Act (2004). The HTA will be responsible for licensing and regulating tissue establishments and the storage of human material for education, training and research and also anatomical and post-mortem examination. The guiding principles used for such activities will be defined by codes of practice. These will cover issues, such as consent, definition of death, control of existing holdings of human tissues, removal, import and export of human tissue and organs and storage of human tissue with the exception of gametes and embryos, which will remain regulated by the HFEA. According to the Secretary of Health's review of Department of Health's Arm's Length Regulatory Bodies (20 May 2004) the HTA and HFEA will be merged by 2008 to create a new authority, the Regulatory Authority for Tissues and Embryos (RATE).

HESC lines derived in the UK under licence from the HFEA must be deposited in the UK Stem Cell Bank (UKSCB), hosted at the National Institute for Biological Standards and Controls (NIBSC). Established in 2002, this government-backed facility was the first of its kind to be established with the aim of providing a quality-assured repository for hESCs for research and therapeutic purposes (Healy *et al.* 2005). The bank was accredited by the MHRA in June 2004. At the time of writing, 24 lines had been approved for deposition in the bank, of which 5 had been deposited (Glyn Stacey, Personal Communication). Five of these had been derived in the UK. While at present all these lines are regarded as 'research grade' owing to the nature of facilities and procedures in place at the site and time of their derivation, multiple groups across the UK including ourselves are actively engaged in upgrading procedures and facilities designated for this purpose to fulfil existing and emerging regulatory standards for clinical use. International competition to attain these standards is intense, and recently one multinational effort led by the Singapore-based biotechnology company Embryo Stem Cell International, announced the derivation of two new hESC lines in facilities operating to present Good Manufacturing Practice (GMP) as defined in Singapore (for further information, see www.escellinternational.com/).

As embryologists and scientists unaccustomed to thinking in manufacturing terms, the learning curve has been steep. Key challenges have included: (1) the anticipation and implementation of elevated quality standards that are still being defined, (2) addressing the needs of transplantation medicine without compromising assisted reproduction services and (3) securing the necessary resources to achieve elevated standards for a therapeutic objective whose benefits remain speculative. The funding breach to support the latter remains an outstanding issue, with many efforts across the UK moving slowly forward with varied and piece-meal support from either the Medical Research Council, Charitable trusts, Universities and/or regional economic development agencies. Although private–public funding partnerships to support the general translation of stem cell research to the clinic have been discussed, this support has yet to be realized.

Lessons from haematopoietic stem cell transplantation

Current and emerging standards and perspectives on tissue and cell therapies as embodied in the EU directive have culminated from decades of experience in adult organ and tissue transplantation. Best practice which has developed from this experience is defined by standards established by the Joint Accrediation Committee for the International Society for Cellular Therapy (ISCT) and the European Group for Blood and Marrow Transplantation (EBMT), or JACIE (http://www.jacie.org). Arguably, the best present example for the use of cells in therapy is the use of haematopoietic stem cells (HSC) and progenitors for the treatment of blood cell diseases, such as acute and chronic leukaemia, Hodgkin's disease, non-Hodgkin's lymphoma, severe aplastic anaemia and systemic autoimmune disease (e.g. severe rheumatoid arthritis or systemic lupus erythematosus). It is important to note that these treatments have largely relied on primary cell preparations and are not involved in the type of extensive cell culture that hESCs will ultimately invoke. However, this experience is valuable for interpreting and anticipating emerging standards, as noted below.

