Stem Cells – What, Why, Whereabouts and When?

by Pierre Schembri-Wismayer **January 16, 2020**

2006, ARTICLES, ISSUE 4 - 2006, LIBRARY, THESYNAPSE MAGAZINES, THESYNAPSE ONLINE

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Stem cells are a hot issue. The reason for this extreme interest is the promise of regeneration. They are presently making big waves in life sciences research conferences, in ethical discussions, and also, already, in clinical trials. Unfortunately they have also already featured in news items relating to falsification of scientific results [1].

Modern medical science has improved health in leaps and bounds when it comes to prevention of illness through sanitation and vaccination. It has also vastly improved global health with antimicrobial drugs for treating ongoing infections. It is also greatly improving obstetric care (though there is still a lot to do in developing countries).

In the parts of the world with financial resources and access to advanced hospital care, surgery and modern medicines have also achieved tremendous success in curing cancer, heart disease, obesity and other scourges of the modern world.

Such medicines have also allowed us to control to some extent the more chronic degenerative disorders of joints, kidney, lung, liver and brain to allow an acceptable quality of life to their sufferers.

However all of this is work on preventing death of cells and tissues and on controlling symptoms as best one can.

Regeneration, that is, re-establishing structure and function in organs which has irreversibly lost that function, is still largely a dream – a modern dream similar to the fabled elixir of everlasting life.

Medical successes in all the fields mentioned above, through increasing the longevity of patients have created a bigger market yet for regenerative medicine. This is in the population of older people who are not yet ready to die and who are keen to maintain their health and functionality for as long as they are to live.

Those of our patients, and of ourselves who have overcome heart attacks, angina, strokes all wish to continue living to the best of their ability. As do those patients continuing to struggle with diabetes or cirrhosis. As do too, coronary bypass graft patients and cancer survivors.

The present medical facilities for treating the degenerative disorders which accumulate in us as we get older, are a mixed team of talented transplant surgeons, replacing joints, livers, kidneys and hearts with plastic and metal mock-ups or better still with donor organs.

Despite the great skills of these surgeons, their job is limited by the quality, durability and lack of plasticity/healing of artificial implants and by the scarcity and immune rejection of natural organs for donation.

It is into this gap that the promise of stem cell therapy hopes to expand. Whilst the surgery mentioned above is a saving grace at present, I think we all hope for a day when it is largely irrelevant. As a comparison one can consider the relative obsoleteness of gastric ulcer surgery in the present milieu of endoscopy and the arsenal of anti-ulcer drugs.

Stem cells – basic definitions.

So what are stem cells?

Stem cells can be broadly defined in terms of their two most salient features – the capability to self-replicate and the capability to differentiate into a wide range of derivative cell types. Both these features are necessary to make a stem cell and once a cell has these two features it shares the property of stem-ness. A cell of this nature must probably have the capability to perform a functionally polar or non-symmetric division where one daughter cell will produce another stem cell whilst the other daughter cell is programmed to differentiate into a number of more mature cell types. An easy framework in which to understand the stem cell function is in the bone marrow where a single stem cell can divide to produce a self-replica and another daughter cell which can divide to give rise to all the cell types found in blood.

Basic research on stem cells has been ongoing since the 60s when Till and McCulloch first identified colony forming units in the spleen of irradiated transplanted mice.

Different sources of Stem cells

Stem cells are usually defined by the range of cells they can differentiate into and/or according to their source of origin.

Thus one may talk about totipotent embryonal stem cells – these are the initial 8 cells in a morula (early embryo), each of which upon separation is theoretically capable of differentiating into a complete organism. In fact removal of one to two cells at this stage can be used to do genetic studies on an embryo pre-implantation [2](as in certain recent cases of designer babies produced with the aim of providing a bone marrow donor for ill siblings). The ability to remove such cells without destroying/perturbing the remnant group of 6-7 cells contribute to the discussion about the origins of personhood and the possibility of deriving human embryonic stem cell lines from very early embryos.

Following this, further cell divisions of the early embryo lead to a certain amount of differentiation with different cells forming the embryoblast which will give rise to the embryo and the trophoblast which will give rise to much of the placental tissue.

Cells derived from the embryoblast are usually referred to as pluripotent since they can produce almost all tissues but would not be capable inherently of producing a complete conceptus and resultant human being[3]. This is the usual source of human embryonic stem cells.

The more the embryo develops, the less the range of differentiation of its cells(with the exception of those cells destined to become germ cells); at this point, these cells are called multipotent stem cells and will differentiate into tissue specific stem cells. The natural function of these stem cells throughout embryonic development and adult life is to help replace cells lost by depletion or damage.

Embryonic stem cells are thus called because they are derived from the early embryo- the embryoblast. They can be kept proliferating in tissue culture without differentiation (usually under the influence of certain cytokines, particularly Leukaemia inhibitory factor) [4]. This “immortality” raises the possibility of small numbers of human embryonic stem cell lines being used to treat large number of patients over long periods of years.

