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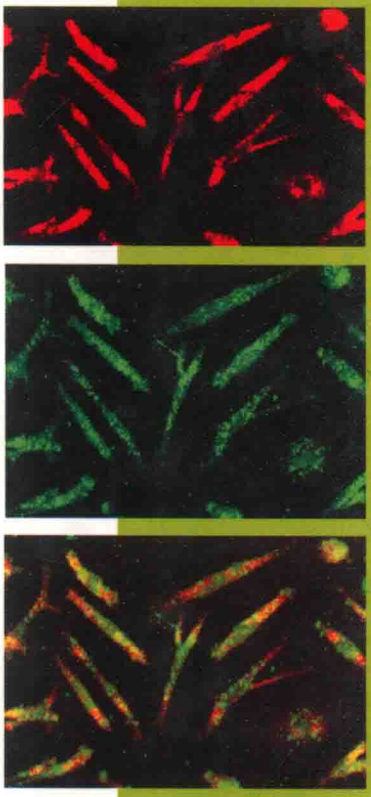

Stem Cell Repair and Regeneration

Imperial College Press

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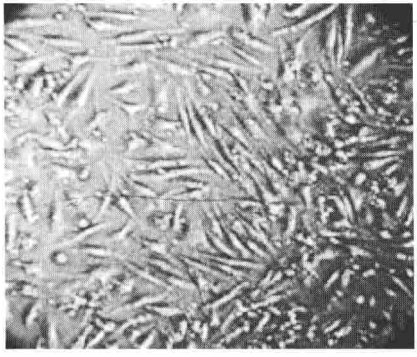

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Stem Cell Repair and Regeneration

PREFACE

The last few years have witnessed an explosion in interest in stem cells, arising from reports that they are capable of developmental plasticity so that they are capable of regenerating any damaged tissue in the body. Whilst stem cells have been hailed as the solution to many serious degenerative and debilitating conditions, it is clear that many issues remain to be resolved—like the relative merits of embryonic versus adult stem cells, and the size of the risk that some stem cell lines may produce tumours when they are transplanted *in vivo*. These issues are being actively debated in public and scientific arenas.

This book resulted from a *Symposium in Stem Cell Repair and Regeneration* (September 2004, Imperial College, London) that provided a forum for the participants to discuss many aspects of stem cell therapy. It is based on the lectures delivered at the symposium and covers a spectrum of interest from fundamental stem cell biology through stem cell manipulation to the potential clinical applications of stem cell therapy. The editors would like to thank the authors for their contributions, which have made possible the publication of this volume.

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1

AN INTRODUCTION TO STEM AND PROGENITOR CELL BIOLOGY

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Introduction

1998 saw the publication of two papers describing the growth *in vitro* of human embryonic stem (ES) cells derived either from the inner cell mass (ICM) of the early blastocyst or the primitive gonadal regions of early aborted foetuses. Work on murine embryonic stem cells over many years had already established the amazing flexibility of ES cells, essentially able to differentiate into almost all cells that arise from the three germ layers. The breakthrough in 1998 was to keep human ES cells in a state of prolonged undifferentiated proliferation by the use of a blocking factor (leukaemia inhibitory factor) that, when removed, allowed the dividing cells to go down specific, directed differentiation pathways. The realisation of such pluripotentiality (see below) has, of course, resulted in the field of stem cell research going into overdrive with the establishment of many new biotechnology companies (http://www.stemcellresearchnews.com/catalog1677.html), stem cell banks and regulatory bodies to oversee their use, with a genuine belief that stem cell research will deliver a revolution in terms of how we treat cardiovascular disease, neurodegenerative disease, cancer, diabetes and the like. However, many people believe that early human embryos should be accorded the same status as any sentient being and thus their 'harvesting' for stem cells is morally unjustifiable.[1,2]

With this in mind, other sources of malleable stem cells have been sought. In the adult, organ formation and regeneration was thought to occur through the action of organ- or tissue-restricted stem cells [*i.e.* haematopoietic stem cells (HSCs) making blood, gut stem cells making gut]. However, we now believe that stem cells from one organ system, for example, the haematopoietic compartment, can develop into the differentiated cells within another organ system, such as the liver, brain or kidney. Thus, certain adult stem cells may turn out to be as malleable as ES cells and so also useful in regenerative medicine. In this chapter I summarise the important attributes of stem cells from a variety of sources, clarify the terms used and try and get beyond the hype that so often accompanies apparent new 'breakthroughs' in medical research. I also emphasise the importance of stem cell biology to the development and treatment of cancer.

