"Stem Cells - From Bench to Bedside" - Neurological Application

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Introduction
The use of stem cell therapy in the treatment of central nervous system disorders is a natural extension of the recently developed concept of 'restorative neurology'. At the same time, the role of neurosurgery in the treatment of neurological diseases is evolving from disease modification and cerebral protection to regenerative therapy. The latter is concerned with the restoration of lost neuronal populations by means of the induction of endogenous neurogenesis and/or the implantation of progenitor stem cells. Conceptually, stem cell therapy may have potential roles in the treatment of a variety of conditions. (Table 1) The so-called 'neurotransplantation' is a particularly exciting field which has attracted considerable attention and research effort. In this review, the author aims to provide an overview of the recent developments in the clinical application of stem cells therapy in the treatment of neurological disorders.

Experimental studies
A wide range of cell sources have been studied as potential candidates for stem cell therapy. (Table 2) The wealth of information generated from in vivo and in vitro experimental studies is beyond the scope of this review. Briefly, stem cells have been shown to survive and proliferate in host animals. Some are able to maintain metabolic activities, and migrate within the neuroaxis, sometimes across the cerebral hemispheres, towards artificially induced lesion sites (e.g. infarction). Differentiation into neurons may occur although the tendency for glial differentiation predominates in most cases. Neuronal 'daughter' cells may form axon-like processes but it remains uncertain if these processes are target-directed and contain functional synapses. Animals with, for example, induced cerebral infarction, have been shown to obtain measurable improvement in European Stroke Scale (ESS) and Modified Rankin’s Score. One key issue is the ability of MSC, which are mature differentiated ‘adult’ cells, to ‘transdifferentiate’ into cells of the neural lineage. This has been questioned by some authorities, who suggested that apparent transdifferentiation was in fact the results of fusion with other mature cells. Further studies are underway to investigate the precise roles of MSC in neurotransplantation.

Clinical studies
Cerebrovascular accidents
Cerebrovascular accidents, including both ischaemic and haemorrhagic 'strokes', are devastating diseases which result in permanent loss of brain function. Stem cell transplantation for stroke requires the repopulation of a combination of cell lineages, including neurons, glial cells and mesenchymal elements. Using the appropriate cell source is of paramount importance.

Initial effort in this clinical application included a pilot study which transplanted porcine lateral eminence basal ganglion cells into human adults with basal ganglion (BG) stroke. Despite mild improvement in some patients, the study was suspended due to adverse side-effects. Further clinical studies on patients with BG stroke have been conducted using human post-mitotic neurons. A Phase-I study using human teratocarcinoma-derived neuroprogenitors demonstrated both safety and technical feasibility. Position Emission Tomography (PET) in the transplanted patients showed enhanced metabolism around the areas of infarction which correlated well with clinical improvement. Post-mortem study in one patient who died from an unrelated cause also harboured donor-derived neurons within the transplanted site.

Kondziolka et al, conducted a Phase-II study, which included 18 patients with ischaemic or haemorrhagic stroke. Post-mitotic human graft neurons were transplanted stereotactically into multiple target sites around the BG. Post-operative immunosuppression was used. None of the patients suffered from any significant morbidities. Although there was no significant change in motor function, measurable improvement in European Stroke Scale (ESS) was observed in the treatment group.

Mesenchymal stem cells (MSC) derived from the bone marrow stromal cells have been investigated as an abundant and autologous source of neural progenitor cells. MSC may be introduced into the central nervous system either intra-arterially or intravenously. Bang et al, conducted a randomised controlled trial using intravenously administered MSC on patients with middle cerebral artery (MCA) infarction. There were no adverse effects and the transplanted patients showed improvement in both Barthel Index and Modified Rankin’s Score. One key issue is the ability of MSC, which are mature differentiated ‘adult’ cells, to ‘transdifferentiate’ into cells of the neural lineage. This has been questioned by some authorities, who suggested that apparent transdifferentiation was in fact the results of fusion with other mature cells. Further studies are underway to investigate the precise roles of MSC in neurotransplantation.

Spinal Cord Injury (SCI)
Neurotransplantation for SCI has received increasing attention in the past several years. Again, MSC has emerged as a promising cell source. In a pilot study using human MSC graft, Sykova et al, have demonstrated improved function in SCI patients. The results also suggested that patients in the subacute stage might do better than those in the chronic stage of SCI. The mode of delivery may also affect the outcome, with intra-arterial delivery being more efficacious than intravenous administration of MSC. Artificial ‘vehicles’ for graft cells have been developed for stem cell transplantation in animals, including a three-dimensional hyrdogel, which has
been shown histologically to integrate well within the SCI injury site.

Contrary to the classical teaching, neurogenesis is now believed to occur in human adults in the subgranular layer of the hippocampal gyrus and the subventricular zone. Stem cells from the subventricular zone migrate to the olfactory bulb, where there is continuous proliferation of neural progenitor cells. The olfactory mucosa is therefore an attractive graft site for neurotransplantation since it contains both multipotent neural stem cells as well as olfactory ensheathing cells, which may potentially enhance remyelination of the injured spinal cord. It also has the advantage of being autologous (i.e., non-immunogenic) and harvesting can be achieved using minimally invasive surgical techniques through the nasal passage.