Stem cells with a limited pluripotency can be derived from human fetuses lost at different stages of pregnancy and may have been responsible for the partial success of fetal brain transplant surgery for Parkinsons’disease [5].

All other stem cell types commonly in discussion/study are known as adult stem cells, and here are usually named according to their source of origin – umbilical cord stem cells, bone marrow stem cells, neuronal stem cells, mesenchymal stem cells etc[6].

Plasticity and therapeutic cloning.

As explained above, embryonic stem cells have the possibility of deriving all different sources of tissue.

Adult stem cells, up till recently believed to be tissue-specific stem cells, have generated a lot of interest (and a good amount of controversy too) in the past few years due to recent studies showing a good deal of plasticity.

Plasticity can be defined as the capability of a stem cell derived from one tissue producing cells of a number of different tissues. The extent of plasticity is controversial and is thought to depend on the environment of these stem cells, including the extent of surrounding tissue damage.

A lot of what is known about stem cell plasticity comes form animal studies and also clinical studies of sex-mismatched organ transplants where different tissues in the recipient (usually of a bone marrow transplant) were assessed for cells containing sex-mismatched cells in other tissues.

Bone marrow stem cells, probably the most well studied stem cells, have been shown in various studies to give rise to numerous other different types of cells, including muscle cells, cardiac muscle cells, liver cells, lung cells, bone cells, cartilage cells, fat cells and even neuronal cells[7, 8]. These are derived from either the haematopoietic stem cell or the mesenchymal stem cell founding bone marrow.

The therapeutic potential of this phenomenon causes a lot of interest to ethicists, scientists and clinicians alike. The option of efficiently re-programming cells derived directly from the patient (rather than depending on a few lines of embryonic stem cells, maintained in tissue culture) is one we all look forward to with hope. It would reduce most problems with transplant rejection, and the ensuing problems of immunosuppressive regimens and their associated complications.

The mechanism of therapeutic cloning holds a lot of potential for the ultimate re-programming of adult cells into totipotent stem cells. This introduces a lot of ethical issues, due to the creation of a novel zygote-like cell (and therefore potentially a new human being). Theoretically, however, it would also create a source of every cell-type potentially needed for transplantation procedures and regeneration therapies, from any one of the patient's skin or blood cells.

Making human embryonal cell lines from adult cells through the process of therapeutic cloning is presently an unachieved goal of stem cell research. It holds great potential but as it is seen as ethically problematic, human cloning having been outlawed in several national and international declarations[9, 10]. The process of therapeutic cloning – creating a novel zygote- like cell to develop person-specific stem cells, unfortunately tends to be hijacked by the fact that this research could also result in the much more controversial reproductive cloning.

,Most mammalian embryos created through cloning do not in fact develop into mature animals, in fact the great great majority die at a very early stage of development as was the case with the experiments which eventually produced Dolly – she was one success story from 300 plus attempts[11].

This is due to a number of characteristics of the embryonal development, including the process of imprinting, only a few of which are understood to any extent[12].

Due to the very low likelihood that a zygote created through cloning would ever develop into a human being, even in the best circumstances in the womb, one may argue whether it may be possibly acceptable on moral grounds to accept the process of therapeutic cloning.

Normally, embryonic stem cells are derived from a clone once it has been allowed to develop into a blastocyst [13]. This in itself engenders certain aspects of personhood to those of us (including myself) who believe that human life starts from the zygote formation, despite the fact that the chances of development into a person are presently non-existant. It may be possible in the future to directly derive the therapeutic clone from the initial nuclear-transferred cell. This may possibly reduce some of the ethical conundrums associated with therapeutic cloning by not allowing anything similar to an early embryo to ever develop.

Clinical Therapeutic implications of stem cell biology

Lots of interesting clinical results have already been seen from stem cell transplantation and many others await us in the near future.

Bone marrow transplants are a form of stem cell transplantation which has been curing patients of aplastic anemia, leukaemia, and various other diseases for many decades.

This has more recently been supplemented with similar procedures of cord blood transplants and G-CSF-mobilised peripheral stem cell transplants.

Heart disease is one of the front runners in the field of stem cell clinical trials, where bone marrow injected into the heart of patients during or after myocardial infaction has resulted in an improvement of ejection fraction and other cardiac function parameters[14].

The great interest is that unlike all other previous therapies available, this therapy results in a return of function to the post-infarct heart – a finding that holds much promise.

Other areas of ongoing clinical research into stem cells include retinal, pancreatic and skeletal diseases [15-17]

With the perpetual lack of donor organs, the capability of developing new organs from one's own stem cells (or those of donors) provides a new frontier in medicine.

Tissue engineering is a whole new branch of medical research which is developing rapidly to make the most of advances in stem cell research as well as biomechanics and other technologies.