Stem Cell Research Comes of Age

Morbidity and mortality as a result of malfunctions in vital organs plague even the most technologically advanced societies. Because of a dearth of transplantable organs there is a growing hope that stem cells may be the answer to mankind's prayer to be able to replace tissues worn out by old age and ravaged by disease. Indeed, it is impossible to open a newspaper today without seeing yet another apparent 'breakthrough' in stem cell research, the more optimistic hoping for an elixir of life — the promise of immortality. More realistically, regenerative medicine is already delivering results, for example, biotechnology companies like Osiris Therapeutics, Inc. are making 'off-the-shelf' products from human mesenchymal stem cells for bone (OsteoCel^{TM}) and joint (Chondrogen^{TM}) repairs. This type of tissue repair uses the body's own three-dimensional matrix and growth factor milieu. More difficult will be the realisation of the holy grail of tissue engineering — the creation of whole complex internal organs, such as the liver and kidney, outside the body.

ES cell lines are invariably derived from the ICM of 5-day-old embryos (blastocysts) or foetal gonadal tissue (Fig. 1). Blastocysts are usually from *in vitro* fertilisation programmes that would have otherwise been discarded, though some have been deliberately created. These blastocysts are composed of about 100 cells, of which 30–40 make up the ICM. A misconception is that ES cells are 'totipotent', *i.e.* they have the potential to form an entire human being. This is incorrect because they have been separated

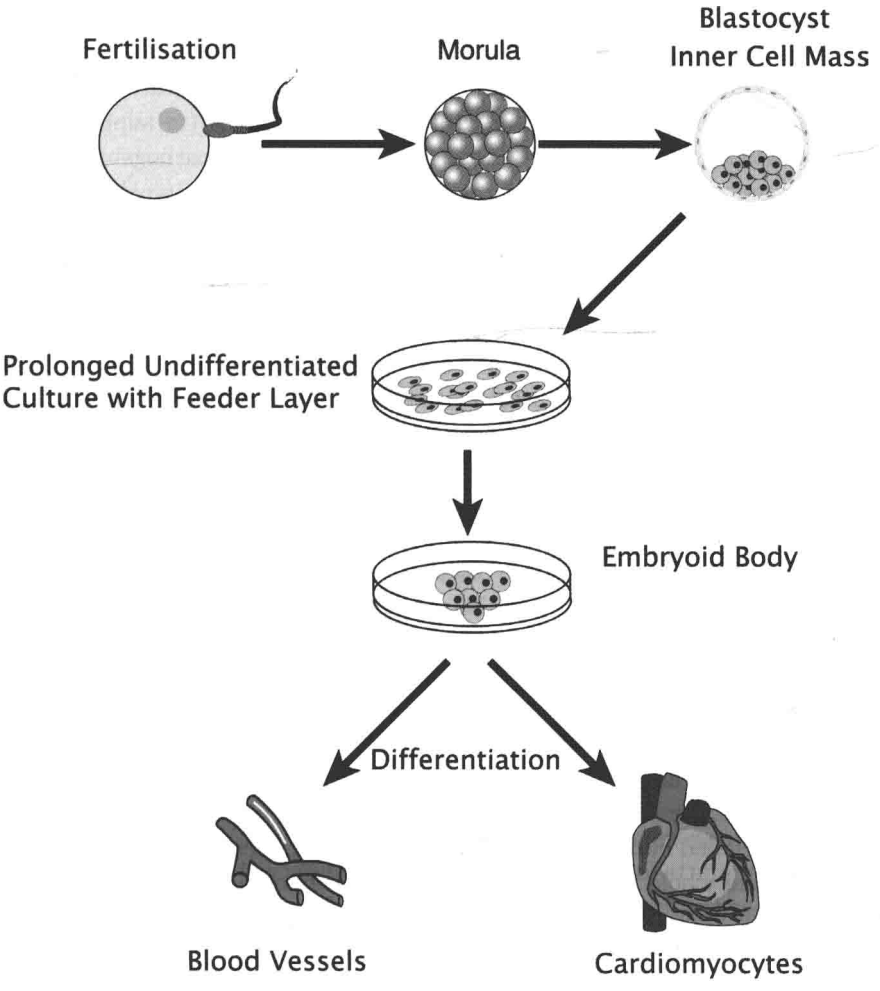

Fig. 1. Scheme for the generation of new cells and tissues from ES cells.

