Adult Stem Cell Therapy – Imagining Futures in Cell Biology

Professorial Lecture

by

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SUMMARY OF PROFESSORIAL LECTURE

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With clinicians from the Princess Alexandra Hospital we are currently undertaking a small clinical trial of cell transplantation therapy by removing cells from the paraplegics and transplanting them into their own injured spinal cord. The transplanted cells are not "stem cells" but "olfactory ensheathing cells", cells that promote regeneration of the olfactory sensory neurons and guide the growth of their connections from the nose to the brain. The promise of “stem cells” is that they have the potential to repair or replace *any* part of the body damaged or lost by injury or disease. "Embryonic stem cells" are isolated from human embryos and are capable of making all the cells in the body. "Adult stem cells" are isolated from adult tissues and give rise to multiple cell types and are commonly thought to be involved in tissue repair. The most widely studied adult stem cells are the haematopoietic stem cells, the cells in the bone marrow that give rise to blood cells. Adult stem cells also exist in several brain regions and there is great interest in developing ways to stimulate these brain stem cells to repair the brain, for example after stroke or Parkinson’s disease. The olfactory sensory neurons in the nose are continually replaced throughout life from a stem cell that we have shown recently can develop into many different cell types, apart from olfactory sensory neurons. This source of adult stem cells holds potential for cell therapies in which a patient’s own cells are transplanted back into their own body, obviating the technical and ethical issues of embryonic stem cells and the problems of transplant rejection by the immune system. This talk will discuss the biology of olfactory stem cells and propose how they might be used for cell transplantation therapies in the future.

Published article relevant to Professorial lecture

Stem Cells - A Beginner's Guide

by

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Introduction[1]

The promise of the new "stem cell" biology is that it is hoped that one day these cells might be used to replace parts of the human body lost through injury or disease. It may be possible for humans to grow new parts, just as lizards grow new tails or amphibians, new legs. Transplanting bone marrow stem cells to replace those lost in cancer therapy is already clinical practice and clinical trials are underway to repair the heart using these cells. The creation of new pancreatic cells for diabetes is under intensive research in animal studies, and treatments for brain diseases such as Parkinson's disease and Alzheimer's disease are thought possible. These developments in biotechnology promise great advances in treating human ailments, but at the same time as raising challenging ethical dilemmas for society. In this paper some of the terminology of stem cells is presented and the biology of these cells is introduced. The focus here is therefore not on the ethical issues explicitly but rather presents the scientific background to a debate on the ethics of this, exciting and rapidly advancing science. In particular this chapter introduces the concepts of "embryonic and adult stem cells" and their therapeutic potential, both dreamed of and currently embodied.

Embryonic stem cells

The advent of the microscope in the seventeenth century allowed Robert Hooke and Anton von Leevanhoek to observe cells for the first time. Their observations eventually led to the concept that all organisms are made of single units called "cells". We know now that some organisms are only a single cell (for example bacteria) but even single celled organisms often live in colonies, sometimes with other single celled organisms, resulting in more complex structures and cell specialisations. Slime moulds, for instance, are a fungus that can live as separate cells but which come together in specialised structures called "fruiting bodies" to reproduce. Lichens are colonies of algae and bacteria that live in large colonies that superficially resemble plants. Larger organisms are comprised of many cells, all of which are genetically equivalent but which live together in a large "colony", the multicellular organism, in which each cell type is highly specialised and dependent on all the others for survival. So, all organisms in nature are comprised of cells. Multicellular organisms, such as humans, are distinguished from single-celled organisms, such as bacteria and algae, by the specialisation of the cell types and their inter-dependence such that the reproduction of the organism is left to specialised "germ cells". Thus "growth" and "reproduction" of the multicellular organism are separate functions whereas they are equivalent in single-celled organisms - proliferation of the cells represents both processes in such organisms.

