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Stem cells in regenerative medicine: introduction

Dusko Ilic[1], and **Julia M. Polak**[2]*

[1]*Kings College London School of Medicine, Division of Women's Health, Assisted Conception Unit, 11th Floor Tower Wing, Guy's Hospital, Great Maze Pond, London SE1 9RT, UK, and* [2]*Faculty of Medicine, Regenerative Medicine, Room 144, Roderic Hill Building, Department of Chemical Engineering, Imperial College, South Kensington Campus, London SW7 2AZ, UK*

Background: Considerable amount of information about the potential of stem cell therapy in regenerative medicine is available today. Scientific meetings and publications in specialized journals enable experts in stem cell science and regenerative medicine to follow worldwide cutting-edge research. However, controversial information plaguing the media and the Internet lead patients to believe that stem cells are the long-awaited panacea even though there are little or no stringent factual data available yet.

Sources of data: PubMed database systematically searched in the period 4–6 January 2011.

Areas of agreement: Stem cell-based therapy is a future of regenerative medicine.

Areas of controversy: Based on unsubstantial claims fueled by media, patients are frequently seeking advice about the risks and prospects of specific therapeutic regimes from their physicians. Reports in specialized journals written in a scientific vocabulary are difficult to evaluate for many primary-care physicians. Hence, physicians are reluctant to provide advice or endorse treatment options for cell-based therapies.

Areas timely for further development: We wish to fill the gap and offer physicians suitable guidance. By giving a comprehensive overview of different types of stem cells and their potential in a simple language, here we are introducing a series of articles written by world-renowned experts on regenerative medicine about the current status and prospects of the field from the point of view of the standard level of patient safety and efficacy for the healthcare industry.

Keywords: stem cells/regenerative medicine/patient safety/personalized medicine

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**Correspondence address.* *Faculty of Medicine, Regenerative Medicine, Room 144, Roderic Hill Building, Department of Chemical Engineering, Imperial College, South Kensington Campus, London SW7 2AZ, UK. E-mail: julia.polak@imperial.ac.uk*

Introduction

Reading and interpreting the literature in a jargon-intensive field of science are very difficult for non-scientists. A brief overview of basic knowledge in the field of stem cell science provided in this manuscript should enable readers, not familiar with the field, to follow and understand more easily the issues that will be outlined and discussed in the series of commissioned articles on the current status and prospects of regenerative medicine and stem cell biology.

Definition and potential use

Stem cells are cells that are able to renew themselves through mitotic cell division and differentiate into a diverse range of specialized cell types.

Their use in therapy and regenerative medicine as well as in toxicity screening and drug development is widely anticipated.

Classification based on differentiation potential

All stem cells can be classified, depending on their differentiation potential, into five groups: toti- (omni-), pluri-, multi-, oligo- and unipotent.

Totipotent or *omnipotent* cells can differentiate into embryonic and extraembryonic tissues and generate a complete and viable organism. A fertilized egg is an example of a totipotent cell.

Somewhat less powerful, though possibly more useful for regenerative medicine, are *pluripotent* cells. They can self-renew and differentiate into any of the three germ layers, ectoderm, endoderm and mesoderm, from which all tissues and organs develop. Embryonic stem cells are currently the only known natural pluripotent stem cells. As their name indicates, recently created induced pluripotent stem (iPS) cells also belong to this group. They are actually tissue-specific cells reprogrammed to the level of embryonic stem cells. Since their stemness is 'man made' and not natural, they represent a separate category themselves.

Multipotent stem cells can self-renew and differentiate only in a closely related family of cells. Mesenchymal stem cells are a typical example of multipotent cells. Mesenchymal stem cells are loosely packed, unspecialized mesodermal cells set in a gelatinous stroma that

can give rise to connective tissue, bone, cartilage and the circulatory and lymphatic systems.

Oligopotent stem cells can self-renew and differentiate only into closely related cell types. Hematopoietic stem cells that can differentiate into both myeloid and lymphoid lineages would be an example of oligopotent stem cells.

Unipotent stem cells are the least potent. An example would be the muscle stem cells. They can self-renew and differentiate into only one cell type.

Classification based on origin

Based on their origin, stem cells can be grouped into five categories: embryonic, fetal, perinatal, adult (resident or tissue-specific) and iPS. Embryonic and iPS cells are pluripotent, and fetal and perinatal are, in general, multipotent, whereas adult stem cells are usually oligo- or unipotent.

Embryonic stem cells

Embryonic stem cells are derived from blastocyst, a stage of embryo 5–6 days after fertilization. The blastocyst has two groups of cells: inner cell mass, which will subsequently form the embryo, and an outer layer of cells, trophoblasts, which will later form the placenta. To derive embryonic stem cell lines, cells from the inner cell mass are separated from trophoblasts and transferred into a culture dish where, under very specific conditions, they can be maintained and propagated infinitely in an undifferentiated state.