from the supporting trophectoderm, a tissue that protects the ICM, and implanted in the uterus. Legislation regarding the use of ES cells varies around the globe. In countries like the UK and Australia[3] new cell lines can be created from spare embryos with the uncompensated permission of the donors, but in the USA at the moment, in a compromise between proponents and critics of ES cell research, federal funds (taxpayers' money) can only be used on ES cell lines created *before* 9 August 2001 ($\approx$70 existing cell lines), the rationale being that such cells, while exhibiting

pluripotency, do not have the ability to develop into a whole human being, so that the sanctity of human life is not compromised by their use. However, many of these 'approved' cell lines are now not available, and most of the others were grown in the presence of mouse feeder cells (to supply essential growth factors), exposing human cells to potentially pathogenic murine viruses and proteins, thus rendering them unsuitable for clinical therapies. With such considerations in mind, along with the realisation that up to 3000 Americans die every day of diseases that could be combated with ES cells, not forgetting that the nation's best scientists may move abroad to more supportive environments, intense political lobbying is now hoping to reverse this decision. Further impetus has been given to the ES cell lobbyists in the USA by the realisation that other countries, as well as privately funded entities are forging ahead, perhaps with irresponsible research, while research funded by the National Institutes of Health would be (hopefully) carefully regulated and have safety as a major priority. In the UK, the Human Fertilization and Embryology Authority (http://hfea.gov.uk/Home) licences and monitors all human embryo research, including using embryos for stem cell extraction. Moreover, on 19 May 2004 the world's first stem cell bank opened in the UK, jointly overseen by the Medical Research Council (MRC) and Biotechnology and Biological Sciences Research Council (BBSRC) (http://www.ukstemcellbank.org.uk/), acting as a repository and supplier of all types of human stem cells, not just embryonic but also those derived from foetal and adult tissues and discarded cord blood.

Of course, for ES cell research to have a major impact on regenerative medicine, cloned human blastocysts will be needed because replacement cell therapy, like whole organ transplants, must overcome the obstacles posed by immune system incompatibility (graft rejection). Somatic cell nuclear transfer (also called 'therapeutic cloning') offers the possibility of using the patient's own genome to generate ES cells and so overcome this problem. Therapeutic cloning involves taking a cell from the patient and inserting it into an enucleated egg from an anonymous female donor (not as easy as it sounds), nurturing it to the blastocyst stage and then harvesting the ES cells from the ICM. Each cell would be almost identical in genetic terms to the cells of the patient who would be treated with them, and the first successful demonstration of human ES cells derived in this manner has now been published.[4] Many argue that cloning embryos for regenerative medicine is not exactly therapeutic for the embryo (true!), and really what is happening is placing society on the slippery slope to reproductive cloning (see below).

Nuclear transfer technology has in fact been with us for over 50 years, and scientists such as John Gurdon were able to clone frogs by transplanting nuclei into enucleated frogs' eggs. Unsurprisingly, cloning was more successful when relatively primitive cells like blastula cells were used, rather than adult skin or gut epithelial cells. However, it was the birth of Dolly the sheep in 1996 that attracted so much media attention, being the first mammal to be cloned from a cell (a mammary cell) extracted from an adult. Scientists and lawmakers in particular make an important distinction between 'therapeutic cloning' and 'reproductive' cloning, the latter being described as implanting a cloned embryo into a woman's womb — a practice that is strictly illegal in most countries, including the UK. Nevertheless, maverick fertility expert Severino Antinori has recently claimed to have several women pregnant with cloned embryos under his care, though few have taken these claims seriously. Apart from the moral boundary between therapeutic and reproductive cloning, the most vociferous criticism of human reproductive cloning comes from scientists themselves finding that almost all cloned animals develop one or more abnormalities.[5,6] So, while no one really doubts that ES cells are likely to be the most flexible of all stem cells, the ethical issues surrounding their use have prompted the search for alternative adult sources.

Adult Stem Cells

According to some (Michael Fumento; http://www.fumento.com/biotech/stemcell.html) there is a 'stem cell cover-up', a deliberate attempt to downplay the therapeutic value of adult stem cells in order to divert more attention (money) to ES research — this has been called 'stem cell wars'. While I do not wish to get into this conspiracy theory, adult stem cells, in particular, bone marrow transplants, have been used to treat diseases like leukaemia since the 1970s.

