

Review

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Tissue engineering and cell based therapies, from the bench to the clinic: The potential to replace, repair and regenerate

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Abstract

The field of Regenerative Biology as it applies to Regenerative Medicine is an increasingly expanding area of research with hopes of providing therapeutic treatments for diseases and/or injuries that conventional medicines and even new biologic drug therapies cannot effectively treat. Extensive research in the area of Regenerative Medicine is focused on the development of cells, tissues and organs for the purpose of restoring function through transplantation. The general belief is that replacement, repair and restoration of function is best accomplished by cells, tissues or organs that can perform the appropriate physiologic/metabolic duties better than any mechanical device, recombinant protein therapeutic or chemical compound. Several strategies are currently being investigated and include, cell therapies derived from autologous primary cell isolates, cell therapies derived from established cell lines, cell therapies derived from a variety of stem cells, including bone marrow/mesenchymal stem cells, cord blood stem cells, embryonic stem cells, as well as cells tissues and organs from genetically modified animals. This mini-review is not meant to be exhaustive, but aims to highlight clinical applications for the four areas of research listed above and will address a few key advances and a few of the hurdles yet to be overcome as the technology and science improve the likelihood that Regenerative Medicine will become clinically routine.

Introduction

Many diseases and or physical defects due to injury result in the loss of specialized cells within organ systems and lead to organ system dysfunction. For example, Parkinson's disease results in the progressive loss of dopaminergic neurons within the substantia nigra region of the brain leading to motor deficits that result in dystonia and dyskinesia. Injuries, such as meniscal tears and spinal cord injury, can also result in the degeneration and loss of tissue leading to physical defects that can affect normal behavior. Additionally, insulin dependant diabetes mellitus (IDDM), multiple sclerosis (MS) and other autoimmune disorders lead to a loss of tissue that disrupts normal metabolism and bodily functions. The potential

to treat these conditions with cell-based therapies holds promise for tissue/organ repair with the ultimate goal to regenerate and restore normal function. Several cell types will be discussed and are defined as; tissue specific differentiated cells, such as chondrocytes; progenitor cells isolated from specific tissues, such as bone marrow stem cells or neural stem cells; and embryonic stem cells that are derived from the inner cell mass of the developing blastomere.

Autologous Cell Therapy

Tissue specific differentiated autologous cells (as opposed to autologous progenitor cells, see below) harvested from an individual, cultured ex vivo to expand, and reintro-

duced into a second site for repairing damaged tissue with "self" is ideal from an immunologic perspective. Several pre-clinical models as well as clinical applications are currently being explored and include chondrocytes for cartilage repair [1-12], keratinocytes and/or dermal fibroblasts for burn and wound repair [13-17], myocytes for myocardial repair [18-23], retinal pigment epithelial cells for age related macular degeneration [24-27] and Schwann cell transplantation to restore myelin in CNS lesions. [28-32]. The two most developed autologous cell therapies that have advanced from the laboratory to the clinic involve the repair of cartilage using autologous chondrocytes and the treatment of burns with autologous cultured keratinocytes.

Grande et. al., first demonstrated that autologous chondrocyte cultures could be utilized to repair articular cartilage defects in the rabbit knee [11,12]. Subsequently, this technique has been applied to the clinical treatment of articular cartilage defects [1,4,7,33] and has now evolved into an FDA approved therapy supplied by Genzyme Biosurgery <http://www.fda.gov/cber/approvltr/autogen082297L.htm>. Genzyme Biosurgery has also developed an autologous keratinocyte culture procedure and currently markets Epicel® as a treatment for burn victims http://www.genzymebiosurgery.com/prod/burn/gzbx_p_pt_burn.asp. Although repairing "self" with "self" is attractive and doesn't require immunosuppressive drug therapy for graft maintenance, there are limitations related to the harvesting of tissue and expanded tissue culture. Typically, harvesting the original source tissue from the patient requires a surgical procedure which minimally is a biopsy, but could also require a large resection of the tissue. The concern is causing a second site defect that leads to pain, discomfort or a deficit that effects behavior, hopefully to a lesser extent than the original. The ability of the adult tissue to expand in tissue culture to generate sufficient numbers of cells is also a potential limitation. Additionally, primary cell cultures can become senescent or dedifferentiate during the culture period. Another limitation is that this type of therapy is only amenable to tissues that can sustain surgical harvesting and ex vivo culturing, emphasized by the fact that only two autologous cell therapies have achieved FDA approval for the medical market.

Autologous progenitor cells harvested from an individual and used for "self" tissue repair is also immunologically ideal. The most widely used source of adult progenitor cells are derived from bone marrow. The mesenchymal compartment within the bone marrow has the capacity to differentiate into many cell and tissue types given the appropriate growth conditions [34]. Early studies using bone marrow stromal cells for tissue repair focused on the repair of bone defects [35] however, more recent studies

have applied bone marrow progenitor cells to repair a variety of damaged tissue types, including cartilage [36-38], myocardium [39,40], liver [41], spinal cord injury [42-44] and most recently diabetes [45]. However, these differentiation studies are still in the experimental stage. The potential to differentiate "self" progenitor cells into a variety of tissues is extremely promising for the field of Regenerative Medicine, however continued experimentation is necessary to understand the differentiation processes and to be able to reproducibly guide these cells into the appropriate tissue, prior to clinical application.

