

REVIEW

Regenerative medicine. Opportunities and challenges: a brief overview

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Regenerative medicine is a new multi-disciplinary field aiming at the repair or replacement of disease body parts. The field is progressing at an unprecedented pace and although the opportunities are immense, many hurdles lie ahead. This brief review analyses the opportunities and challenges faced by regenerative medicine.

Keywords: regenerative medicine; tissue regeneration; opportunities and challenges

1. INTRODUCTION

The newly recognized multi-disciplinary field of regenerative medicine aims at the replacement, repair or restoration of normal function to disease organs/tissues by the delivery of safe, effective and consistent therapies composed of living cells, administered either alone or in combination with specially designed materials (Langer & Vacanti 1993).

The concept of tissue regeneration is by no means new—going back a long time as illustrated by the famous legend of Prometheus. Prometheus was a champion of human equality. He stole fire from Zeus, which he then gave to the mortals. As a punishment for his crime, Zeus bound him to a rock and sent a giant eagle to eat his liver. However, his liver re-grew every night and the eagle had to return again and again.

Tissue regeneration is also a primitive event, occurring in many organisms such as newts where it is well known that a sectioned limb will be completely regenerated after a six to eight-week period.

In humans, regenerative medicine such as solid organ transplantation and cell therapy have been practised for many years, for example, kidney transplantation was first performed in 1954 (Murray & Holden 1954) and bone marrow transplantation since 1968 (for review see Appelbaum 2007).

The field covers what was thought originally to be separate therapeutic areas: cell therapy and tissue engineering (creation of *in vitro* tissues/organs for subsequent transplantation as fully functioning organs or as tissue patches) among others (Baron & Storb 2008). Therapeutic examples include replacement

(transplantation), repair (exogenous cell therapy) or regeneration (mobilization of endogenous pools of stem cells).

2. CELLS AND MATERIALS ARE THE CORNER STONE OF REGENERATIVE MEDICINE

2.1. Cells

A variety of cells types have been and are currently used in regenerative medicine (Buttery & Shakesheff 2008). Before the isolation and identification of human stem cells, cells isolated from adult tissues were employed. These were used in combination with natural or man-made materials and provided an insight into the mode of action of biomaterials, as regards to their regenerative properties (Xynos *et al.* 2000*a,b*).

Mouse stem cells were isolated in 1981 (Evans & Kaufman 1981) after immunosurgical ablation and human embryonic stem (ES) cells in 1998 (Thomson *et al.* 1998).

Since then, interest has focused on the potential use of these cells for regenerative medicine because these cells are able to differentiate into lineages of the three germinal layers (endoderm, mesoderm and ectoderm; Guillot *et al.* 2007). In parallel, much work has also been carried out to demonstrate the potential of the so-called ‘adult’ stem cells for regenerative medicine. These cells also identified as ‘niche-specific’ stem cells reside in every tissue of the body although some of these cells are better recognized and characterized than others (e.g. bone marrow stem cells, cardiac stem cells, stem cells from the umbilical cord, including placenta, amniotic fluid and Wharton’s jelly; Weiss & Troyer 2006). ‘Adult’ stem cells have a more limited

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One contribution to a Theme Supplement ‘Translation and commercialization of regenerative medicines’.

differentiation potential than embryonic stem cells as they occur further down the developmental pathway.

In principle, these cells could be removed from a patient, incorporated into a tissue construct and put back into the same individual when repair becomes necessary, thereby removing the need for immunosuppression. Clearly, adult-derived progenitor cells need to be investigated and their clinical usefulness established. However, as with mature cells, problems with accessibility, low frequency (e.g. there is roughly one stem cell per 100 000 bone marrow cells), restricted differentiation potential and poor growth, limit their usefulness for tissue engineering. 'Adult' stem cells derived from the bone marrow have been used for more than 40 years for the treatment of haematological disorders. Throughout the 1950s and 1960s, it was shown that transplantations of 'haematopoietic stem cells' (HSCs), isolated from the bone marrow, could reconstitute the depleted bone marrow following irradiation. This culminated in 1963 when Mathé demonstrated the long-term survival of a leukaemia patient treated with HSCs (Mathe *et al.* 1963). Bone marrow transplantation is now a routine medical procedure (Appelbaum 2007).