Scientists interested in human and animal development have been fascinated for years in unraveling the mystery of how the many cell types in complex, multicellular organisms could arise from a single cell, the fertilised egg. In organisms such as frogs, the single egg gives rise to the whole organism directly. In mammals the situation is more complex because the developing embryo must first of all attach

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to the wall of the uterus in order to gain sustenance and grow. As a consequence, the majority of cells formed in the first few hours or days of the mammalian embryo, the “blastocyst”, are specialised for attacking the mother’s uterine wall and attaching to it, rather like a cancerous growth, to form a blood supply and the membranes which surround and protect the embryo in the womb. Only a small number of cells in the blastocyst are reserved to give rise to the embryo proper. These cells are known as "embryonic stem cells". Thus an embryonic stem cell is, conceptually at least, a non-specialised cell whose progeny will give rise to the thousands of different cell types that make up the mature organism.

Embryonic stem cells are typically isolated from the human blastocyst about five days after fertilisation, the time at which they are also used for IVF, and when they are still independent of the mother: implantation does not take place until eight days after fertilisation. The stem cells must be dissected free of the rest of the blastocyst, the rest of which will develop into the placenta (to attach to, and feed from, the mother’s uterus) and the membranes which surround and protect the embryo as it develops inside the womb.

It is not easy to grow human embryonic stem cells. Once they are isolated from the blastocyst they do not simply grow in a standard broth of culture medium, they require contact with other cells. Typically this requires a layer of cells called "fibroblasts" which are specialised, differentiated cells found, for example, in the skin. These are grown into a sheet on the bottom of a culture dish and then exposed to with gamma irradiation that stops them dividing further. The human embryonic stem cells grow on top of the irradiated fibroblasts in a growth medium supplemented with blood serum from a fetal calf. The embryonic stem cells grow in clumps that, after 2-4 weeks, are chemically or mechanically dissociated, removed from the first culture, diluted and grown again on a new layer of fibroblasts. This process is repeated several times until a large number of similar cells is achieved. Several sequences like this produce colonies of apparently homogeneous cells that can be further dissociated and expanded in number, thus creating a "cell line". The feeder layer of fibroblasts is typically derived from mouse and all the current embryonic stem cell lines available around the world were created in this way. This raises both scientific and ethical issues. Incubating human cells with mouse cells may allow viruses and other pathogens to “jump” between species thereby introducing new diseases into humans. This is thought to be a very low probability but nevertheless remains a risk. (Despite this risk, clinical trials transplanting cells from pigs into humans are underway in the United States. In fact, several laboratories are developing genetically engineered pigs designed to “fool” the human immune system when transplanted as cells or organs.) Recently a method was developed to grow human embryonic stem cells using human fibroblasts. This obviates the risk of mouse cells for embryonic cell lines derived in the future but the currently available cells are considered inappropriate for transplantation.

Adult stem cells

In recent years it has been realised that adult tissues may also harbour stem cells but here the terminology begins to get a bit mixed up. Often, references to "stem cells" in

adult tissues do not connote "multipotent" stem cells which give rise to multiple tissue types, like embryonic stem cells, but rather mean "cells that give rise to several cell types restricted to a tissue type". Thus has arisen the terms "haematopoietic stem cells" giving rise to the blood cells, "epithelial stem cells" giving rise to the skin, "neural stem cells" giving rise to some cells in the brain, and so on. Adult stem cells then are commonly understood to be "undifferentiated" cells residing in adult tissues that can give rise to the cells of a single tissue type. They also maintain their own presence, a property known as "self-renewal".

The best studied of the adult stem cells are the haematopoietic stem cells that give rise to all the cell types of the blood and immune system. These cells are thought to be small, inconspicuous, undifferentiated cells which live in the bone marrow. Several attempts have been made to isolate these stem cells but there is still debate about their exact identity. Surprisingly, recent research has demonstrated that haematopoietic stem cells can give rise to cells other than blood. In fact, they have been coaxed into becoming epithelial cells, liver cells, muscle cells, heart cells, and even brain cells! In mice these adult stem cells have been used to repair the injured heart and injured skeletal muscle, and to re-stock the bone marrow stem cells after radiation that is normally lethal. In other words adult “stem” cells, once thought to be restricted to producing only a few restricted cell types, are being shown to produce a much wider range of cell types, that is, they may in fact be “multipotent”.