Even if attention is paid to ethical, religious and political issues that relate to human embryonic stem cells, there are still a number of obstacles to be resolved before these cells can be widely used for cell-based therapy. Two issues that stand out the most are differentiation protocols and genomic stability.

Differentiation

Manipulating cell culture conditions by adding or removing various growth factors or changing a substrate on which they grow would cause embryonic stem cells to lose their pluripotency and undergo differentiation. It is still unknown how to manipulate systematically and reliably the culture conditions in such a way. The yield of differentiated cells varies depending on their type. With current knowledge, encouraging differentiation of embryonic stem cells toward cells of

ectodermal origin such as retinal pigment epithelium or oligodendrocytes will yield a much higher percentage of desired cell phenotype than attempts made to differentiate embryonic stem cells into cells of endodermal origin such as the hepatocytes. Still, the final product, such as the oligodendrocytes or retinal pigment epithelium, has to be purified in order to be used for therapeutic purposes. Since embryonic stem cells can proliferate definitely, there will be enough starting material to produce sufficient number of any cell type regardless of how low the yield of particular differentiated cell type is.

Genomic stability

Chromosomal instability is typical for almost any cells maintained for a long time *in vitro*. Embryonic stem cells are not different. Extended culture time very often results not only in chromosomal imbalance, but also in structural chromosomal abnormalities, which may or may not result in undesirable consequences for the recipients of stem cell therapies. Therefore, there is a constant need to improve the cell culture conditions in order to make the cells safe for clinical use.

In spite of all the issues, oligodendrocytes and retinal pigment epithelium derived from embryonic stem cells are already undergoing or just about to undergo high-profile well-controlled clinical trials in the USA and the UK. Depending on their outcome, either way, the attitude toward embryonic stem cells is likely to change. Mesodermal cells, cardiomyocytes, derived from embryonic stem cells recently hit the market as the testing model for drug toxicity.

Fetal stem cells

Fetal stem cells are obtained from the embryos of terminated pregnancies. They are not as potent as embryonic stem cells and they are somewhat less controversial. Fetal stem cells cannot divide indefinitely in cell culture without being coerced. Whether such an intervention is safe depends on regulations, and the regulations differ from country to country. The majority of neural stem cell lines available today are of fetal origin, and several of them are undergoing clinical trials in the USA and the UK. In other countries that have less regulated rules about patient safety and experimental procedures, fetal stem cells are frequently used for controversial medical purposes. For example, at the Beijing Xishan Institute for Neuroregeneration and Functional Recovery, cells from aborted fetuses were injected into more than 1500 patients to treat spinal cord injury and a variety of central nervous system diseases. This and other similar approaches are not documented with the measurable data that would support the claims about their

efficacy. Moreover, these procedures have not been reviewed by either leading researchers or appropriate ethical committees.

Perinatal stem cells

Based on their origin, perinatal stem cells can be divided into three groups: stem cells from amniotic fluid, placenta and umbilical cord. Placenta has three sources of stem cells: amnion, villi and blood, whereas umbilical cord stem cells come from two sources: cord blood and Wharton's jelly (Table 1).

Amniotic fluid

Amniotic fluid, which completely surrounds the embryo from the 4th week onwards, is filtered out from both maternal blood and fetus itself. The cells found in amniotic fluid are mostly fetal epithelial cells that flaked off from fetal skin. Several reports suggest the presence of multipotent stem cells in amniotic fluid. These cells cannot divide infinitely and have the characteristics of mesenchymal stem cells. However, they are impractical for the broader use of stem cell-based therapy and are unlikely to be the subject of clinical trials in the future. The main reason for this is the stem cells can be obtained from the amniotic fluid only by amniocentesis, a procedure that carries ~1% risk of miscarriage.

Placenta

In general, when one is talking about placenta as a source of stem cells, it is about placenta at the end of pregnancy or terminal placenta. Although it sounds as an attractive source of stem cells, we should not dismiss the fact that the terminal placenta is, from a physiological viewpoint, a worn-out organ with no further use. Stem cells isolated from both amnion and placental villi are characterized as mesenchyma-like and hematopoietic progenitors. They cannot divide indefinitely *in vitro*; however, there are attempts being made to expand them

Table 1 Perinatal stem cells.

Perinatal stem cells	
Amniotic fluid	
Placenta	Amnion
	Villi
	Blood
Umbilical cord	Cord blood
	Wharton's jelly

sufficiently for allogeneic transplantation. Several clinical trials with these cells are ongoing in the USA and Europe, focusing on the therapy for critical limb ischemia.