Following this it was noticed another cell type in bone marrow explants initially called the 'fibroblast colony-forming cell' (Friedenstein *et al.* 1974). This was later shown to be a stem cell. They are now referred to as marrow stromal cells or mesenchymal stem cells (MSCs). These cells resemble cells of the connective tissue (fibroblasts) and in contrast to HSCs, can be easily grown in cell culture dishes. MSCs can differentiate into mesoderm-derived tissues among others while HSCs can re-constitute the haematopoietic system. By changing the composition of the medium in which they are grown, MSCs can be selectively differentiated into bone cells (osteocytes), fat cells (adipocytes) and cartilage cells (chondrocytes). This property has made them an attractive choice for bone and cartilage tissue engineering (Wise *et al.* 2009), especially since they may be used to treat the person from whom they were isolated as an 'autologous' transplant. There is increasing evidence in the literature that these cells may also differentiate into other lineages. Adult MSCs also have limitations, they can only divide a finite number of times (depending on the age of the donor), which limits their supply, and they may accumulate genetic changes over time.

Embryonic stem cells with their relentless capacity to proliferate and differentiate on all cell lineages of the three germinal layers are considered to have a significant potential for regenerative medicine. However, ethical issues and the fact that these cells will be allogeneic in origin when administered to the individual, lessen their potential. An alternative source of pluripotent stem cells offering less ethical concerns has recently been proposed.

In 2006, Takahashi & Yamanaka (2006) showed that skin cells from both embryonic or adult mice can be reprogrammed into induced pluripotent stem cells (iPS) by the use of what is now known as the Yamanaka's factors: Oct 4, Sox 2, Myc and Klf4. This fantastic discovery was later demonstrated to be

possible using human skin (Takahashi *et al.* 2007; Yu *et al.* 2007).

These discoveries caused considerable excitement, as researchers envisioned that these iPS cells will not have the ethical baggage that ES cells have and can be made patient-specific. However, the use of oncogenic virus made the iPS cells rather unsafe for clinical applications. Improvements from the original methods appear regularly in the literature. These include virus-free methods to avoid insertional mutagenesis, use of a combination of vectors to deliver seven reprogramming genes including the Yamanaka cocktail, direct use of protein products from reprogramming genes and the 'hit and run' strategy using a sequence of all Yamanaka's factors, separated by spacer elements with a transposome vector called piggy BAC (for review see Clarke & van der Kooy 2009).

In addition, much efforts are being directed in an attempt to produce patient-specific iPS cells free from alterations in their genomic DNA and after ensuring that the patient's genetic defect (previously existing) is corrected (Park *et al.* 2008). Comparative studies between ES cells and iPS cells have recently been carried out. These comparative studies demonstrate that these cells do not behave identically (Feng *et al.* 2010; Hu *et al.* 2010; Stadtfeld *et al.* 2010):

- the potential to differentiate into specific lineages is markedly superior in ES cells;
- iPS cells frequently show apoptosis and senescence signs.

Chin *et al.* (2009) compared the genome-wide expression patterns in mice ES cells and iPS cells and discovered a small stretch of DNA in the long arm of chromosome 12. In this region, two previously unreported genes and a series of mRNA sequences were consistently activated in ES cells and silenced in iPS cells.

2.2. Cell expansion

Two-dimensionally grown static cultures, results in the differentiation of only a small number of cells, are cumbersome, time-consuming and labour intensive. Furthermore, they lack mixing and monitoring. Ideally three-dimensional cultures should be carried out to form a cohesive, organized, perfused and functional tissue (Polak & Mantalaris 2008). This aim has been greatly aided by the development and the use of bioreactors, supplying nutrients, oxygen, removing catabolites, monitoring pH and applying mechanical stresses to stimulate the formation of extracellular matrix (Dvir & Cohen 2008).

A bioreactor is a device that reproduces the physiological environment (including biochemical and mechanical functions) specific to the tissue that is to be regenerated. Bioreactors can also be used to apply mechanical strength during maturation of the tissue and for studying and understanding the mechanical factors influencing tissue regeneration.

In order to obtain a large number of identical cells of a specific phenotype, encapsulated cells should be seeded into a three-dimensional scaffold and the

construct cultured in a controlled environment where by nutrients can be provided and waste products removed. Three-dimensional dynamic culture conditions are likely to provide an environment more akin to an *in vivo* situation (Placzek *et al.* 2009). Encapsulated undifferentiated cells will grow indefinitely in the bioreactor whereby upon administration of specific growth agents cells can also be maintained differentiated and in large quantity for an unlimited period (Siti-Ismael *et al.* 2008).