Donor consent, selection and screening

While the original scope of UK Human Fertilisation and Embryology Act (1990) and resulting HFEA code of practice addressed patient consent for the use of gametes and embryos in IVF treatment, storage by cryopreservation, disposal and the licensing of research in human reproduction and development, the production of a tangible asset such as stem cells has raised new issues in the consenting process, the impact of which are still being addressed. Based on experience with consenting for blood products, including HSCs, there is now a greater recognition for a need to provide information and obtain instruction regarding disposal of tissues, their commercial exploitation and feedback of information relevant to the health status of the donor. As noted in the first EU Cells and Tissues technical annex (summarized in Table 2), more rigorous selection and microbiological screening of gamete donors is required, consistent with the present requirements of the UK Blood Services & NIBSC Guidelines for the Blood Transfusion Services (the Red Book). Donors will be required to complete a health and medical history questionnaire. Furthermore, on the basis of family history, the donor's first degree relatives may have to be free of non-trivial autosomal dominant or X-linked disorders with age of onset extending beyond the age of the donor (such as Huntingdon's chorea), autosomal dominant disorders with reduced penetrance (such as Marfan's syndrome or Alport's disease) and autosomal recessive disorders with high prevalence in the general population (such as cystic fibrosis, Factor V Leiden and haemochromatosis). A family history of familial disease with a major genetic component (such as cleft lip, spina bifida or congenital malformation), non-trivial Mendelian disorder (such as haemophilia, hereditary hypercholesterolaemia or neurofibromatosis), familial disease with a reliably indicated major genetic component (such as juvenile diabetes mellitus, severe hypertension or rheumatoid arthritis) or of chromosomal rearrangement may also preclude contribution to therapeutic grade cell lines. Consideration will also need to be given as to the extent to which the potential for later development of disease in donors of embryos might impact upon the clinical safety or utility of a cellular therapeutic product. The significance of this point is underscored by stipulations in the EU cells and tissues directive for maintenance of full traceability of tissues from donor(s) to recipient patient(s) for at least 30 years following the last clinical use of any therapeutic product derived from those cells.

In recognition of the need to improve and consolidate provision of information to assisted conception patients and consenting for embryo donation for stem cell research, the MRC has funded the co-ordination of this effort across multiple centres. This has resulted in the formation of the human Embryonic Stem Cell Co-ordinators group (hESCCO; http://ukstemcell.net). Till date, this group has worked to standardize donor information and consent forms and the consenting process in accordance with HFEA guidelines, and establish a secure database to provide solid evidence of public confidence in embryo donation for stem cell research, the latter in concert with social scientists.

With publication of EU directive 2006/17/EC, there is now more clarity on microbiological testing that is required of donors of cells or tissues (summarized in Table 2). With the exception of additional testing dependent on the donor's travel and exposure history, it is assuring that the present screening of third party donors of gametes for assisted reproduction of an infertile woman is consistent with these requirements. However, one nuance is an EU requirement for testing at the time of donation. In the context of embryo donation for stem cell research, this could be interpreted to imply repeat blood sampling of donors of the gametes resulting in that embryo closer to the time the embryos are created.

Facilities specification and operation

Considerable attention and concern has focused on the necessity to handle gametes and embryos in an *in vitro* fertilization clinical setting in the same manner as cells and tissues for transplantation. Most notable have been fears of incorporating provisions for the specification and operation of clean rooms described by EC Guide to GMP as contained in Rules and Guidance for Pharmaceutical Manufacturers and Distributors 2002 (Orange guide) and updated regularly in Eudralex (http://ec.europa.eu/enterprise/pharmaceuticals/eudralex/index.htm). Although it is clear that facilities for deriving hESCs need to operate to

these standards, it is less clear that this will be the case for IVF labs. Indeed, a recent consultation document issued by the HFEA on standards for assisted conception units (May 2006) has implied lesser requirements, such as operation in unclassified or lower classification of background air. The present draft of the EU Technical Annex (Draft 29/03/2005) makes provisions for less stringent environments 'where it is demonstrated that the mode and route of application of the tissue or cell to the recipient implies a significantly lower risk of bacterial or fungal infection than cell transplantation (e.g. insemination)'. Until clarity is achieved on this point, IVF centres across the UK supporting hESC derivation have taken a variety of approaches from focusing on the introduction of quality systems of operation to the clean room installation. The former is an absolute requirement of the EU directive, on which practically all other considerations are dependent (see Table 1). Recognition of the importance of quality systems in assisted reproduction treatment in Europe is not new, but until recently has been implemented entirely voluntarily (Wikland & Sjoblom 2000).

