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Review Article

Stem cells for therapeutic use in tissue engineering: a promising tool or an approachable reality?

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CONTENTS

Abstract

The limited potential of the human body to regenerate itself has led scientists to seek new strategies aimed at improving or substituting the physiological mechanisms of homeostasis. Stem cell technology appears to be the most promising way to reach the goal of tissue engineering, *i.e.*, to rebuild injured or damaged human tissues. Unfortunately, many problems remain to be solved before we can transfer results from experimental *in vitro* and *in vivo* models to clinical trials. In this review, the most promising fields of tissue engineering, where clinical trials are ongoing or will soon be initiated, are discussed, with particular emphasis on bone, cardiac muscle and neural regeneration. The goal of this review is to highlight advances in stem cell research, and thereby contribute to the discussion of the use of stem cells in human therapy.

Introduction

The human body has the capacity to repair itself efficiently. Blood and tissues like skin proliferate and regenerate during the entire life of a human being, while other tissues like bone or cartilage can regenerate only small defects due to the limited ability of cells in these tissues to proliferate. Tissues like brain or myocardium have lost this ability because cells either can no longer proliferate or have serious difficulties in reorganizing correct tissue architecture after injury. Within this framework, stem cells are a promising tool for tissue repair because of their extensive proliferation and plasticity, and ability to transdifferentiate, characteristics that theoretically allow them to regenerate the structure of injured tissues (1). In the last 10 years, based on these properties, scientists have focused their attention on developing clinical applications of tissue engineering using stem cell-based strategies. Unfortunately, many problems remain when trying to transfer *in vitro* or *in vivo* research to clinical trials.

One of the main problems with the therapeutic use of stem cells is the identification of accessible sites within the human body where an adequate amount of such cells can be collected. Homologous stem cell transplantation can result in pathogen transmission and requires immunosuppressive treatment like any other tissue or organ transplant procedure, so that the gold standard today is the use of autologous stem cells (2). Surgical access to the collection site is limited by the associated morbidity. Stem cells are present in virtually every tissue of the human body, but collecting these cells from, for example, the central nervous system (CNS) would result in such residual deficits after the collection procedure that possible advantages from the use of the cells collected would be minimal (3). On the other hand, the selected donor site should contain a high percentage of stem cells relative to the volume of tissue collected. Adipose tissue, for example, is not particularly rich in stem cells, but this problem can be overcome by increasing the volume of donor tissue. Unfortunately, in most human tissues, the amount of tissue that can be obtained is very limited in order to avoid excessive weakening of the organ or apparatus of the body. Therefore, improvements in collection and sorting techniques are needed in order to conduct clinical trials of stem cell-based technologies.

The second major problem is the nature of the stem cells collected, which is related to their functional properties; cells must be capable of extensive proliferation in order to repair macroscopic defects and to represent a therapeutic alternative, but proliferation must follow a predetermined and reproducible scheme (4, 5). The cells must undergo controlled proliferation, as well as differentiation, after collection from their physiological source;

activation of these mechanisms must respond to stimuli in the surrounding environment, or otherwise this might result in uncontrolled multiplication or differentiation abnormalities, with the consequence of an alteration in activity. The plasticity that characterizes stem cells should represent an advantage and not a drawback when the cell type is transferred to the host. However, a stable and functional cell type should result after *in vitro* or *in vivo* activation. Cellular proliferation and cell type stability depend directly on the cell originally collected. It is important to define what we mean by "stem cells" and how they are identified, *e.g*., what antigens are used. Although stem cells are probably present in all human tissues, in most cases they have lost much of their initial pluripotency, turning into multi- or even unipotential cells, so that it would probably be more appropriate to call them progenitor cells rather than stem cells (6, 7). The identification of stem cells through an antigen-antibody reaction has been performed using so many antigenic patterns that this raises confusion in managing stem cell-based therapies and makes it very difficult to compare clinical trials performed by different institutions.

The last point to be considered in the therapeutic use of stem cells is their interaction with biomaterials. Building *de novo* tissues to fit a 2-dimensional technology, such as *in vitro* cell culture, in a 3-dimensional organism like the human body is associated with many difficulties and often results in failure. The dynamic process of organogenesis is far from being completely understood, so that the only way to develop a 3-dimensional structure today is to seed the cells in a scaffold, mimicking the volume and shape of the part to be reconstructed and replaced (8). To do this, we need to identify stem cells that are able to interact with biomaterials, and *vice versa*, leading to the correct 3-dimensional tissue architecture that will help to establish cell-to-cell and cell-to-matrix contact. More animal studies must be conducted before clinical trials can be performed. Many of the cancer clinical trials have failed because of the lack of adequate preclinical testing in animals other than mice, as murine biology does not often reflect that of humans despite the similarity between the murine and human genomes.