3. OPPORTUNITIES OFFERED BY REGENERATIVE MEDICINE

3.1. Cells

Regenerative medicine is likely to transform the way we practise medicine. With regenerative medicine, the repair of unhealthy tissue or restoration of bodily functions can be achieved by a ‘once and for all’ treatment thereby differing entirely from the current medical practice using pharmacological or surgical procedures. With conventional pharmacological approaches, the patient is likely to require therapy for a considerable period of time, if not forever. Although cell therapy would appear to be expensive to produce/and or administer, the aim will be to produce a permanent restoration of the organ/tissue’s lost function. Ultimately this is anticipated to be more economical and beneficial than current medical practice.

The opportunities for regenerative medicines are immense especially in light of an ever-increasing ageing population with associated ailments. For example, cells can be used as vehicles for gene therapy and cultured cells can be used to study *in vitro*, a specific disease process or for drug development. The discovery of iPS cells also offers the potential to produce disease models to support new drug discovery as well as patient-specific cells for therapy.

3.2. Biomaterials

Many materials are able to induce a cellular reaction from the host tissue, and hence, do not need the addition of cells. These have been used for regenerative purposes. Materials can be used as cell carriers or as vehicles for the delivery of therapeutic agents or angiogenic factors. Ideally, for implantation, the material should be resorbable and for pharmacy it should be insoluble (Hench & Polak 2002). The advent of nanotechnology has allowed further developments in the field of biomaterials, since appropriately nano-modified surfaces can induce a better cellular response than untreated surfaces and a more sustained, robust and specific cell differentiation, after cells have been placed in contact with these materials (Gentleman *et al.* 2009). This in fact is a rather specific mode of action, which depends on the type of engineered material, its molecular structure, its nano-modified surface and its mechanical properties, among others.

A scaffold should be:

- biocompatible and biodegradable with a controllable degradation rate;
- degradation products should be non-toxic;

- highly porous with an interconnected architecture, of controlled shape, size and alignment to facilitate oxygen, nutrients and waste transfer as well as rapid vascularization and tissue in-growth;
- resistant to stress and strain and hold good mechanical properties;
- be clinically compliant (good manufacturing practice (GMP)).

4. CLINICAL TRANSLATION

Worldwide research in the field is intense and several trials are currently progressing through the clinic (Hall *et al.* 2010). For example, artificially constructed bladders have been successfully implanted into young children (Atala *et al.* 2006) and a trachea built from a patient’s divided trachea and seeded with autologous mesenchymal cells was successfully transplanted back into the same patient (Macchiarini *et al.* 2008).

The mechanism of action of stem cell therapy is still being determined, but the general consensus suggests that the most probable mechanism might be through the release of cytokines and other growth-promoting molecules. Harnessing the potential of these biologics enables one to foresee a future where a ‘once and for all regenerative pill’ might become available. If the field of regenerative medicine continues to progress at its current pace and is able to become well established, it is likely to be a major revolution similar to that witnessed, for example, by the advent of monoclonal antibodies.

There are multiple coordinating efforts in this active multi-disciplinary field such as the UK National Stem Cell Network (www.uknscn.org) and the Alliance for Regenerative Medicine in the USA (www.alliancerm.org) (among others). Furthermore, major pharmaceutical companies are actively investing in stem cell research (e.g. the Pfizer regenerative medicine initiative located in the UK and USA, the GSK alliance with the Harvard Stem Cell Institute (USA)) and in the UK, the first ever public/private partnership (Stem Cells for Safer Medicine (SC4SM)), has been set up to exploit human embryonic stem cells for drug safety testing.

5. CHALLENGES

5.1. Current research undertaken to overcome these

Research into the main components of regenerative medicine is intense worldwide. Particular efforts are being made at overcoming the current challenges that the field is confronted with in order to translate basic science into robust clinical products. Below is a list of the main areas of research to overcome current hurdles.