Lima et al, reported a pilot clinical study on the use of autologous olfactory mucosa graft for the treatment of SCI. The olfactory mucosa was transplanted as solid tissue mass into the site of SCI in seven patients. All patients demonstrated improvement in their motor scores. Some patients also exhibited the return of limb and bladder sensation as well as voluntary anal sphincter contraction. However, without histopathological correlations, the mechanism behind these apparent clinical benefits is unknown.

Neurotransplantation for SCI using olfactory ensheathing cells obtained from aborted foetuses has been extensively reported by some researchers from the China Mainland. More than 300 patients have been treated so far. The treatment approach and study methodology, however, were met with criticism and the value of this approach remains to be proven in future controlled studies.

Parkinson’s disease (PD)
Foetal mesencephalic dopaminergic neurons have been transplanted into the striatum of human patients with PD. The initial clinical benefits were significant, with many patients being able to reduce their L-dopa requirement. Two randomised controlled trials have demonstrated significant benefits in the transplanted group when compared with the control group. The clinical benefits, however, diminished after several months. Subsequent reports also indicated that many patients suffered from ‘off-medication’ dyskinesia.

Despite the set-backs, these pioneering studies lend themselves to guide future cell-based therapy for PD. It appears now that neurotransplantation would benefit mostly young patients with clearly defined dopaminergic lesions (e.g., prior good response to L-dopa) and less severe forms of PD. The composition of the graft, its pre-transplant storage and culturing methods, may affect outcome. Co-treatment with immunosuppressants also appears to be beneficial.

Human embryonic stem cells (ESC) transplantation currently holds great promise for the treatment of PD. This approach involves the pre-differentiation of ESC into dopaminergic neurons prior to implantation. To the best of the author’s knowledge, there is as yet no reported clinical study using this approach. One major concern with ESC is the latter’s potential to form tumours. In animal studies, transplanted human ESC has been found to develop teratoma in the hosts.

Other conditions
Patients with traumatic brain injury, Huntington’s disease and demyelinating conditions (e.g. multiple sclerosis) are also potential candidates for stem-cell therapy. Transplanted oligodendrocyte precursors have also been found to remyelinate. And autologous MSC directly transplanted into the spinal cord has been shown to slow down the clinical deterioration in patients with amyotrophic lateral sclerosis.

Issues in clinical stem cell therapy
Unlike the controlled condition created for defined experimental studies, the diseased nervous system represents a far more complex and multi-dimensional environment for the transplanted stem cells. Many critical issues remain to be solved before stem cell therapy can be soundly applied in patients. These include the timing of transplantation (acute / subacute / chronic), the site of implantation (intra-lesional / penumbra), the mode of delivery (intra-arterial / intravenous / intralesional), the function of the graft, the mechanisms behind functional changes, and the role of immunological response and immunosuppression. Oncogenicity, infection and ethical issues are other major concerns.

Conclusion
Stem cell therapy is an exciting and rapidly developing field. Experimental studies have provided us with a solid foundation for the development of future treatment. The small number of reported clinical studies demonstrated both safety and feasibility although none has so far demonstrated significant and persistent clinical benefit. There is little doubt, however, that stem cell therapy will continue to develop and bring upon us a new era of treatment approaches to neurological disorders.

Table 1. Neurological conditions potentially amendable to stem cell therapy

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<td>Cerebrovascular accidents</td>
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<td>Spinal cord injury</td>
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<td>Parkinson’s disease</td>
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<tr>
<td>Brain trauma</td>
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<td>Demyelinating disease</td>
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<td>Huntington’s chorea</td>
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<td>Metabolic diseases</td>
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Table 2. Examples of potential cell-sources for stem cell therapy

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<th>Source</th>
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<tr>
<td>Embryonic stem cell</td>
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<td>Adult neural progenitors (e.g., olfactory bulb)</td>
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<tr>
<td>Adult mesenchymal stem cell (e.g., bone marrow)</td>
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<td>Immortalised cell-lines (e.g., human teratocarcinoma)</td>
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<tr>
<td>Xenograft from animal (e.g., porcine neurons)</td>
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<tr>
<td>Embryonic stem cell</td>
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<tr>
<td>Human foetal neural progenitors</td>
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<td>Donor-derived umbilical cord stem cell</td>
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References
Rebuilding the Degenerated Intervertebral Discs by Bone Marrow Stem Cells

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Low back pain and intervertebral disc degeneration

Low back pain (LBP) has an annual prevalence ranging from 15% to 45%1, which is the most common cause of activity limitation in people younger than 45 years and the second most frequent reason for visits to physicians. It is also the fifth-ranking cause of admission to hospital and the third most common reason for surgical procedures2. Degeneration of intervertebral disc (IVD) is commonly associated with LBP and sciatica3. There is a lack of consensus concerning the aetiology of IVD degeneration but it is thought to be multifactorial as aging, mechanical factors, cigarette smoking, and genetic factors may contribute to its occurrence4. Despite the unclear cause, the earliest signs of the degeneration can be detected through magnetic resonance imaging (MRI) and radiographs, which include decreased water content in the disc, reduction of disc height, and bulging of the disc.