The purpose of this review is to shed some light on the most promising fields where stem cells have been used in clinical applications of tissue engineering.

Bone regeneration

The need for bone has been increasing in Western countries in the past few decades as a result of the longevity of the population. In the elderly, fractures tend to occur more frequently because of bone weakening due to a decrease in calcium content in the extracellular matrix (ECM) and to slower bone remodeling mechanisms (9). The periostium and the bone marrow stromal fraction are rich in bone progenitors, and since the early 1990s bone has been one of the targets of clinical tissue engineering (10). On the other hand, the characterization of osteoblast genesis (11, 12) has led to the strategy of inducing bone differentiation by stimulating quiescent progenitors located in the tissue itself. The advantage of this approach is the use of autologous precommitted cells, thereby decreasing the risks of *ex vivo* stimulation.

Following the report of Pittenger *et al*. (13), bone marrow stroma was also identified as a source of multipotent cells, in addition to being the main source of osteoblasts. Osteogenic progenitors have also recently been identified in dental pulp and adipose tissue (14, 15), opening up a wide range of sources for the collection of adult autologous stem cells for bone tissue engineering. However, some of the issues discussed previously have limited many clinical applications to single case reports (5, 16).

The use of bone progenitors or bone-committed cells in clinical trials falls into two main areas: scaffolding and the local administration of drugs with bone-inducing activity (17). Scaffolding is considered the safest way to replace a bone defect, acting, at least initially, as a weight-bearing bone substitute. From a biological point of view, the importance of scaffolding in bone development resides in the need for a 3-dimensional architecture for bone genesis. The scaffold used in clinical trials can either be resorbable or nonresorbable (18). A nonresorbable scaffold, after its *in vivo* placement, will be colonized by host cells, acting as an architectural infrastructure remaining *in situ* (19, 20).

Both resorbable and nonresorbable scaffolds must have osteoconductive activity in order to be colonized, and in same cases may also have osteoinductive activity, stimulating cells to release growth factors (21). These polymers have been used in some trials as drug delivery systems (22-24). Bone tissue engineering consists of preplating the stem cells onto the scaffold *in vitro* before transplanting the manufactured bone replacement. The limit of this approach is the obstacle that nonresorbable scaffolds in particular could represent for ECM deposition, altering tissue morphology (25). Resorbable scaffolds are preferred due to the possibility of using them as shape tutors during the *in vitro* phase and the initial part of *in vivo* placement, when they would bear the load stress of the bone until new bone is obtained from the differentiated stem cells.

Many issues remain to be resolved. Which cells do we use? Is it more appropriate to use periostium progenitors or mesenchymal stem cells? Do they have to be differentiated *in vitro* or should they be transplanted in an undifferentiated stage? How long should they be cultured under selected conditions before transferring the complex scaffold/stem cells *in vivo*? Once the stem cells have committed to osteoblast lineage, can the cell type be considered stable?

Coupling drugs with bone-inducing activity to the scaffold itself may be the answer in some cases, using the scaffold as a delivery system for these drugs (26, 27). This method initially seemed promising, but protocols have not yet been standardized because of the difficulties in integrating recombinant human proteins with the local tissue environment (28-30).

The use of bioreactors could be another way to simulate physiological conditions during bone morphogenesis, avoiding the use of drugs within the tissue. Mechanical forces, like shear or fluid stress (31), may provide better results, because of the importance that mechanical stress has in bone differentiation and remodeling.

Cardiac regeneration

Heart failure is one of the most common diseases in the modern world, affecting approximately 23 million people. After years of intensive research, however, the single most effective treatment is still cardiac allograft transplantation, with all the problems related to the shortage of donors and the need for immunosuppression. The need for an alternative therapy has aroused interest in stem cell therapy, especially following the findings of Anversa *et al*., whereby transplantation of autologous bone marrow-derived cells (BMDCs) in a mouse model of heart injury resulted in the integration of the transplanted cells into damaged tissue and the generation of *de novo* myocardium (32).

Cardiomyocytes are specialized cells electrically coupled to beat synchronously, and due to gap junctions, the electrical stimulus can be transmitted to all cells simultaneously. When an ischemic event occurs, fibrotic tissue substitutes cardiomyocytes, leading to cardiac hypertrophy and progressive heart failure. In order to re-establish cardiac functionality, transplanted stem cells should integrate into damaged tissue, differentiate to cardiomyocytes, form gap junctions and beat together with pre-existing cardiomyocytes. Furthermore, they should promote angiogenesis by a paracrine effect or by transdifferentiating into endothelial cells.