6. TRANSITION FROM BENCH TO BEDSIDE. CURRENT HURDLES FOR THE CLINICAL APPLICATION OF STEM CELL RESEARCH

- Robust lineage specific differentiation of stem cells with the desired functional attributes. Safety and

- efficacy being the two main requisites for the translation of research into robust clinical products.
- Determination of what would be best for cell therapy: use of precursors or terminally differentiated cells.
 - Further development of suitable surface markers to identify pluripotent/multipotent/ precursors cells.
 - Understanding the important role of the micro-environment/host tissue.
 - Further development of natural or man-made materials using nanotechnology to improve their surface and enhance specific cell attachment, proliferation and differentiation.
 - Development of biomaterials together with stem cells for drug discovery or as therapeutic agents.
 - Reduction of currently found karyotype changes during prolonged culture conditions.
 - Development of specific clonal assays for the selection of cells with appropriate karyotype.
 - Enhancement of angiogenesis, relevant to large *in vitro* constructs or after cell engraftment.
 - Development of robust cell delivery systems and assessment of best route of administration, determination of optimal cell number and most appropriate timeframe for product delivery.
 - Change from currently used laboratory practice of two-dimensional cultures to a more natural three-dimensional methodology, using good laboratory practice (GLP) facilities.
 - Development of optimal methods for automated cell expansion using non-invasive sensors to assess cell viability.
 - Development of appropriate imaging methods to trace cell fate, engraftment and cell survival.
 - Overcoming immunological barriers when allogenic products are employed.
 - Enhancement of graft vascularization, including further development of suitable materials and or bioreactors.

7. REGENERATIVE MEDICINE: THE WAY FORWARD

Surgery and drug therapy are currently accepted options for clinical practice. Large numbers of patients are treated with drugs, which are typically self-administered. It is possible to foresee that with cell therapy selected patients will be treated by specialist involvement that will require the training of a new generation of medically qualified personnel and healthcare auxiliary staff.

Regenerative medicine is a new field and hence the regulatory landscape is evolving. It is not as yet clear whether regulatory agencies, including the Food and Drug Administration Agency (FDA) and the European Medicinal Agency (EMA), will consider stem cell therapy as a biological or a device. The FDA has set up the Office of Combination Products and the Office of Cellular, Tissue and Cell Therapies. Furthermore, and unlike the landscape in a single country such as the USA, in Europe, the EMA may recommend guidelines, but whether these will be adhered to by the members states remains to be seen.

It is clear that product consistency, uniformity and stability are of paramount importance. Safety requisites should include toxicity, tumour formation (applicable only to ES and iPS cells) and immunogenicity. In those instances where the transplanted cells become fully incorporated into the tissue, unwanted/unexpected effects must be considered in advance. Cell therapy must offer a better clinical outcome than current therapies and must be cost-effective in order to be accepted by healthcare sectors such as the National Health Service. Furthermore, clinical trials must be carried out within acceptable clinical practice and due ethical considerations. Exalting the promise of regenerative medicine to vulnerable patients is unacceptable. The International Society of Stem Cell Research has recently issued useful guidelines (<http://www.issrc.org>).

Cell therapy is likely, at least initially, to be expensive. Both product development and clinical trials require considerable levels of funding. The cost of the product is considerable if one is to account for the cost of growth factors and small molecules needed for viable cell preparations, in addition to the cost of medical care, both direct (healthcare sector) and indirect (carers and others).

8. CONCLUSIONS

The field of regenerative medicine is here to stay, as exemplified by the nascent but exponential growth of examples of translation from bench to bed side (e.g. cardiac (Green & Alton 2008), limbal regeneration (Kolli *et al.* 2010), bladder (Atala *et al.* 2006) and tracheal (Macchiarini *et al.* 2008) implantation). The current hurdles are by no means insurmountable and, therefore, it is reasonable to assume that we can look forward to a more mature and highly rewarding field.

The opportunities for regenerative medicines are immense especially in light of an ever-increasing ageing population with associated ailments. For example, cells can be used as vehicles for gene therapy (Kawamura *et al.* 2009) and cultured cells can be used to study *in vitro*, a specific disease process or for drug development. The discovery of iPS cells also offers the potential to produce disease models to support new drug discovery as well as patient-specific cells for therapy (Hollander & Wraith 2008). As regards to biomaterials, again this field is intensely researched; the advent of nanotechnology has allowed the development of specially designed nanosurfaces that encourage cell attachment, cell growth and differentiation (Hench & Polak 2002; Wise *et al.* 2009).

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