Human haematopoietic stem cells can be obtained from bone marrow by placing a needle through the hipbone (pelvis) and down into the bone of the thigh (the femur). They can also be released by a drug from bone marrow and subsequently isolated from blood samples. Despite this relative ease of harvesting, they prove difficult to isolate and grow in culture, partly because they are uncommon, being about only 1 in 100,000 cells in bone marrow. Such is their success in animal experiments that a clinical trial is underway using bone marrow stem cells to repair the heart after a heart attack. The advantage of this procedure is that the patient's own stem cells are used, obviating the technical problems of immune rejection. And of course any ethical problmes raised by embryonic stem cells are also avoided. I will therefore discuss two other sites where adult stem celss can be found.

Stem cells in the adult brain

An interesting stem cell is located in the brain. For many years there has been an understanding, even a dogma, that brain cells cannot be replaced, especially nerve cells (“neurons”). The brain, it was thought, was formed during early life and had no capacity to repair itself. This makes sense because self-repairing tissues are usually those exposed to the environment such as the skin and gut, and the liver, which is particularly vulnerable to ingested toxins from the food. The liver is well known in its restorative capacity – even after removal of two thirds of its volume, it can regrow to its original size! The liver appears to grow according to metabolic “need” and to “know” its appropriate maximum size so that children can receive transplants of pieces of adult liver without the liver filling up the inside of the child recipient’s body. On the other hand, the brain is protected from the external environment by the skull, and even protected from the internal environment by the so-called “blood-brain

barrier". This barrier, comprised of specialised structures to regulate flow of nutrients and toxins from the blood into the brain, is effective against many drugs, for example, which can find their way into most other tissues of the body. The assumption has been that the brain is in a privileged and protected position in the body, and would seem to have no "need" to produce new cells as none would normally be destroyed by the environment. "We are born with all the brain cells we will ever have" is the notion that has permeated even the popular wisdom.

About thirty years ago it was noticed that there are dividing cells in the brains of mice. Because of the nature of the observations they were dismissed as evidence of new neurons being formed because they might have been other cells types, such as "endothelium" the cells that line the blood vessels, or they may have been "glial cells". Glial cells, or "glia", are the support cells of the nervous system and are ten times more numerous than neurons. In other words, the notion of new nerve cells was too much of a heresy to overthrow the accepted wisdom without stronger evidence than simply the presence of dividing cells. This did not come until about 10 years ago when a group in Canada managed to isolate cells from the adult mouse brain, grow them in culture, show that they could divide and then differentiate into both neurons and glia. This evidence was too strong to resist and the accepted wisdom was turned on its head, so to speak, but this did not yet show that new neurons and glia are produced in the brain. Further experimentation was needed to show that the dividing cells in the brain can produce neurons (or glia) and that these neurons incorporate themselves into functional circuits. The evidence is accumulating that newly formed neurons can indeed migrate away from the site of formation into sites of functional circuits and they look like functional neurons, but it is exceedingly difficult to prove that they are actually functional, and no one has yet provided convincing proof.

There is debate about whether there is continuing production of new neurons in the adult primate brain. The first study failed to show any cell division in the monkey brain, but that study was criticised because it examined the brains of pregnant females and for technical reasons the dividing cells may have been missed. This was followed by new evidence demonstrating that there were dividing cells in the adult monkey brain, in the same regions seen in mouse and rat. Similarly it has now been shown that there are also dividing cells in the adult human brain, in the hippocampus. These experiments are difficult to do; they relied on patients suffering from cancer who gave their permission to be injected with a chemical that incorporates into the nuclei of dividing cells. When those volunteers died their brains were examined for dividing cells. Dividing cells were found in the hippocampus indicating that cell division occurs there even though definitive proof was not gained that these turn into neurons. There are, it appears, stem cells in the brain, though we do not know for certain precisely what they do.