Properties of adult stem cells

A hierarchy of potential

As we have already seen, the appeal of ES cells is the fact that they are pluripotent, able to differentiate into almost all cells that arise from the three germ layers, but not the embryo because they are unable to give rise to the placenta and supporting tissues. On the other hand, most adult

tissues have multipotential stem cells, cells capable of producing a limited range of differentiated cell lineages appropriate to their location, *e.g.* small intestinal stem cells can produce all four indigenous lineages (Paneth, goblet, absorptive columnar and enteroendocrine), CNS stem cells have trilineage potential generating neurons, oligodendrocytes and astrocytes,[7] while the recently discovered stem cells of the heart can give rise to cardiomyocytes, endothelial cells and smooth muscle.[8] However, describing tissue-based stem cells as 'multipotential' may be incorrect if, as it appears, some adult stem cells, when removed from their usual location, can transdifferentiate into cells that arise from any of the three germ layers (so-called plasticity). The least versatile are unipotential stem cells, cells capable of generating one specific cell type. Into this category we could place epidermal stem cells in the basal layer of the interfollicular epidermis that produce only keratinised squames and certain adult hepatocytes that have long-term repopulating ability.[9] Some would argue that there is no such thing as a unipotential stem cell, and really these cells should be called 'committed progenitors'. While there is no doubt that in some tissues, *e.g.* the gastrointestinal tract and haematopoietic renewal systems, there are committed stem cells (progenitors) with more limited division potential than their multipotential stem cells, in the epidermis these unipotent cells do have a large clonogenic capacity capable of producing large sheets of cells for the treatment of burns patients.

Self-maintenance

Stem cells are usually relatively undifferentiated, not having the functional specialisations of the progeny that they give rise to. Perhaps the single most important property of stem cells is their ability to self-renew. They are normally located in a protective environment (niche; French recess), and in a tissue such as the small intestine, where the cell flux is in one direction, they are found at the origin of the flux. In the heart, they are located in areas of the least haemodynamic stress. Although only a small percentage of a tissue's total cellularity, stem cells maintain their numbers if, on average, each stem cell division gives rise to one replacement stem cell and one transit amplifying cell (an asymmetric cell division). The interactions with the stem cell niche are crucial to this process (Fig. 2) and the controlling factors are rapidly becoming elucidated. In the *Drosophila* ovariole, a stem cell niche known as the 'germarium' has been defined, and here germline stem cell (GSC) number is maintained by the close apposition of GSCs with