Amnion is better known for its use in the therapy of eye injuries and a few other pathologies rather than as a source of stem cells. There are no clinical trials or attempt made to use stem cells from amnion in cell-based therapy.

Placental blood has an enriched population of stem cells that are also found in cord blood. Therefore, theoretically, stem cells from placental blood could be purified and cryopreserved to enrich the stem cell population isolated from the umbilical cord. In reality, this is quite rarely or never done, because the procedure is extremely impractical and laborious.

Umbilical cord

Stem cells from the umbilical cord are probably the most talked about. Parents around the world are paying large sums of money to preserve cord blood from their newborns believing that they are providing an additional level of insurance for their children in case of a life-threatening disease. Stem cell banking has become a lucrative business, and private cord blood banks are mushrooming and flourishing around the world. There is no doubt that the stem cells from cord blood can be used to treat hematopoietic system disorders. However, little attention is paid to the dosage of stem cells (number of cells per kilogram of body weight). Given the limited number of cord blood stem cells present in one umbilical cord and limited expansion ability, not only adults but also children older than 5–7 years cannot be treated even if they have their cord blood preserved. To obtain sufficient number of stem cells, they have to turn to public banks.

According to a number of reports and advertisements from private companies, cord blood stem cells can also be used to treat diseases unrelated to the hematopoietic system. For example, using stem cells harvested from umbilical cord blood, a Chinese company, Beike, has treated over 5000 patients to date. The company claims an 85% rate of improvement in a variety of conditions, from spinal cord injuries to autism. However, there are no convincing measurable data proving that the treatment indeed helped. The rate of improvement is based on the statements made by former patients, who are, very often, desperate and seeking marginal improvements in their quality of life. However, not all stem cells therapies performed in China are of dubious quality. The Chinese government is heavily investing into stem cells and regenerative medicine, whereas China's Ministry of Health is trying to bring and enforce rules about therapies currently administered by clinics and hospitals to ensure patients safety. And reassuringly, contributions of

Chinese authors to scientific journals on topics of stem cells and regenerative medicine increased from only 37 in year 2000 to 1116 in 2008. The number is exceeded only by the contributions of scientists from the USA, the UK, Japan and Germany.

The International Society for Stem Cell Research (ISSCR) strongly condemns the administration of unproven stem cell therapies, and it has written a handbook to help doctors and patients make informed decisions about available stem cell therapies. The booklet is available for free download from the official ISSCR website.

Recently, attention has been brought to Wharton's jelly, a gelatinous substance within the umbilical cord, largely made up of mucopolysaccharides (hyaluronic acid and chondroitin sulfate), that contains some fibroblasts and macrophages. Mesenchymal component (fibroblasts) might have stem cell potential. Although there are no compiling data, private companies have started to offer banking of cells from Wharton's jelly.

Here, we should mention the sibling cord blood-banking program, available in the USA, which offers cord blood preservation at no cost to expectant parents who have a child who may be in need of umbilical cord blood stem cell transplantation. The program released more than 100 family-related cord blood stem cell units for transplantation to siblings, mostly sickle cell anemia and thalassemia patients.

Adult stem cells

Adult stem cells, also known as *resident stem cells* or *tissue-restricted stem cells*, like all stem cells, share two characteristics:

(i) self-renewal; they can make identical copies of themselves for long periods of time;

(ii) they can give rise to mature cell types with a specific morphology and function.

These cells reside within organs/tissues of an adult, and they play a crucial role in tissue regeneration and self-renewal. Each of the different tissues of the body has its own natural life cycle. Continuous regeneration of our body is enabled through proliferation and differentiation of its resident stem cells; for example, human epidermis is renewed every 3–4 weeks, gastrointestinal epithelium within less than a week and endometrial epithelium once a month. Adult stem cells are oligopotent in the best case or unipotent in most of the cases. Although proliferation of these cells *in vitro* is very limited, they are a subject of great interest. Many laboratories around the world, non-profit and for-profit, are trying to elucidate how to stimulate proliferation of these

cells and how to mobilize them in case of injury and make them repair a damaged organ or tissue. Depending on their location, certain adult stem cells are easier to access and isolate than others. Whereas we can easily isolate stem cells from peripheral blood, bone marrow or adipose tissue, it would be extremely difficult to do this with cardiac or pancreatic resident stem cells. The major goal of adult stem cell field is to find out how to make resident stem cells from one tissue repair another tissue or organ. Would it be possible, for example, to repair a damaged heart or a broken bone with adipose stem cells? If so, how can we get a sufficient number of adipose stem cells to do this? There are hundreds of clinical trials using different types of adult stem cells around the world with variable success. In some cases, the cells are simply taken from one part of the body and injected into another, whereas in other cases, the cells are expanded before being transplanted/re-injected into the patient. It is still difficult, if not impossible, to discern benefits from placebo effects or simple stimulation of healing mediated by an endogenous stem cell population, which is initiated by the injection of stem cells from other parts of the body.