Stem cells in the nose

The focus of my own research is the biology of the olfactory epithelium, the sense organ of smell, which is found at the back of the nose. This organ is of special interest to me because the sensory neurons are continually replaced throughout adult life. There is a continual cycle of regeneration and repair of the neural connections

between the nose and the brain, a process known as “neurogenesis”. This continuing neurogenesis occurs in human adults and provides unique opportunities for those interested in cell transplantation therapy. For a start the olfactory glial cells are unusual because they encourage the regeneration of the sensory neurons and facilitate their reconnection with the brain. Animal studies, including our own, show that olfactory glia also encourage regeneration and reconnection when transplanted into the injured spinal cord. After olfactory glia transplantation, animals with severed spinal cords can even regain movement in affected limbs.

We have started a small clinical trial to test the effect of transplantation of a olfactory glia into the injured spinal cord in human paraplegics. This is not stem cell transplantation but it illustrates the possibility of harvesting cells from the nose, growing them *in vitro*, and returning them to the same patient via transplantation into another site. Our recent experiments indicate that within the adult olfactory organ are true stem cells that give rise to the new sensory neurons. These olfactory stem cells are “multipotent” with the potential to differentiate into many different cell types including, but not limited to, neurons and glia. Stem cells isolated from the human nose could potentially provide an accessible source of cells for transplantation therapies. Olfactory tissue can be obtained under local anaesthetic, with few side effects and no loss of the sense of smell.

Stem cell transplantation therapies

In the last 20 years an understanding has advanced rapidly of how the embryo normally develops *in utero*. This includes the identification of many signalling molecules released by cells to influence the development of cells around them. The signals, or "growth factors", are important regulators of cell development and many are now available commercially. With the use of different growth factors in the growth medium, embryonic stem cells from the mouse have been directed into developing into cells from pancreas, blood vessels, bone, blood, muscle, heart, fat, brain, skin, and immune system. These experiments in the laboratory "dish" provide evidence of the "developmental potential" of embryonic stem cells. In other words, it seems that human embryonic stem cells, even when removed from the embryo, have the ability to produce many different cell types and thus may differentiate into these cells when transplanted into the diseased or injured patient. This developmental potential brings to life long-held dreams of growing human replacement organs, although as yet human embryonic stem cells have not shown the same developmental potential as mouse cells.

There has been some progress in demonstrating the success of mouse embryonic stem cells in transplantation studies: they have been used to assist repair of the injured spinal cord and injured heart muscle. A major barrier to the use of embryonic stem cells for tissue repair is that, like any "foreign" tissue graft, they are the target of the patient's immune system. This can be reduced by finding a close immunological "match", but this will require a "bank" of thousands of embryonic stem cell lines with which a patient's immune type can be compared. Immune suppressant drugs are available which reduce tissue graft rejection, but this treatment is not ideal because it raises the risk of infectious disease in the recipient.

What other types of cells, tissue or organs are contemplated for stem cell therapies? Already stem cell therapies are being used after radiotherapy or chemotherapies for cancer. These treatments kill the rapidly dividing cancer cells but can also target the blood-forming cells, the "haematopoietic stem cells", many of which form part of the immune system. Haematopoietic stem cells are harvested from the bone marrow before cancer therapy and injected back into the same patient to assist recovery of the immune system. Cancers of the blood, such as leukaemia, cannot be treated this way because their own haematopoietic stem cells may be the cause of their cancer. In these cases haematopoietic stem cells from another person can be used. Such transplants are being done today, but they are limited by the difficulty in finding transplanted cells that are matched immunologically with the patient. Family donors can help here, but (as above) it is envisaged that a bank of donor cells would need to be set up in order to match all possible patients. This bank would need cells from tens of thousands or millions of donors and patients from minority ethnic groups and indigenous groups would be difficult to match. In the future, an alternative therapy could use a patient's own stem cells, isolated and grown from another tissue before radiotherapy or chemotherapy, and transplanted into the bone marrow afterwards, making a perfect immunological match.