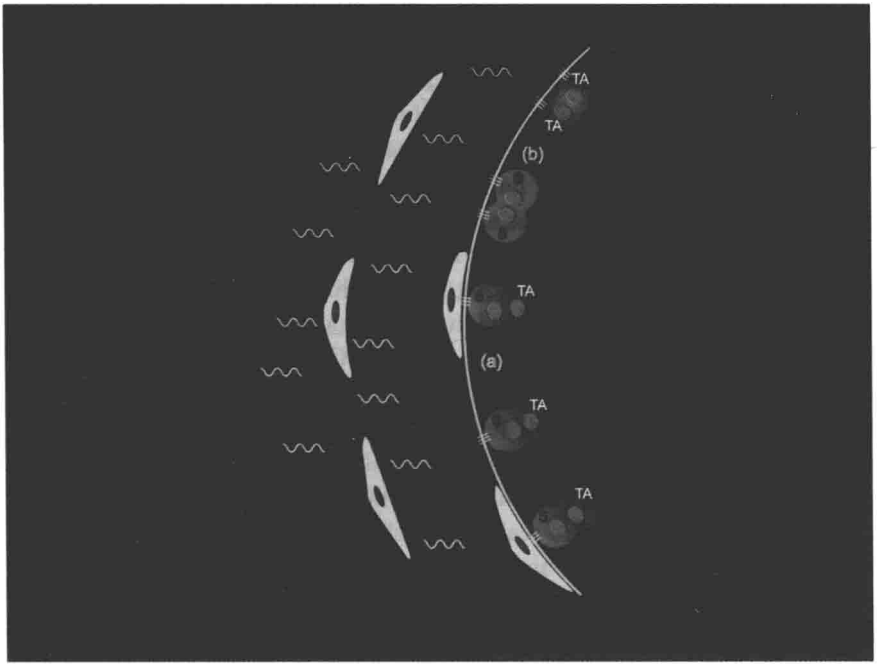

Fig. 2. The stem cell niche. The niche (microenvironment) is likely to control many facets of stem cell behaviour including the rate of division, the orientation of mitotic axes and the type of division (symmetric vs. asymmetric). The effectors are likely to be secreted soluble factors (growth factors), integral membrane proteins that require cell–cell contact, such as the receptor Notch and its ligand Delta, and cell adhesion molecules such as integrins that maintain contact with the extracellular matrix.

cap cells; Armadillo (fly β-catenin) and decapentaplegic (DPP) [a homologue of mammalian bone morphogenetic proteins (BMPs)] signalling are involved (10). Likewise, in the *Drosophila* testis, GSC number is strictly controlled by the interaction with so-called hub cells[11] — in both ovariole and testis; disruption of DPP signalling and/or Armadillo/Adenomatous Polyposis Coli (APC) interactions can result in supernumerary GSCs due to alterations in the orientation of the mitotic axes (Fig. 2). In mammals too, cadherin/catenins and BMP signalling are also involved in the maintenance of haematopoietic stem cell number through interactions with osteoblasts.[12]

Proliferation, clonogenicity and genomic integrity

Stem cells are slowly cycling but highly clonogenic. Teleologically, it would seem prudent to restrict stem cell division because DNA synthesis can be

error-prone. Thus, in many tissues stem cells divide less frequently than transit amplifying cells. In the intestine, stem cells cycle less frequently than the more luminally located transit amplifying cells, and in the human epidermis the integrin-bright cells have a lower level of proliferation than the other basal cells. In hair follicles, the hair shaft and its surrounding sheaths are produced by the hair matrix that is itself replenished by the bulge stem cells. As befits true stem cells, the bulge cells divide less frequently but are more clonogenic than the transit amplifying cells of the hair matrix. Combined with an infrequently dividing nature, stem cells would also appear to have devised a strategy for maintaining genome integrity. Termed the 'immortal strand' hypothesis or Cairns hypothesis, stem cells can apparently designate one of the two strands of DNA in each chromosome as a template strand, such that in each round of DNA synthesis while both strands of DNA are copied, only the template strand and its copy are allocated to the daughter cell that remains a stem cell.[13] Thus, any errors in replication are readily transferred (within one generation) to transit amplifying cells that are soon lost from the population. Such a mechanism probably accounts for the ability of stem cells to be 'label retaining cells' after injection of DNA labels when stem cells are being formed.[14]

Adult stem cell identity

In many tissues and organs the identity of the stem cells has remained either elusive or at least equivocal, and the search for true stem cell markers has become frenetic. Some have argued that stem cell markers are like the spots on a Dalmatian dog, useful for identification, but not appearing to play an essential role in dog (stem cell) function. However, in the bone marrow the recognition of cells with the properties of self-renewal and multilineage differentiation potential is well advanced. In fact, such cells were recognised operationally back in 1961 by Till and McCulloch as cells that gave rise to multilineage haematopoietic colonies in the spleen (colony forming units — spleen). In the human bone marrow the sialomucin CD34 is a haematopoietic cell surface antigen that has been extensively exploited for the selection of long-term repopulating cells with multilineage potential, although not all HSCs express this marker. In the mouse, HSCs are known as KLS cells (c-kit$^+$lin$^-$Sca-1$^+$). An alternative method of enriching for HSCs exploits the fact that some cells have evolved a cellular protection mechanism against toxic metabolites and xenobiotics. This mechanism involves the expression of efflux pumps that belong to the ATP-binding

cassette superfamily of membrane transporters, and such cells are able to efflux a combination of Hoechst 33342 and Rhodamine123, thus appearing at the bottom left corner of a dual parameter fluorescent activating cell sorter (FACS) analysis — hence, called the side population (SP). There are SP cells in many other tissues that might well correspond to their multipotential stem cells.[15]

In the basal layer of the interfollicular epidermis, clusters of likely stem cells highly express melanoma chondroitin sulphate proteoglycan, along with the β-1 integrin, the receptor for type IV collagen (a component of the underlying basement membrane).[16] In the central nervous system, neural stem cells and probably their transit amplifying descendants express both the intermediate filament nestin and an RNA-binding protein known as musashi 1. Musashi 1 was first identified in *Drosophila* and thought to be responsible for the asymmetric divisions of sensory organ precursor cells; it may also be a marker for intestinal crypt stem cells.[17]