We will briefly mention three of the easiest-to-access, and therefore the most 'popular', sources of adult stem cells: bone marrow, fat and menstrual blood.

Bone marrow

Cell-based therapy with stem cells from bone marrow is known and in use for more than 50 years. In 1958, French physician Georges Mathé treated six physicists from Yugoslavia who became ill from radiation exposure during a nuclear reactor accident with the first successful bone marrow transplant not performed on identical twins. The bone marrow transplant used in the treatment of hematopoietic diseases is probably the only safe and controlled stem cell-based therapy in use today.

As in the case of cord blood, a number of for-profit organizations are attempting to capitalize on increasing treatment with bone marrow stem cells. They are not necessarily restricted to exotic locations such as Dominican Republic or China. X-Cell, Germany-based company, offers treatment with autologous bone marrow stem cells for cerebral palsy, spinal cord injuries, diabetes mellitus (types 1 and 2 as well as sequelae) and neurological diseases/disorders such as Parkinson's and stroke, multiple sclerosis, amyotrophic lateral sclerosis and Alzheimer's as well as arthritis, heart disease and eye diseases such as macular degeneration. Does it work? We do not know this for a fact as yet. As in the case with cord blood stem cell transplants, the reported rate of improvement is based on the statements made by former patients, who introduce a degree of bias because of the costs involved and the possible placebo effects.

Adipose tissue

Adipose tissue is relatively rich in resident stem cells. More than 50% of nucleated non-fat cells present in liposuction-extracted fat have stem cell markers. Can these stem cells repair, for example, a damaged heart? Clinical trials are underway. However, the basic problem, whether is it possible to get a sufficient number of cells for therapy, remains unanswered. Clinical studies have suggested that with the current methods of delivery, only a small percentage, about 2%, of infused cells remain in the heart. This phenomenon is also seen in other organs. Advancements in technology made liposuction a simple and affordable low-risk low-cost procedure and, for sure, the notion that cosmetic surgery might result not only in better physical look, but also in life-saving benefits, would be readily accepted by the public and gain popularity in time.

Menstrual blood

It is only a matter of time before stem cells will be found in menstrual blood and a commercial menstrual blood banking will be developed. Although undeniably a small fraction of cells with proliferative capacity can be found in menstrual blood, the questions as to how potent they are and how much can they be expanded are still unanswered. There are no ongoing clinical trials, and it is unlikely that the menstrual blood will become a prevalent source of stem cells for clinical use. Simply, obtaining a sterile sample of desired volume might be quite a difficult task.

iPS cells

Changing the fate of differentiated cells from one to another cell type was the focus of many laboratories for a long time. In 2006, a group of Japanese researchers were able to perturb the stable state of differentiated cells and revert them to the level of embryonic stem cells. These new types of pluripotent stem cells were named iPS cells. In addition to being an exciting research and drug development tool, iPS cells have therapeutic potential for custom-tailored cell therapy, because they can divide *in vitro* indefinitely and can be differentiated into any mature cell type of the human body. iPS cells, therefore, could circumvent the lack of matching tissues for organ transplantation and requirement for lifelong treatment with immunosuppresants. However, a number of technical issues should be resolved before the reprogramming technology could be applied in translational medicine. The biggest obstacles are the following:

(i) Need for reprogramming factor delivery system that would have no adverse effects on genome.

(ii) The xeno-free cell culture conditions before, during and after reprogramming in a laboratory compliant with current good manufacturing practice.

(iii) Quality control. Data that should come from basic research are still insufficient. There are a number of parameters that could be tested, including karyotype and biochemical markers, gene and protein expression, cellular impuritiy profile, biological activity, potency or DNA methylation. However, should they be tested? What is a safe minimum? It is still unclear which tests are needed and which values would be in a permissive and safe range without being too superficial and porous or being in excess.

Conclusions

Individuals with lifelong threatening conditions and hoping for an improvement, cure or amelioration of symptoms see in stem cells a new option for protecting, maintaining and improving their health in the foreseeable future. We believe that it is the responsibility of the physicians to be the front-line resource to the patients and interested members of the public for the issues surrounding the stem cell-based therapy. A luxury of ignorance cannot be afforded.

This comprehensible guidance through the elementary stem cell biology to clinical applications contains information that is needed to understand the more specialized articles in the field of stem cells and regenerative medicine.