As mentioned above, the heart is also a target for stem cell therapies, based on observations in animals, which showed that transplanted haematopoietic stem cells would "home in" on damaged heart muscle, lodge there, and differentiate into cardiac muscle and blood vessels and so repair the heart. This extraordinary behaviour does not normally seem to happen after a heart attack in humans but these experiments suggest that it might occur if enough cells are transplanted. Again, the preferred treatment is to transplant a patient's own haematopoietic stem cells, to avoid immunological rejection, and clinical trials are now underway in patients with heart disease to test the effectiveness of this treatment.

Another cell type that is the object of research efforts is the -cell of the "islets" of pancreas, which normally produce insulin. Type I diabetes results from a loss or malfunction of these cells and incapacity to produce insulin, a hormone essential for regulating the levels of glucose in the blood. -cells make good candidates for stem cell therapy because they don't necessarily have to be formed into a complex tissue in order to function. Potentially transplanted cells need only be in contact with the blood to perform their function, they need not even be in the pancreas, unless it is shown that the other cells of the pancreas are necessary for maintaining the health of the -cells. Mouse embryonic stem cells have been induced to form -cells *in vitro* and to produce insulin when stimulated by glucose. The same has not been shown of human embryonic stem cells nor any adult stem cells but the potential is demonstrated by the animal experiments.

The skin is another tissue of great interest. For many years now skin grafting has been commonplace initially using donor skin and subsequently skin grafted from the patient's own body. The donor skin is used in emergency conditions to close the wound to prevent infection and dehydration. This skin will be rejected by the immune system and slough off eventually but its application can give technicians time to grow up layers of skin generated from pieces of skin taken from elsewhere on the body.

Recent experimentation seeks to isolate skin stem cells with the hope of discovering a "molecular soup" in which to grow them to very quickly produce large amounts of graft-able skin from small amounts of starting material. Alternately molecules might be found to accelerate the growth of new skin at the edge of the wound.

Researchers are exploring whether embryonic stem cells might be useful in repairing the spinal cord after injury causing paraplegia or quadriplegia. Long thought unrepairable, even spinal cord injury is succumbing to modern biotechnology. Already in clinical trials are transplantation therapies based on "glial" cells and embryonic stem cells are being used in animal experimentation. Other researchers are investigating drugs, hormones and even gene therapies for spinal cord injury. It is possible that in the future paraplegia might be cured by a combination of therapies that include stem cell transplantation.

Embryonic versus adult stem cells in human therapies

If adult stem cells prove to have the same developmental potential as embryonic stem cells there would be no obvious scientific or medical reason for the use of embryonic stem cells in human therapies, though the latter could be easier to obtain. Even if adult stem cells do not have unlimited developmental potential, given the large number of sources of adult stem cells it is possible that either an appropriate stem cell can be found in the injured tissue or organ, or that another stem cell might be coaxed into differentiating into the desired therapeutic cell type.

In most cases an “autologous” cell is preferable than a “foreign” cell that will interact with the patient’s immune system. Thus it would always be preferable to derive stem cells from the patient. There may be times when this is not practical, for example in emergencies, like burns, when there is no time to derive the cells of interest. There may be other times when the patient’s own cells carry a genetic mutation which is the cause of the disease being treated. In the future such mutations might be corrected using genetic engineering before transplantations of the patient’s cells, or it might be easier to transplant cells from a immunologically matched donor.

In my view it is difficult to envisage any therapies in which it would be necessary to use embryonic stem cells *if adult stem cells could be used*. This remains one of the big uncertainties in the field at present: the relative potentials of embryonic versus adult stem cells. It must remain speculation and the focus of further investigation. There is a good case to be made for continued research on embryonic stem cells even if they will not be used in future therapies. The strongest case is that they will help us understand the cellular and molecular events of early embryonic development and the formation of tissues and organs. This will have long-term implications for future therapies designed to overcome diseases and disorders of development such as schizophrenia, and autism. These future therapies will not be based on transplantation but on manipulation via drugs or nutrition. Another case for embryonic stem cell research is that research on how to manipulate the developmental outcome of embryonic stem cells will inform the research into adult stem cells.