In general, IVD changes gradually after birth in terms of its structure, cellular content, and biochemical properties. Foetal or neonatal disc comprises of two cartilaginous layers called end-plates (EP) which sandwich a middle gelatinous structure called nucleus pulposus (NP). The NP is confined by a fibrous ring called annulus fibrosus (AF) to withstand mechanical load while at the same time provide mobility to the vertebral segments. The NP and AF contain abundant extracellular matrix, particularly collagens and the vertebral segments. The NP and AF contain abundant extracellular matrix, particularly collagens and proteoglycans. In humans, the NP becomes dominated by chondrocyte-like cells and transforms into hyaline cartilage-like tissue as the IVD matures6-9.

Currently the pathophysiologic mechanisms of IVD degeneration are not clear, although a role of mechanical load and inflammatory pathways have been implicated in the degeneration, in the same way as osteoarthritis6-12. The process of degeneration comprises of loss of proteoglycan rich matrix and water in the disc, which are important to maintain a hydrostatic pressure to resist disc collapse because of axial loading. The consequential loss of load-resisting capacity results in structural failure of the IVD, leading to microfracture and disc herniation. Current treatments for severe IVD degeneration and back pain do not address the underlying problem, but rather bypass it by spinal fusion, thus rendering the segment immobile and putting more stress on adjacent mobile segments. Treatments that preserve motion are therefore desirable. Some trials suggest that an artificial disc replacement can successfully relief pain and preserve motion in the short and medium term. However, such surgical procedures are associated with complications and a chance that the segment may undergo auto-fusion in the long term13.

New ways to treat IVD degeneration

A number of research groups have been designing biological methods to heal or regenerate the IVD in a natural way. At present, biological therapies under pre-clinical investigations aim to restore proteoglycan level or synthesis within the degenerated IVD. For instance, the use of intradiscal adenovirus-assisted gene therapy14-16 or delivery of growth factors17-21 has been shown to preserve the architecture of disc tissue and/or increase collagen and proteoglycan synthesis in animal models. On the other hand, cell therapy approaches, such as stem cell-based tissue engineering, have been used to treat articular cartilage defects in animal models with satisfactory results12. In view of similar characteristics to osteoarthritis, the feasibility of using stem cells in arresting or even reversing the degeneration of intervertebral disc has recently been investigated.

Rebuilding the disc with adult stem cells

Stem cells are defined as unspecialised cells capable of long-term self-renewal and differentiation into specialised cells. The maintenance and renewal of totipotent embryonic cells is suggested to be dependent on the special micro-environmental niche23. Adult stem cells are the undifferentiated cells in adult tissues that retain differentiation potential to become cell types of their origin and their use is far less controversial than foetal or embryonic stem cells24. Adult stem cells normally play a role in local tissue turnover but are not involved in differentiating factors as they can differentiate into tissue of another type (trans-differentiation) in vitro or in vivo25. Bone marrow has been the primary source for two adult stem cell populations: the haematopoietic stem cells (HSCs) and the stromal mesenchymal stem cells (MSCs). Recent animal studies have demonstrated the therapeutic effect of MSCs in tissue repair through either local implantation or via systemic delivery26-28. Therapeutic effects of autologous MSCs in IVD regeneration have recently been demonstrated pre-clinically in small animal models, suggesting they can overcome and counter the degeneration process to some extent. Using a rabbit model of induced degeneration, collagen-embedded MSCs injected into the degenerated IVD can survive over a 4-week period and the proteoglycan content can be enhanced in the discs after implantation29. In addition, they can preserve annular structure, re-establish a disc nucleus pulposus-like structure, as well as partial restoration of disc height and disc hydration30,31. The mechanism of the regenerative effects has not yet been sufficiently evaluated but is believed to take place due to the differentiation of MSCs into disc cells, or the MSCs have acted as helper to induce endogenous repair.

Possibility of allogeneic application and cell pre-modulation

Extending the concept of stem cell therapy further, investigators have exploited the use of allogeneic stem cells as this has the added advantage of off-the-shelf availability. Moreover, as the cause of disc degeneration is thought to be multifactorial, the use of allogeneic stem cells may eliminate potential autogenic precipitating factors of degeneration such as genetic predisposition13,32 or the diminished potency of stem cells due to natural aging33. In fact, IVD is suggested to be immune-privileged due to its avascular nature. Moreover, immune rejection is even less likely for allogeneic MSCs since MSCs are capable of escaping from alloantigen recognition34,35. In recent studies in rabbit and mouse models it has already indicated that allogeneic MSCs transplantation can arrest or reverse IVD degeneration and without any immune rejection, suggesting that allogeneic MSC therapy holds promises in IVD regeneration.

Enhancement for the stem cell therapy has also been investigated by pre-loading biological signals to MSCs prior to implantation in order to “direct” MSCs differentiation, or otherwise turn MSCs into signal carriers to stimulate host cell differentiation36,37. For instances, studies have attempted to stimulate MSCs to directly differentiate into disc-like cells using cytokines or genes encoding cytokines prior to implantation38,39, or by co-culturing them with differentiated cells40,41. It is believed that priming MSCs with cell-to-cell signals, cytokines, or genes coding the morphogens can provide additional...
The current findings about the potential use of MSCs in IVD regeneration are encouraging. However, many issues still have to be addressed prior to clinical trials. Firstly, a clearer understanding of the aetiology of disc degeneration is necessary. Presence of endogenous developing factors may render any therapy ineffective or only temporary. Secondly, little is known at present about how to address prior to clinical trials. Firstly, a clearer understanding of the aetiology of disc degeneration is necessary. Presence of endogenous developing factors may render any therapy ineffective or only temporary. Secondly, little is known at present about how to proceed to human clinical trials.