Irrespective of lab specification, it is clear that settings supporting IVF and hESC derivation will require quality systems comparable with those required by the MHRA for tissue establishments. This will entail a fully audited quality management system, such as the ISO9000 system (http://www.iso.org/iso/en/iso9000-14000/index.html) or other systems meeting the requirements of the HTA. These would feature:

- Pre-commissioning validation and regular monitoring of equipment and facility performance.
- Validation and review of all measurable processing parameters against defined specifications.
- An extensive documentation system covering management policies, staff training, operating procedures and records.
- Formal contracts with all third party suppliers of materials and services.

Based on the experience provided by the quality-assured production of blood cells and products, a number of other considerations are worth noting. These include:

- Failsafe and backup systems for critical equipment and storage.
- Data registry encompassing consent/authorization; donor history, examination and testing; and record of procurement. This would be fully auditable and password-protected in compliance with the Data Protection Act.
- Electronic systems to uniquely code and track each cell line. These should have inbuilt access controlled safety features, which would preclude inappropriate release of cells for clinical purposes e.g. if cell lines are bacteriologically contaminated, the viral markers from the original donors are reactive, the medical history is not acceptable, etc.

Evaluation of hESCs in clinical trials

Although the clinical evaluation of hESCs may be a few years away, future-proofing present standards is necessary. Many of the same factors will need to be defined and optimized as presently met by transplantation of fetal and adult cells and tissues. This includes definition of the optimum cell lineage for transplantation, the route and timing of administration, 'dosage' of cells and whether single or multiple administrations are required, definition of clinical subsets of disease, interplay of other medications, etc. Appropriate investigatory and clinical end points need to be defined, as well as the necessity and ethical appropriateness of randomizing or blinding the study (Cesaro 2004, Dunnett & Rosner 2004). The extensive dependence of hESCs on culture systems to expand and differentiate them, presently achieved by reliance on research grade or animal sourced reagents, arguably makes this a greater issue for the therapeutic use of these cells than for cells sourced from other tissues. Reagents need to be quality assured and traceable, and exposure to animal cell products could theoretically brand a stem cell product as a xenobiotic, impacting on the patient follow-up in a clinical trials.

At present, clinical trials of experimental therapeutics need to be carried out in compliance with: (1) the EU Directive on clinical trials (2001/20/EC) and (2) the Research Governance Framework for Health and Community Care. These cover the responsibilities of the Sponsor, approval of the trial by the relevant competent authority (MHRA) and Ethics Committee(s) and the conduct of the study according to the principles of Good Clinical Practice. In addition, any clinical trial must be reviewed and monitored by an independent trial monitoring committee. Any experimental therapeutic product would be manufactured in compliance with GMP standards and systems would need to be in place for the recording and investigation of any adverse events occurring during the study and ongoing pharmacovigilance. Written informed consent would be obtained for all participants before commencement on the study and specific documented procedures would be in place for the handling of donor and patient trial samples and archive material. Patient and donor related data would be handled in accordance with the Data Protection Act 1998 and strict confidentiality would be maintained. The issue of compensation in the event of non-negligent harm would be addressed in line with Ethics Committee requirements and appropriate indemnity established.

Evolution of defined culture systems supporting hESC isolation

Whilst the emphasis of existing and emerging regulatory standards is on quality assurance and traceability of all donations and procedures, a critical parameter in optimizing the safety of hESCs for therapy is improvement of the culture systems supporting them. Ultimately, these systems need to produce sufficient quantities of functionally normal cells that are tolerable, or can be made tolerable to the recipient. Cells produced by these systems also need to be free of, or have minimal risk of contaminants that could harm the individual, or result in the transmission of disease to the general population. A significant concern for the latter is the potential for cross-specific or zoonotic transmission of unknown pathogens associated with exposure of cells in culture to animal tissues or undefined tissue products. Historically, zoonoses have accounted for some of the most virulent diseases known to man, including anthrax, hantavirus, Q-fever, and more recently variant Creutzfeldt-Jakob disease (CJD) arising from bovine spongioform encephalopathy (Weiss 2003, Parker *et al.* 2006). Thus a critical first step for the communal safety of stem cell technology is the evolution of completely humanized and animal product-free culture systems.