Therapeutic cloning

"Therapeutic cloning" is a term used to describe another approach to supply stem cells for cell, tissue or even organ transplantation. The experiments that resulted in Dolly the cloned sheep demonstrated that it is possible to "clone", make a copy of, an adult animal by taking a skin cell from an adult animal, grow it *in vitro* for a period, extract the nucleus, inject the nucleus into an egg which has had its nucleus removed, and then implant into a mother using *in vitro* fertilisation (IVF). This procedure is theoretically possible in humans and it has been claimed to have been done, with the embryo kept alive for a few cell divisions. It is legal in several countries, including Singapore and the USA, provided that US government funds are not used.

The proposal is to use therapeutic cloning to make a copy of a person and extract stem cells from the clone for transplantation back into the person to replace diseased cells or repair damage. Another scenario involves leaving the embryo to develop further and "harvesting" organs from it after they have developed. Therapeutic cloning is illegal in many countries because of the many ethical issues it raises. There are some technical issues as well because the "clone" is not an exact copy unless the person's own mother's egg is used in the cloning procedure. This is because the mother's egg contributes genetic material to the offspring in the form of "mitochondrial" DNA. The mitochondria are cellular components that are essential for energy metabolism in the cell. They contain their own DNA that resides in the cell outside the nucleus. Thus mothers pass more than just their nuclear DNA to the offspring. It is not known whether such differences in mitochondrial DNA are large enough to render a cell different enough to raise an immune response after transplantation and lead to graft rejection.

“Heal thyself” – tissue self-repair

One area of research that is gaining increased interest is the concept of stimulating adult stem cells to repair the tissue in which they reside. It appears that many, if not all, adult tissues contain “stem cells” or cells that act like stem cells when removed from the body and grown in vitro. If so, why doesn’t the body automatically repair all tissues and organs? Why doesn’t the heart heal itself, or the brain? We don’t know. Perhaps they do and we don’t notice anything unless the damage is massive and beyond self-repair. Certainly this is an emerging focus for research and a possible avenue for future drugs aimed at stimulating self-repair.

It is of interest that cells from several brain regions and the spinal cord are proposed to be able to divide and differentiate into neurons. "Stem cells" have been isolated *in vitro* from pieces of human brain removed during surgery for epilepsy and for Parkinson's disease. This raises the possibility that "dormant" stem cells might be present throughout the nervous system, which just need "activation" to repair the damaged or diseased brain. Experiments in rats have shown that “dormant” brain stem cells can be activated and can migrate to a region of brain damage. There are several laboratories around the world that are looking for ways to activate these dormant cells with the hope of applying to human diseases, including Parkinson's disease, Alzheimer's disease and Huntington's disease.

Conclusions

Stem cell biology is a rapidly advancing frontier. New research continues to uncover new secrets of the human body leading to new capabilities to manipulate these secrets to improve the health and welfare of humankind. With these new capabilities come new ethical challenges unthought of ten years ago: such as balancing the rights of unborn embryos against the rights of unhealthy children and adults; such as balancing the potentials therapeutic cloning for cell, tissue or organ transplantation against the potential for creating cloned human beings. Is it right to continue embryonic stem cell research when there is a possibility that adult stem cells could be used instead?

My view is that both embryonic and adult stem cell research should continue in parallel. As future candidates for cell transplantation therapy, neither is yet an obvious choice because of the uncertainties in producing embryonic stem cells and unproven capabilities of adult stem cells. From a scientific point of view it makes sense, at this stage of understanding, to investigate both types of stem cells. We still don't yet know how to manipulate the biology of either stem cell to produce differentiated cell types on demand to produce specialised cell for transplantation, or to grow organs containing multiple cell types. History shows that knowledge flows between fields and informs the research into all stem cells. It is my belief that eventually most cell transplantation therapy will be done with adult stem cells if they show the developmental potential of embryonic stem cells. Even if adult stem cells only have limited abilities, restricted to the tissue from which they derive, it might be more appropriate in most circumstances to use adult stem cells, avoiding the problems of immune rejection and obviating the ethical issues surrounding the use of embryos. Stem cell transplantation is upon us. With further knowledge of stem cell biology it may be possible to avoid transplantation altogether and eventually stimulate self-repair.

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