In summary, many challenges await to be addressed before recommending mesenchymal stem cell therapy as a treatment option of IVD degeneration. Nonetheless, as the science of stem cell progresses in leaps and bounds, we believe that such technology would benefit many potential back pain sufferers in the not too distant future.

References
Reflection on Some Ethical Concerns of Human Stem Cell Research

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Introduction

Research using pluri-potent human embryonic stem cells (ESC) has raised hopes that tissues incapable of self-regeneration when damaged, e.g. brain, heart, pancreatic islet cells etc., can now be replaced by coaxing ESC to become the damaged tissue, potentially curing heart disease, Parkinson's disease, multiple sclerosis, spinal cord injuries and diabetes. However, the use of ESC has also sparked an intense worldwide moral debate. This paper reviews and discusses the ethical issues of ESC research.

Sources of stem cells (SC)

(A) Stem cells derived from non-embryonic sources

For the purpose of ethical analysis, it is convenient to classify human SC as (A) non-embryonic origin and (B) embryonic origin (Table 1), and only embryo-derived stem cells are of ethical concern because of derivation ontosis the desired SC type of human embryos. The sources of non-embryonic SC include (1) primitive foetal gonadal and somatic tissues, (2) adult somatic tissues including bone marrow, adipose tissue, central nervous system, muscle, (3) placenta and umbilical cord blood, and the ethical issues they raise are relatively simple. To harvest SC from aborted human foetuses, the pregnant woman's decision to terminate the pregnancy is transmuted into a decision to donate foetal tissue for research by undergoing two consent processes. She should not be allowed to designate any SC transplant recipient(s). Adult somatic tissues and placental/umbilical cord blood have become popular among those who for ideological reasons prefer not to destroy human embryos. The disadvantages of adult SC include the limited quantity of SC that can be harvested from the small number of tissues containing SC. More importantly, it is believed that adult or placental/cord blood SC have only limited potential for further differentiation as they are restricted by their own lineage determined by the tri-laminar (ectoderm, mesoderm and endoderm) embryonic differentiation. Hence they are described as multi-potent rather than pluri-potent. Their applications for self-renewal and cell-line cultures are also more limited than ESC. However, some recent studies have shown that adult SC from bone marrow and other tissues may cross lineage lines e.g. haematopoietic stem cells 'trans-differentiate' to become liver, heart and brain cells.1,2,3 In 2002, Verfaillie et al. described a new adult SC from bone marrow that could produce cell types of all three embryonic lineages. Dubbing it a multi-potent adult progenitor cell (MAPC), Verfaillie speculated that it may serve as a universal repair mechanism for the adult body.4 Some researchers have postulated that perhaps cellular trans-differentiation is a 'function' possessed by all SC and other primitive cell types capable of genome reprogramming to provide different phenotypes,5 hence the most ethical approach to SC research is to explore both embryonic and non-embryonic sources of SC or SC-like progenitor cells in order to maximise their therapeutic potentials.6

(B) Stem cells derived from embryonic sources

The most potent SC are found in the inner cell mass of the embryo at the blastocystic stage between 4-6 days after fertilisation. When cultured in the proper medium, these cells can multiply indefinitely as immortal cells, and under proper coaxing conditions they can be directed to differentiate into any one of the 220 cell types of the human body. They are the "uncommitted" progenitors of the subsequent 3 germ layers of the embryo.7 The major disadvantage and most contentious moral issue of human ESC is related to the obvious fact that their harvesting entails embryo destruction. Furthermore, ESC can be harvested from at least 3 different sources of human embryos and the moral assessment for each is significantly different: (1) surplus' embryos produced by in vitro fertilisation (IVF) for infertility treatment (IVF-surplus embryo); (2) embryos produced by IVF specifically for research purposes including harvesting SC (IVF-research embryo); (3) embryos produced by somatic cell nuclear transfer (NT) specifically for research purposes including harvesting SC (NT-embryo).

A hierarchy of moral contentiousness of human stem cells

Depending on the source of human SC, the moral consideration for their uses varies and there is a rough hierarchy of increasing moral contentiousness in the following order: (1) SC from umbilical cord blood; (2) adult or somatic SC; (3) SC from foetal gonadal and somatic tissues; (4) existing ESC lines; (5) ESC from IVF-surplus embryos; (6) ESC from IVF-research embryos; (7) ESC from NT-embryos using human ova; (8) ESC from NT-embryos using animal ova. Table 2 shows that SC with increasing moral contentiousness are permitted by a decreasing number of countries. For example, most countries including Hong Kong do not prohibit the use of human SC from cord blood, adult and foetal somatic tissues as long as proper informed consents are obtained from donors and safeguards against commercialization of tissues are in place. The use of existing ESC lines and ESC from IVF-surplus embryos are also tolerated by many countries, but the number of countries allowing the creation of IVF-research embryos' drops significantly, and the number of countries that permit the production of NT-research embryos' drops even lower. In Hong Kong, the use of human SC is governed by the Human Reproductive Technology Ordinance (Cap 561) which implicitly or explicitly permits the use of adult somatic SC as well as ESC from 'surplus' IVF embryos, but creating embryos by IVF or NT for the sole purpose of harvesting SC or embryo research is prohibited.7

Ethical principles guiding the use of SC

Some principles are applicable to both 'embryonic' and 'non-embryonic' SC and some are exclusively related to ESC only.