Eight years on from the first successful hESC derivation by Thomson *et al.* (1998) all successful efforts to isolate and expand these cells have continued to involve direct exposure to one or more undefined animal sourced blood or cell products. This includes the use of blood complement to 'immunosurgically' lyse blastocyst outer trophectodermal cells in order to recover embryo inner cell mass (ICM), mitotically inactivated mouse embryo fibroblasts (MEF) or MEF-derived extracts for embryo cell attachment and outgrowth, and supplementation of media with bovine serum or serum-derived products (Reubinoff *et al.* 2000, Lanzendorf *et al.* 2001, Hovatta *et al.* 2003, Mitalipova *et al.* 2003, Cowan *et al.* 2004, Heins *et al.* 2004, Klimanskaya *et al.* 2005, Ludwig *et al.* 2006). However, many studies have demonstrated the capacity to obviate direct exposure to one or more of these factors. Thus, at least two groups demonstrated hESC derivation from whole embryos without animal immune complement to isolate ICM (Heins *et al.* 2004, Simon *et al.* 2005). In place of MEFs, human fibroblasts derived from neonatal foreskin and adult placentas have also been exemplified to serve as feeders for the outgrowth of embryo ICM and whole blastocysts respectively (Hovatta *et al.* 2003, Simon *et al.* 2005). The prospect of feeder-free isolation has also been exemplified by growth of embryo ICM on extracellular matrix (ECM) extracted from MEFs (Klimanskaya *et al.* 2005) and most recently on a combined preparation of human sourced collagen IV, fibronectin, laminin and vitronectin (Ludwig *et al.* 2006). In our own recent efforts, we have isolated six new hESC lines from whole blastocysts, without exposure to animal immune complement, on an ECM substrate of purified human laminin and transitional reliance on mitotically inactivated human fibroblast feeder cells. Most notably, one of these lines was isolated using a serum-free medium (SFM) containing only human sourced and recombinant proteins (De Sousa *et al.* 2005, Fletcher *et al.* 2006). To our knowledge, this is the first exemplification of a new hESC line derived without direct exposure to any animal cell product, and as such represents another significant sequential step in the generation of conditions suitable for the derivation of therapeutic grade stem cells with reduced risk for transmitting zoonotic pathogens.

In the absence of a detailed understanding of cell-culture requirements, serum, normally of bovine origin, is a common media supplement. It provides a range of growth factors capable of stimulating both growth and differentiation and is a valuable nutritional source of protein such as albumin, which has the added side benefit of facilitating cell attachment to substrates. Accordingly, almost all efforts to derive hESC till date have involved supplementation of minimal essential medium with bovine-sourced serum (Thomson *et al.* 1998, Reubinoff *et al.* 2000, Lanzendorf *et al.* 2001, Hovatta *et al.* 2003, Mitalipova *et al.* 2003, Pickering 2003, Stojkovic *et al.* 2004) or bovine serum replacement (bSR; marketed as KNOCKOUT SR, Invitrogen) (Cowan *et al.* 2004, De Sousa *et al.* 2005, Klimanskaya *et al.* 2005, Simon *et al.* 2005), of which albumin is a major component (Price *et al.* 1998). The SFM used in our own recent work was based on *X vivo* 10 (Cambrex, Wokingham Berkshire, UK), a product marketed for human therapeutic applications involving haematopoietic cells, to which basic Fibroblast Growth Factor (bFGF) alone was added (De Sousa *et al.* 2005, Fletcher *et al.* 2006). This was based on its capacity to support hESC self-renewal in SFM as well as bSR supplemented media under feeder-free culture conditions (Xu *et al.* 2001, Li *et al.* 2005). Although the base composition of this media is proprietary, human insulin, transferrin and albumin are specified in available product information. Recently, Ludwig *et al.* (2006) published details of their own animal protein-free media supporting hESC derivation. In addition to containing a broad range of inorganic salts, trace minerals, energy substrates, lipids, amino acids and vitamins, this media contains a number of human growth factors and proteins, including bFGF, TGF-β1, GABA, pipecolic acid, glutathione, insulin, transferrin and albumin. Previous studies developing feeder-free culture environments to support established hESC lines have suggested that TGFβ1 and leukaemia inhibitory factor (LIF), and the bone morphogenetic protein (BMP) antagonist Noggin, synergize with bFGF to suppress differentiation and sustain self-renewal in non-conditioned medium supplemented with bSR (Amit *et al.* 2004, Xu *et al.* 2005). How universal variations in culture environment and handling are for deriving and sustaining hESC lines remains to be formally