The option of introducing nerve cells to sites of neuronal injury following accidents or vascular events also opens up new frontiers into an as yet restricted field, resulting in rehabilitation of seriously disabled patients.

Ethical and Safety issues

I will not be discussing the ethical issues with embryonic stem cells and personhood in detail here. However other ethical issues relating to stem cell therapy should be noted.

A possible cause for concern with stem cell therapy involves the risk of cancer [18]. Whilst this was hardly considered till a few years ago, nowadays, the literature teems with papers about tumour stem cells.

Mouse experiments involving the injection of stem cells or their progenitors clearly show the link with teratomas [19].

In the case of embryonic stem cells, prolonged *in vitro* culture can be associated with genetic changes making embryonal stem cells similar to embryonal carcinomas [20]. This raises important questions about safely using embryonic stem cell lines propagated for a long time in vitro as a source of donor stem cells.

Although the amount of therapeutic studies using embryonic stem cells is presently very small, and no such statistics can be calculated, there is theoretically an increased risk of cancer developing in stem cells.

The increased plasticity of adult stem cells and the possibility of creating patient-specific stem cells through processes similar to therapeutic cloning may make this a mute point in the near future.

Regarding tumour stem cells, the author's personal opinion is that tumour cells, upon becoming immortal obtain much of the properties of stem-ness. However, many papers now specifically describe a specific sub-population of tumour stem cells[21, 22]. Whichever of these positions is the more accurate, there is little doubt that the more primitive a cell, the more propensity it has for malignant transformation. Due to this, detailed and extensive studies following transplantation of stem cells (autologous or heterologous) will be required before the procedure will be accepted as one with minimal associated risk.

Stem cell collection and banking

With all this stem cell-related research ongoing throughout the world, are there any measures worth taking up locally?

In the author's opinion, the obvious and relatively easy option is to start up public cord blood banking. In fact a proposal document had been submitted to the health authorities by the author on behalf of a private charity a number of years ago.

Cord blood banking has been developed over the last decade or so in a number of countries around the world, including Italy, the Netherlands and the UK. Recognition of the usefulness of this resource were heralded by titles such as "turning garbage into clinical gold" in some of the world's most prestigious scientific journals [23].

Cord blood banking can be separated into private and public banking. Private/individual banking normally involves the preservation of the cord blood from a child's placenta at birth and keeping those blood cells for the child in question. This involves an initial payment and sometimes a recurrent payment to cover cryopreservation. Since the blood is only tested for infective organisms and does not need to be cross-matched against other individuals, it is relatively cheap to bank such blood.

In 1999, the American pediatric association issued a recommendation stating "Families may be vulnerable to emotional marketing at the time of birth of a child and may look to their physicians for advice.No accurate estimates exist of the likelihood of children to needtheir own stored cells. The range of available estimates is from1:1000 to 1:200 000. Empirical evidence that childrenwill need their own cord blood for future use is lacking. Therealso is no evidence of the safety or effectiveness of autologouscord blood transplantation for the treatment of malignant neoplasms. For these reasons, it is difficult to recommendthat parents store their children's cord blood for future use"[24].

This was before the recognition of the different types of stem cells found in cord blood and their much greater plasticity potential. However even much more recently, the Canadian Society of Obstetricians and Gynaecologists issued the following amongst a long list of recommendations "5. Altruistic donation of cord blood for public banking and subsequent allogeneic transplantation should be encouraged when umbilical cord blood banking is being considered by childbearing women, prenatal care providers, and(or) obstetric facilities. 6. Collection and long-term storage of umbilical cord blood for autologous donation is not recommended because of the limited indications and lack of scientific evidence to support the practice. [25].

Public banking is much more expensive on a per unit basis but provides a resource for the whole health service. Due to the relative immunological naivety of cord blood, a perfect 6/6 major HLA match is not required for successful transplantation. 4/6 matches are often successful.

Studies by the Turin cord blood bank have in fact found that with just 500 units ($1/10^{th}$ of the amount of cord units which could be collected in a year in Malta) would be able to successfully cross match about 90% of the Italian population, ie more than 50 million people[26].

Until recently, cord blood was only found adequate for transplant into children and small adults of less than 50kg body mass, due to a need for more stem cells to adequately replace bone marrow in a larger individual[27].

Recent studies however are suggesting a wider range of potential recipients due to a number of modifications including the simultaneous transfusion of more than one cord blood unit into the same patient [28] as well as ex-vivo expansion of the stem cell population [11, 29].

So Cord blood banking might just be the most useful stem cell-related health investment for the local health authorities. Private public partnerships may also provide a useful option, especially to allay costs. Here, public health authorities could take over cord blood units banked privately for

individuals after a fixed time period or after private individuals decide to stop paying cryopreservation costs, thus forfeiting ownership. By performing HLA typing and by recording these units in a database, they will slowly build up a local stem cell therapeutic resource, with the private sector having initially footed the start-up cost.

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