(A) Principles useful for both 'embryonic' and 'non-embryonic' SC

(1) Principles of beneficence and non-maleficence: there is an obligation for the doctor to provide treatment with a net benefit rather than harm to the patient. This principle implies that the risk/benefit ratio for the therapeutic use of SC should meet the community's prevalent medical standard, and there should be a reasonable chance of medical benefit for the patient as a SC recipient. The dictum: 'first do no harm' has special relevance for SC therapy. The principle also regulates the interests of SC donors.

(2) Proportionality principle: uses and procurement of tissues enriched in SC or, in the case of ESC, destruction of human embryos, should serve important and worthwhile goals and purposes e.g. to treat serious or life-threatening illnesses and not for trivial reasons or satisfaction of scientific curiosities.

(3) Subsidiarity principle8 stipulates that when the same results can be obtained through two different methods, the least offensive or problematic method should be employed, assuming a consensus of what is 'the least offensive or problematic'. In the context of SC research, this implies that: (a) SC or ESC should only be used if no reasonably suitable alternatives exist; (b) SC research should be first done on animals before humans; (c)
somatic SC should be used before ESC; (d) Surplus embryos should be used before research embryos are created.

(4) Principle of Informed consent (IFC): is a key principle and process in SC research and therapy. For example, in U.K., IFC for donating and receiving embryos and other tissues rich in SC is statutorily required by the Human Tissue Act (2004) and the HFE Act (1990). The following are some of the most important items of the IFC process for embryo donation to harvest ESC: 10

(a) Donors should be approached as early as possible, usually before ovary stimulation, to allow sufficient time to think over all issues carefully;
(b) Comprehensive information must be given in a readily accessible form, and potential donors must be allowed to make a free and informed decision;
(c) Donor couple must have given in principle consent for the use of embryos in research; and they may be required to re-consent immediately prior to the time of ESC derivation;
(d) Donors can vary or withdraw the terms of their consent without giving any reason, at any stage until the point that the embryos are used for research;
(e) Donors should be informed that SC/ESC lines will be successfully derived from donated tissues/embryos only in a few cases;
(f) Stem cells lines created may continue indefinitely and may be used in many different research projects;
(g) Donors cannot interfere with the subsequent research conducted with SC/ESC lines derived;
(h) Donors understand that the derived SC/ESC lines may be used in research projects by other researchers;
(i) Donors understand that any SC/ESC lines derived from their donated tissues/embryos, except when they are found to carry genetic defects, may potentially be used for treatment (including cell replacement therapies) purposes in the future;
(j) Researchers accessing embryos or SC/ESC lines will not have access to any identifying information of the donor;
(k) Donors either agree or disagree to be contacted in the future for information obtained from SC/ESC line studies that are of direct relevance to their own or family’s health;
(l) Embryo research will not lead to any direct medical benefit to the donors;
(m) Donors will not share in any actual or potential financial benefits derived from the commercial uses of SC/ESC lines or patents of medical discoveries;
(n) Donors know how the research is funded and may benefit which will accrue to researchers and/or their departments.

(B) Principles useful exclusively for ESC
Ethical issues that are closely related to the derivation and uses of ESC derived from the 3 different sources of embryos are:

- The moral status of the embryo (applicable to all sources of embryos);
- Moral distinction between 'IVF-surplus embryos' and 'IVF-research embryos';
- Biological and moral differences between 'IVF-embryos' and 'NT-embryos';
- Slippery slopes from therapeutic to 'reproductive' cloning;
- Exploitation of economically disadvantaged women as ova suppliers.

(1) The principle of respect of the embryo's moral right to life
For many, the central ethical concern in human ESC research is the moral status of human embryos. The prevalent divergent views have been influenced by a variety of concepts of human nature and personhood held by different cultures, religions and philosophies. The extremely conservative view regards the destruction of human embryos as equivalent to the killing of human persons and should not be allowed in almost any circumstances. This conservative position can be defended on either religious or non-religious grounds, with the most popular religious ground being the sanctity of all forms of human life including the embryo, espoused by Judeo-Christian, Islamic, Hindu and Buddhist adherents. Non-religious grounds typically include a special regard for the embodied basis of human nature and are critical of the ESC technology for reducing human embryos to transferable and transplantable "post-human" body parts. Alternatively, moderately conservatives regard using embryos to derive ESC as intrinsically unethical because the technology 'instrumentalisces' human life and exploits a vulnerable class of persons, namely, human embryos. Countries adopting this position include Ireland, Italy, Germany and the United States. In the U.S., moral objections to ESC take the form of restricting government funding only to certain existing ESC lines derived prior to August 9, 2001. In Germany, embryo research is essentially banned, but the importation of existing SC lines is permitted.