evaluated and could be dependent on genetic or epigenetic heterogeneity in the cell lines themselves. Another issue affecting the derivation and expansion of undifferentiated hESCs appears to be the requirement to maintain cell association during passaging. This is reflected initially by a requirement for manual dissection and freezing of cells at high density in order to ensure recovery of cells post-thawing. This is further reflected by poor cloning efficiencies of less than 1% of established lines on or off of feeders (Amit *et al.* 2000, 2004).

While our and others recent success (Fletcher *et al.* 2006, Ludwig *et al.* 2006) in isolating hESCs in media containing only human-sourced recombinant and purified proteins is significant, indirect exposure to animal-sourced tissues used in reagent preparation remains an outstanding safety issue for therapeutic objectives. An example of this is the fact that culture of human fibroblast feeders still normally involves exposure to Low Serum Growth Supplement (LSGS; Cambrex, Berkshire, UK) containing 2% (v/v) fetal bovine serum. Fortunately, this serum is sourced from transmissible spongioform encephalopathy (TSE)-free herds in New Zealand that have been certified by the MHRA and comparable health agencies through the world. However, there is of course no guarantee that this source does not carry other unknown pathogens. Similarly, procedures used by manufacturers in the purification of human proteins such as albumin or ECM proteins from tissues undoubtedly still utilize animal-sourced reagents (i.e. trypsin or collagenase), since these are the most commonly available to manufacturers of research grade material. The future production of therapeutic grade stem cells will thus necessitate reliance on therapeutic grade reagents, whose production may in turn necessitate implementing novel strategies for organic synthesis of molecules or the use of recombinant gene technology in evolutionarily distant species (i.e. plant and insect).

A perception of a prevalence of TSE in the UK in the form of CJD has been an issue which has resulted in US bans on the therapeutic use of blood cell products derived from prospective donors that have resided or been transfused in the UK (i.e. http://www.fda.gov.cber/gdlns/cjdvcjd/pdf). This has the potential to impact on the worldwide acceptance of UK-derived hESCs irrespective of their compliance with emerging UK and EU standards. However, according to recent WHO guidelines on Tissue Infectivity Distribution in transmissible spongioform encephalopathies (September 2005), no detectable TSE infectivity has been reported in reproductive tissues such as testis, prostate, semen, ovary, uterus, placental fluids, fetuses and embryos (http//www.who.int/bloodproducts/tse/WHO%20TSE%20Guidelines%20FINAL-22%20JuneupdatedNL.pdf). Thus, while this remains the case, there is no rationale for discriminating against UK-derived hESCs or products providing that any human or animal serum or serum products used in their culture are obtained from TSE-low risk or free sources.

Conclusion

To date, no human embryonic stem cell line has been derived in the UK that fulfils existing and emerging quality assurance requirements stipulated by EU directives and UK legislation. However, it is apparent that significant progress is being made on this objective across all fronts. Clarity on the regulatory requirements is gradually emerging with opportunities to anticipate undefined specifications existing provided that adequate resources are available to address physical and quality assurance requirements. Taking as a whole, UK efforts to update its regulatory oversight of cells and tissue transplantation, and the support that exists for the establishment and co-ordination of stem cell research, banking and clinical use, it is reasonable to conclude that the UK remains globally competitive to achieve therapeutic objectives. A fundamental distinction between the prospective therapeutic use of hESCs and present transplantation of adult cells and tissues is the absolute requirement of the former for quality-assured cell culture methodology. Although it is not essential that the first hESC lines produced in quality-assured facilities be isolated in animal cell product free conditions, recent progress, for example, by Ludwig *et al.* 2006 makes it conceivable that no direct exposure can be guaranteed. However, it may be sometime before all of the necessary therapeutic-grade reagents are available to eliminate the possibility of indirect exposure to animal sources of unknown pathogens.

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