In 2002, Strauer *et al.* published the first clinical trial using bone marrow stem cells to repair myocardial infarction in 10 patients. Autologous bone marrow mononuclear cells (BMCs) were transplanted via balloon catheter along with standard therapy into the damaged heart, and significant improvement in comparison with the control group was observed. The size of the infarcted region or cardiac performance (left ventricular end-systolic volume and contractility, myocardial perfusion of the infarct region) was improved after 3 months of follow-up (33).

Several clinical trials have subsequently been performed, demonstrating the safety and feasibility of BMDC transplantation using either unselected, c-Kit+ or Sca-1+ BMDCs administered via transcatheter or surgically (34). None of these studies reported arrhythmias, postoperative compliance, tumor formation or ectopic tissue formation (33, 35-37). A recent study by Perin *et al.* also demonstrated beneficial effects for transendocardial injection of autologous BMDCs in 5 patients with severe ischemic heart failure who were candidates for allografting. Follow-up at 2 and 6 months showed no significant reduction in the infarcted area, but significant improvements were recorded in the outcome of exercise tests, and 1 patient no longer required a heart transplant (36).

Despite these promising results, the small number of patients, variability in the cell type of the cells injected and the route of administration, the use in combination with standard therapy and the lack of a blinded placebo control group make it difficult to evaluate the efficacy of transplantation therapy (34). Furthermore, discussion is still open as to the real role of BMDCs in cardiac repair. Some scientists sustain that cells normally committed to a hematopoietic cell type are unable to undergo transdifferentiation to cardiomyocytes and explain the benefits of cell therapy in terms of cell fusion, a paracrine effect and stimulation of angiogenesis (38-41). Other scientists, including Dr. Anversa, support the transdifferentiation theory, arguing that within the BMDCs a subpopulation of mesenchymal stem cells (MSCs) exist that are able to differentiate into cardiomyocytes when treated *in vitro* with 5-azacytidine and that transdifferentiation also occurs *in vivo* within the cardiac microenvironment (32, 42-44).

Clinical trials have also been performed using autologous skeletal myoblasts (SkMs) (45-47). SkMs are not proper stem cells but progenitors located in the skeletal muscle that are activated to repair injury to damaged tissue. These cells, obtained from a patient biopsy, can be easily expanded *in vitro* and then transplanted. Moreover, these cells are resistant to ischemia (48).

The first experiment demonstrating the ability of SkMs to repair injured cardiac muscle in a dog model of cryoinjury was published by Marelli *et al*. in 1992 (49), and the first clinical trial was performed by Menasche *et al.* in 2001 (46), followed by many others, often in addition to standard treatment**.** All these studies showed a significant improvement in New York Heart Association (NYHA) class and ejection fraction (34). Postmortem immunochemistry studies showed integration of transplanted cells into the damaged tissue of the host, but failed to demonstrate expression of cardiac markers. Furthermore, some patients had arrhythmia as a side effect (46, 47).

Autologous SkM transplantation has generated some clinical interest but its application is limited by significant cellular mortality after transplantation, by the delay of 3-4 weeks from biopsy to implantation due to the need to expand these cells *in vitro*, and by arrhythmias.

The use of biopolymers is also under evaluation; PLGA (polylactic-*co*-glycolic acid), PGA (polyglycolic acid) and PLA (polylactic acid) have been tested (50), but research is now oriented to more plastic and elastic polymers like collagen because of their adaptability to an organ like the myocardium, which continually contracts (51).

In conclusion, stem cell therapy for cardiac repair appears to be a very promising tool for application in the near future, but a better understanding of the role of transplanted cells within injured myocardium is needed, and the best cell type and the best time to inject stem cells after injury need to be determined. Furthermore, larger double-blind clinical trials are needed to assess the real efficacy of stem cell therapy.

Neural regeneration

The pathological hallmark of neurodegenerative diseases is a loss of neuronal cells. In disorders such as Parkinson's disease, stroke, amyotrophic lateral sclerosis (ALS), Huntington's disease, multiple sclerosis (MS) and spinal cord injury with a genetic, autoimmune or traumatic basis, different cells may be lost. Current therapies are not able to restore neural functionality and side effects often represent a limitation of appropriate treatments.

The main feature of neural tissue is the complex interconnection among the cells that allows the transmission of electrical and chemical signals. Thus, in order to reestablish functionality, transplanted or endogenous stem cells must integrate into the parenchymal architecture, differentiate into the proper neural cell type and establish connections with host cells. Finally, they need to functionally integrate into the host neural circuitry.