A second and extremely liberal view regards the early embryo possessing no more moral status or right-to-life than a clump of cells or isolated human tissue, and harvesting of ESC from human embryo is an important medical innovation that is morally justifiable. Countries that adopt this liberal position include Sweden, Belgium, U.K., China, Korea, Japan and Singapore, and they have implemented permissive policies allowing not only the use of 'IVF-surplus' embryos from infertility treatment programmes, but also the creation of IVF and NT (except Sweden) embryos for the sole purpose of research or harvesting ESC.

Between the two extremes is the 'middle-of-the-road' view that accords the human embryo an "intermediate" moral status: the human embryo is neither a full human person nor mere human tissue, but it possesses a unique status due to its potential to develop into a person. It deserves respect and protection, but not to the extent that goes with full personhood; under some circumstances, the use of human embryos for worthy medical research and harvesting ESC for therapy may be morally justifiable. But even within the 'middle-of-the-road' camp, no consensus exists about the precise ways to respect or protect embryonic lives and under what circumstances can their lives be sacrificed, and this adds to the difficulty of making ethical public policy in a pluralistic society that respects diverse fundamental beliefs and does not want to be held hostage to any single view of embryonic life.

(2) Moral distinction between 'IVF-surplus' and 'IVF-research' embryos
Three arguments have been commonly employed to defend using 'IVF-surplus' embryos to harvest ESC:

(a) 'reproductive intentionality' argument, (b) 'nothing is lost' argument, and (c) 'avoidance of waste' argument.

'Reproductive intentionality argument' asserts that 'IVF-surplus' embryos are intentionally created for reproduction, and as "surpluses" they are not destined for implantation and have no potential to develop to birth. Since the original intention has been defeated, using 'IVF-surplus' embryos to harvest ESC is deemed morally acceptable. The argument serves a good purpose to show that the difference in intentionality between reproduction and research is morally significant, but as...
an argument to support the use of 'surplus' reproductive embryos, it is a weak argument on its own. For if human embryos possess moral values and rights, 'IVF-surplus' embryos should raise questions about why they are in surplus, rather than what alternative 'uses' we can make of them. As an alternative argument to defend the use of 'IVF-surplus' embryos, Gene Outka has made use of a more classical ethical principle of 'nothing is lost' that justifies the killing of innocent life under two conditions: (1) the innocent will die in any case, and (2) other innocent lives will be saved. Since unimplanted 'IVF-surplus' 'will die in any case, nothing more will be lost by their becoming subjects of research, and for patients with Alzheimer and Parkinson diseases receiving ESC therapy, less will be lost, or, at least, someone may benefit. However, the argument has not addressed the fundamental question why embryos should be in surplus and their 'loss' can be morally justifiable if embryos have been created with reproductive intention and possesses moral values and rights or status. Outka has also neglected to include other important 'losses' related to the loss of the embryo's life in his utilitarian calculation. For, if the practice of destroying surplus human embryos becomes prevalent, the 'loss' of people's sensitivity to the value of human life must be counted as loss to the society. Lastly, the principle of 'nothing is lost' presupposes that some other innocent lives will be saved, and so far ESC therapy cannot provide this guarantee. Others have tried to buttress the 'nothing is lost' principle with an 'avoidance of waste' argument, suggesting that greater use is made of the embryo's life (or death) by contributing its SC for research or therapeutic applications. Such a utilitarian view to think about human life and death that no decent society would contemplate. It seems that if 'IVF-embryos' created for reproductive intention have moral values or rights at all, the most ethical solution is to avoid having 'surplus-embryos' at all.

(3) The biological and moral differences between deriving ESC from IVF- and NT-embryos

An important argument in favour of creating 'NT-embryos' is to provide autologous ESC for transplant that avoids rejection problems and graft-versus-host diseases, and potentially to produce 'designer stem cells' unique to a particular patient. The most prevalent objection is a 'slippery slope' effect that promoting 'NT-embryos' may unintentionally promote reproductive cloning. However, skeptics wonder whether banning 'NT-research embryos' ('therapeutic cloning') will actually stop over-zealous scientists to clone human beings ('reproductive cloning'), given the incredible temptation inherent in the possibility of human cloning that even the law may not be able to deter.

Contrarily, we believe that there are several important differences between an 'IVF-embryo' and a NT-embryo that support the argument that it is more advantageous to use cloned embryos than IVF-embryos. Firstly, an IVF-embryo is a newly human being produced by nature's fertilisation that in virtue of its unique genome possesses a distinct genetic identity and ontological individuality. We concur that 'IVF-embryos should not be created a priori for any end other than reproduction, otherwise the embryo becomes a means and not an end itself." On the other hand, NT-embryo is artificially produced by nuclear re-programming without creating a 'new' life with a unique genome. As one puts it: "SCNT resembles tissue culture...[it is an engineered culturing of the nucleus of a somatic cell, accomplished by implanting this nucleus into an enucleated ovum..." It can be seen as merely a 'copy' of the cloned cell, and if the NT-embryo is gestated in a woman's uterus, it will become a cloned human with blurry genetic and social identity. Secondly, the intention to produce the two kinds of embryos is different. The intention to create IVF-embryos can be reproductive or therapeutic or both. But the production of NT-embryos is only for research or therapeutic uses since reproductive cloning is legally banned. If I suffer from a massive heart attack, and if it is technical possible for the doctor to use my own somatic cells and eggs donated by my wife and to produce ESC and heart cells that save my life, reproductive cloning has never entered into my intention save is hard-put to argue that because in the process a human 'embryo' has been produced that potentially can become a human being I should be denied of the treatment.