Molecular control of stem cell behaviour

It appears likely that the local microenvironment, through a combination of cells and extracellular matrix components will govern all aspects of stem cell behaviour. This has led to the concept of the stem cell niche (Fig. 2) that supports and controls stem cell activity. In the intestinal mucosa the pericryptal myofibroblasts that ensheath the crypts serve as niche cells secreting Wnt proteins. One of the most striking observations was made through targeted disruption of the *Tcf*-4 gene. Tcf-4 is a partner protein for β-catenin, and the heterodimer transactivates a number of genes involved in cell cycle progression: the absence of Tcf-4 results in the small intestinal crypts failing to maintain a proliferative zone.[18] In turn, Wnt signalling is kept in check by BMPs, also produced by pericryptal mesenchymal cells.[19] Paradoxically, activation of the Wnt pathway through mutation of the *APC* gene is the earliest recognizable abnormality in human colonic carcinogenesis.[20] In the CNS and haematopoietic system, a key regulator of stem cell renewal appears to be Bmi1, a member of the Polycomb family of transcriptional repressors. Bmi1 targets genes such as $p16^{Ink4a}$ and $p19^{Arf}$ preventing stem cell senescence by respectively maintaining cyclinD/Cdk4 signalling and Mdm2 destruction of p53.[21] Bmi1 is, in fact a downstream target of sonic hedgehog (SHH) through the latters activation of the Gli family of transcription factors. SHH acts on the receptor complex of patched (PTCH) and smoothened (SMO), blocking the inhibitory

influence of PTCH on SMO, resulting in SMO signalling activating Gli and transcription of its target genes like Bmi1. In the skin, mutations in *PTCH* characterise human nevoid basal cell carcinoma (BCC) syndrome (also known as Gorlin's syndrome), and clearly SHH signalling in follicular outer root sheath cells leads to BCC, a tumour characterised by a marked lack of features of terminal differentiation.[22] The Notch family of receptors is also critical for stem cell self-renewal, particularly in HSCs[23]; engagement of ligands of the Delta and Jagged families causes cleavage of the intracellular portion of Notch and its translocation to the nucleus where it acts as a transcription factor. Constitutive Notch signalling is a powerful signal for leukemogenesis (reviewed in Ref. 24).

Adult stem cell plasticity

A large body of evidence now supports the idea that certain adult stem cells, particularly those of bone marrow origin, can engraft alternative locations (*e.g.* non-haematopoietic organs), particularly when the recipient organ is damaged, and transdifferentiate into cell types that function appropriate to their new location (Fig. 3). Hence, there is considerable excitement in using

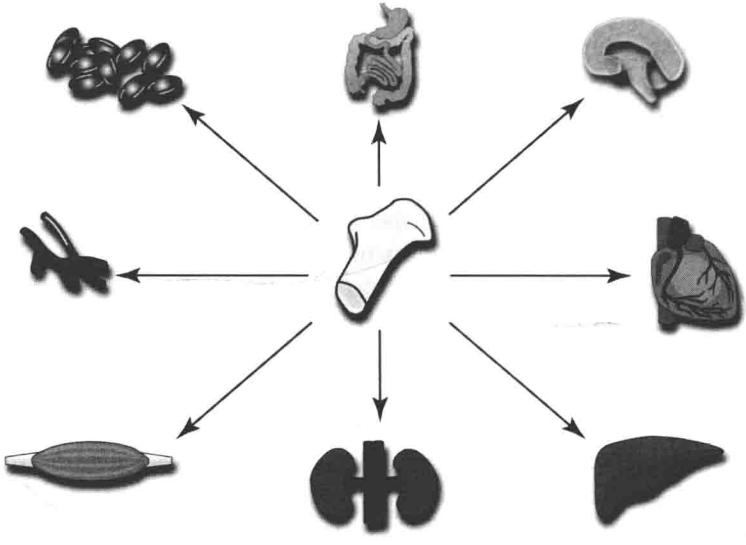

Fig. 3. Adult stem cells, particularly those from the bone marrow, may under certain circumstances migrate to damaged organs, engraft and transdifferentiate into cells of that organ.