Several kinds of cells, such as embryonic stem cells, BMDCs and human neural stem cells, have been differentiated *in vitro* and *in vivo* into dopaminergic, cholinergic or glial neurons (52). Numerous preclinical studies in mice and rats showed that these cells can engraft the injured tissue of the host, differentiate and in some cases contribute to functional improvement (53-57).

Few clinical trials have been performed to assess the role of stem cell therapy in curing neurodegenerative pathologies (58-60). In a clinical trial of human fetal mesencephalic tissue transplantation in patients with Parkinson's disease, cells engrafted in the host and reinnervated the striatum for 10 years; the graft normalized dopamine release and some patients were able to withdraw from levodopa (61, 62). In a clinical trial in patients with stroke, precultured autologous MSCs administered peripherally migrated into the damaged area and produced improvement in functional recovery (63). In clinical trials of intrastriatal human fetal tissue transplantation in Huntington's disease, the graft integrated and established connections with host neural tissue, resulting in improvements in cognitive and motor deficits (64, 65).

However, despite the clinical evidence some issues have yet to be resolved. The rate of neural differentiation is still too low. Preclinical trials in mice and rats have not shown clear improvements in the outcome of the disease model tested (66). Also, the most suitable kind of cell (neural stem cells, embryonic stem cells, BMDCs), the rate of administration and the most appropriate injection protocol have yet to be established. Moreover, side effects have been reported. In a clinical trial of human fetal mesencephalic tissue transplantation, some patients with Parkinson's disease showed dyskinesia that was probably due to patchy and uneven reinnervation or to nondopaminergic activity in the graft (66-68). A recent report by Hofstetter *et al.* pointed out the risk of allodynia in neural stem cell transplantation for spinal cord injury due to astrocytic differentiation and aberrant axonal sprouting (69). Furthermore, some improvement in pathologies such as stroke, spinal cord injury and Alzheimer's disease appears to be attributable to a trophic mechanism rather than functional integration (66, 70).

Biopolymers for peripheral nerve regeneration have also been evaluated in animal models with promising results. PLGA tubes and Vycril appear to be a suitable scaffold for Schwann cells, demonstrating the ability to restore nerve function in a rat model of sciatic nerve resection (71).

Thus, although stem cell therapy has became a powerful tool in the hands of scientists, for neural application there is still a need for a better understanding of the differentiation and grafting properties of each kind of cell, the crosstalk mechanisms between stem cells and native cells, the trophic mechanisms involved in neuroprotection and neurodifferentiation, and especially the behavior of stem cells in pathological conditions.

Conclusions

As demonstrated by many reports, clinical trials on stem cell technology need to be improved before it can become an effective therapy. Animal models are not completely useful for elucidating tissue responses after transplantation of complex structures and systems such as stem cells. Until the mechanisms of self-renewal and differentiation have been elucidated, such therapy may cause more damage than benefit. The risks associated with this procedure often cannot be correctly assessed before treatment and may outweigh the morbidity associated with the pathology. Obtaining ethical committee approval is particularly difficult because many questions about these procedures remain unanswered.

Autologous stem cell transplantation may be a promising means of avoiding problems associated with immunosuppression. Increasing the number of stem cell collection sites within the human body may be a desirable goal, but it is not certain that adult stem cells recovered from different sites will have the same characteristics and will be suitable to replace any cell type. What we refer to as adult stem cells are often only progenitors, or undifferentiated cells that maintain a multipotential capacity, or the potential to become more than one term-differentiated cell type. The pros and cons of using as many progenitors as possible must be determined in order to manage a range of cellular tools for multiple clinical applications of tissue engineering. This will have two immediate advantages. With the identification of more than one collection site for stem cells, we could better manage surgical access and use alternative sources in case of degenerative or traumatic pathologies. The second advantage would be to reduce the need for external stimulation of stem cells in order to alter their fate; these manipulations are difficult and the results questionable. After the step from a controlled *in vivo* environment to a complex *in vivo* system, we cannot be sure that the changes made in cellular commitment will last forever. In other words, we need to reduce manipulations to a minimum for clinical trials. Furthermore, performing clinical trials in the absence of sufficient basic data could bring an

end to a promising field of research due to unsuccessful outcomes. More time is needed to better understand stem cell biology before trying to translate it into medical practice with unpredictable results.

Until then, however, we cannot ignore the possibilities that stem cells offer for improving clinical care, but we must explore the use of this technology as much as possible, while trying to avoid excessive enthusiasm that could expose us to massive disillusion. Although the first heart transplant was performed in 1967, 10 years passed before it became a reproducible procedure. Increasing clinical trials in fields where data have demonstrated the utility of stem cells is important to make their therapeutic use a reality and corroborate basic research.

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