There is another important reason why it is easier to justify producing ESC from NT-embryos and not IVF-embryos: the former has far less reproductive potential than the latter. This requires a brief explanation of the epigenetic phenomenon. The genomes in the male and female gametes are modified differentially during gametogenesis and are epigenetically distinct. In the course of producing an IVF-embryo by natural fertilisation, the epigenetic differences of the parental genomes are retained, and this epigenetic memory persists in the developing embryo to adulthood. In NT, the nuclear re-programming is faulty in the sense that the epigenetic differences in the nuclear genomes are lost. This 'epigenetic memory loss' creates nearly insurmountable "biological barriers" for the cloned embryo to develop properly, if at all, or to survive to adulthood without serious and multiple developmental abnormalities. However, normally functional ESC can be harvested from a NT-embryo without its "epigenetic memory". In short, the therapeutic potential of NT-embryo is equivalent to IVF-embryo, but the reproductive potential of the two are vastly different. In the reproductive sense, the 'NT-embryo' is not a biologically normal embryo and that renders its use morally problematic. To this "anti-thesis" that purports to produce embryos that lack the capacity to develop into babies, this is a similar effort to ensure that it will be more ethically acceptable to produce ESC using ANT-embryos than IVF-embryos.

**Conclusion**

The use of human stem cells will remain a contentious issue for the foreseeable future. This paper discusses some ethical issues for the different sources of SC. As new methods to harvest SC are proposed, they will have to be assessed separately. Each community will have to decide for itself which source(s) of SC will conform best to the prevalent values and norms adopted by the community. Since SC technology is relatively new, clinicians, scientists, ethicists and the general public should keep an open mind to future scientific discoveries and be sensitive to their moral implications.

**Table 1 Sources of human stem cells**

(A) Stem cells not derived from embryos (minimally contentious)
- Umbilical cord blood
- Somatic (adult) stem cells (bone marrow, blood, skin, brain, adipose tissue)
- Foetal germ cells and "adult" stem cells from foetuses

(B) Stem cells derived from human embryos (highly contentious)
- Existing ESC lines derived from IVF 'surplus' embryos
- ESC - IVF 'surplus' embryos
- ESC - IVF 'research' embryos
- ESC - NT embryos created w/ human ova
- ESC - NT embryos created w/ animal ova
Table 2 Hierarchy of moral contentiousness of different sources of human SC and countries permitting the use of different SC

<table>
<thead>
<tr>
<th>Sources</th>
<th>Permitted countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Umbilical cord blood</td>
<td>US, UK, Germany, Sweden, Belgium, France, Finland, HK, China Mainland, Japan, Singapore, South Korea, Australia, Canada</td>
</tr>
<tr>
<td>2. Somatic (adult) stem cells</td>
<td>US, UK, Germany, Sweden, Belgium, France, Finland, HK, China Mainland, Japan, Singapore, South Korea, Australia, Canada</td>
</tr>
<tr>
<td>3. Foetal germ cells &amp; somatic stem cells</td>
<td>US, UK, Germany, Sweden, Belgium, France, Finland, HK, China Mainland, Japan, Singapore, South Korea, Australia, Canada</td>
</tr>
<tr>
<td>4. ESC - IVF surplus embryos</td>
<td>US, UK, Sweden, Belgium, France, Finland, HK, China Mainland, Japan, Singapore, South Korea, Canada</td>
</tr>
<tr>
<td>5. ESC - IVF created &quot;research&quot; embryos</td>
<td>US, UK, Sweden, Belgium, France, China Mainland, Japan, Singapore, South Korea, Canada</td>
</tr>
<tr>
<td>6. ESC - NT created w/ human ova</td>
<td>US, UK, Belgium, China Mainland, Japan, Singapore, South Korea, Australia, Canada</td>
</tr>
<tr>
<td>7. ESC - NT created w/ animal oocytes</td>
<td>???</td>
</tr>
</tbody>
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References

17. Outka G. The Ethics of Human Stem Cell Research. Kennedy Institute of Ethics Journal 2002; 12(2) at p.194
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Unrelated Stem Cells Source

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Stem cells are primal undifferentiated cells that are characteristic to have self renewal (the ability to go through numerous cycles of cell division while maintaining the undifferentiated state) and unlimited potency (ability to differentiate into other cell types.) With these enormous and unlimited potentials, stem cells have been the focus in basic and clinical medicine for the past few decades. More recently a new branch of medicine, known as regenerative medicine, has evolved because of its overwhelming potentials to change the fate of many human diseases by being used to repair specific tissues or to grow organs. Indeed exciting reports have already appeared in the medical literature since in the mid to late 90s. Some of them such as making use of embryonic stem cells in clinical conditions like Parkinson’s disease and spinal cord damage even appeared in the headline of newspaper or TV. However, having fascinated by these future applications, there are still a lot of unresolved issues behind. It is in particular related to the underlyng ethical issues in the collection, expansion and clinical use of the stem cells in human beings.

In human, there are two categories of stem cells - embryonic stem cells, derived from blastocysts and adult stem cells, derived from umbilical cord blood or bone marrow. The former, though has the greatest potential in future application (expansion and differentiation into different types of tissues) is the most controversial because of the ethical issue in the collection, expansion and use. On the other hand, the latter has its clearly defined and long history of clinical use i.e. the use of haematopoietic stem cells (HSC) derived from adult or cord blood for bone marrow transplantation (BMT). Haematologists and oncologists have been making use of HSC and BMT in treating a large number of benign and malignant blood diseases, some immunodeficiency and inherited metabolic conditions and recently on autoimmune diseases. This is reflected by the exponential growth in the number of BMT performed in the last two decades. However, with the recent development of stem cell biology, there are still ongoing arguments in particular on the use of stored cord blood for insurance purpose or application in regenerative medicine.

Although it is now known that we are able to isolate and obtain stem cells from other tissues such as human embryo, oocytes and adipose tissue, the presentation will limit only to source of unrelated HSC for BMT only. In BMT setting, HSC can be obtained from the patients, siblings and family members, unrelated healthy donors and cord blood (related or unrelated). While HSC from related donors are usually preferred in BMT because of the lesser chance of BMT related complications and usually relatively easier in availability. However, with a worldwide trend in smaller family size, the possibility to have a matched related donor is getting lower. Without this, these patients have to go for unrelated donors whether it is from an adult or cord blood. Although the less stringent HLA requirement and almost immediate availability from stored cord blood means much more easy to locate a suitable cord blood unit, it is limited by its smaller cell dose rendering it not suitable to an average size adult.

At present, it has been estimated that the global demand for HSC for BMT is about 150,000 - 160,000 annually. Only about one third of patients (55,000) have successfully identified a suitable donor out of which 7900 are from unrelated donors. It is therefore obvious that there is a need to further optimise unrelated marrow donor registries to help more patients in hope of BMT. These include (1) to recruit more unrelated donors in the registries to increase the chance of matching, e.g. the estimated chance in Hong Kong to have a HLA matched unrelated donor is about 1 in 5000; (2) to improve the baseline HLA typing of donors in the pools (from serologic to DNA typing) so that the chance of full matching can be better.
predicted from initial matching results; (3) to increase the umbilical cord blood pool and hence their availability; and (4) to enhance the efficiency of donor clearance and stem cell collection. The last is of particular importance as from time to time, patients may develop relapse or other problems while they are waiting for the BMT. The shorter the time lapse, the better the chance to reach BMT is. Finally, there is a growing need to have more international co-operation so that donor searching and stem cells donation and transport can be enhanced.

In Hong Kong, the Blood Transfusion Service is the organisation providing such service. A centralised cord blood bank was established in 1998 whereas Hong Kong Bone marrow Donor Registry has taken up the previous work by Hong Kong Marrow Match Foundation since 1 September 2005 on donor recruitment, searching, and co-ordination of haematopoietic stem cell donation. Both services have been in full collaboration with local and overseas transplant centres in the provision of unrelated HSC to patients who are unable to have a related donor but require BMT to treat their underlying illnesses. Up to now, there are about 56,000 marrow donors in the registry and 2,000 units of stored cord blood units. It is committed to enhance the outcome of Chinese patients undergone BMT by its strong co-operation between China and Taiwan.

With regard to BMT, safety of donation, donor and recipient remains the most important concern. In the unrelated setting, the donation risk should always be minimal to the voluntary non-remunerated donors whereas the transplantation risk should be acceptable to the recipient. Therefore, it appears to have a need of consensus on requirement of stringent donor eligibility and quality control on the collection, testing and storage and their applications. Quality assurance and accreditation are some of the key issues to consider. Although there is no regulatory agent on the stem cells service locally, both professional and ethical standards have demand our service to adhere to the state of the art standards. Lastly, one should not overlook the importance in ethical consideration of recruitment, donor confidentiality in searching, matching and donation and of course their clinical uses.

Though cord blood has all along been targeted for homologous use (unrelated BMT or directed sibling BMT), there are on-going arguments whether one should store their cord blood for potential future use. At present, reported use of autologous cord blood unit is very low. Recently the World Marrow Donor Association (WMDA) has finalised her policy statement on the utility of autologous or family cord blood unit storage. Basically, WMDA supports the use of cord blood bank for the presently well accepted indication and research (i.e. unrelated stem cell transplantation and directed donation to family members who suffer from BMT treatable disease). Interested readers could retrieve the full document from the WMDA website for their reference. (Ref: http://www.worldmarrow.org/fileadmin/WorkingGroups_Subcommittees/DRWG/Cord_Blood_Registries/WMDA_Policy_Statement_Final_02062006.pdf)

To conclude, haematopoietic stem cells from unrelated donors have emerged as an important source for patients in need of bone marrow transplantation. With the help of national donor registry and international co-operation, the success in identifying a matched donor has been improved. However, there is still further room of improvement as limited by chance of matching and increasing stringent requirement for HLA matching. Members of the public who are healthy are encouraged to join the registry as potential donors.

References