Autologous peripheral blood or autologous bone marrow stem cells are currently used clinically, but not from a commercial stand point. A current search of the FDA web site <http://clinicaltrials.gov> resulted in 108 studies involving autologous peripheral blood or autologous bone marrow stem cells as a treatment therapy, 96 of which were related to cancer treatments. None of the trials are related to stem cell differentiation followed by transplantation.

Allogeneic Tissues and Cell Lines

The use of allogeneic tissue for transplantation is clinically routine due to the development of immunosuppressive drug therapies, such as cyclosporine, FK506 and rapamycin. The use of engineered tissue and specialized cell lines for the treatment of disease and injury is more recent and will also require immunosuppression unless engineering strategies are utilized to make the tissue resistant to immune destruction or through tissue processing to reduce immunogenicity. As is the case for autologous cell therapies, the furthest advances are in the area of connective tissue replacement, cartilage and skin. [46-48] Currently, Apligraf® (Organogenesis, Inc) is used as a dermal replacement for chronic wounds and is composed of neonatal foreskin keratinocytes and dermal fibroblasts [49]. Although earlier studies demonstrated that Langerhan's cell-free epidermal skin cultures were rejected following transplantation [50], Apligraf tissue appears to be uniquely non-immunogenic due to the processing of the tissue [51,52] and represents an exception to the need for immunosuppression during allogeneic transplantation. A similar product, Dermagraft® is also available from Smith & Nephew.

Another interesting allogeneic cell type harvested from cadaveric sources for the treatment of Parkinson's disease are allogeneic cultured retinal pigment epithelial cells that are encapsulated to provide an immune barrier [53]. Titan Pharmaceuticals, Inc has advanced this research into the clinic and is currently conducting a Phase IIb clinical trial with initial positive results [54].

Allogeneic cell lines are also being developed as a source of cells for regenerating damaged tissue due to disease.

The human NT2 cell line was shown to differentiate and develop into neurons in rodent stroke models [55,56] and is currently being tested in clinical trials for the treatment of stroke by Layton Biosciences, Inc [57,58]. Patients receiving the NT2 cell grafts do receive immunosuppression to inhibit immune rejection of the graft [57,58].

The development of allogeneic engineered tissues for commercial purposes has similar limitations as commercial autologous cell therapies with the added complication of immune rejection. Encapsulation, tissue processing, tolerance induction and/or genetic modification will be necessary unless the patient population is receptive to and the severity of the disease warrants the use of immunosuppressive drugs.

Allogeneic stem cells

Allogeneic bone marrow transplantation is used clinically to treat hematologic disorders and cancer, but as is the case for autologous bone marrow transplantation, not from a commercial, tissue engineering standpoint. New clinical trials are focusing on the use of peripheral blood stem cells and specific subsets of bone marrow stem cells for these indications (for example [59,60]). Searching the FDA clinical trial data base identified 117 trials that utilize allogeneic stem cells or bone marrow in the treatment regime. Sorting through those trials, 99 were specific for stem cell therapy. As for autologous bone marrow or peripheral blood stem cell therapies, most were for cancer indications, 84/99. The data base included 9 trials utilizing allogeneic stem cells for anemia/hematologic disorders, 2 trials are designed to treat metabolic storage diseases and there are single trials for each of the following: Granulomatous Disease, HIV patients not responsive to highly active antiretroviral therapy, Mycosis Fungoides and Sezary Syndrome and allogeneic bone marrow rejection <http://clinicaltrials.gov>. Virtually all of these therapies are in combination with some form of immunosuppression and none of the trials are related to stem cell differentiation followed by transplantation.

The discovery and isolation of neural stem cells from fetal [61-64] and adult human brain [65,66] is a significant development in the area of neural cell differentiation that has led to the possibility of producing specialized cells for the treatment of neurologic disorders, such as Parkinson's disease and spinal cord injury. Many groups have studied the differentiation of neurospheres into neuron, glial, and astrocyte lineages, however the work from Goldman's group [66,67] sets a precedent for selection strategies that are most relevant to the field of tissue engineering. Nunes, et. al., and Keyoung et. al., showed that transfected/transduced specific promoter constructs driving green fluorescent protein (GFP) can be used to select specific neural progenitor cells by flow cytometry [66,67]. Expanding on

these techniques could lead to the eventual development of a commercial application of neural stem cells by reproducibly selecting the desired neural phenotype. Demonstrating that specific lineages can be selected genetically with drug selectable or fluorescent expression plasmids lays the ground work for further selection schemes and further genetic modifications, such as modifying the immune response leading to a universally accepted source of human neural tissue for transplantation.

Although stem cells from adult tissues have more plasticity than originally thought, they typically are limited in their capacity to generate all possible tissue and cell types. Stem cells derived from the inner cell mass of the early embryonic blastocyst (ES cells) can proliferate indefinitely [68] and can give rise to virtually any cell type [69]. The development of human embryonic stem cells [70] has raised the possibility that an unlimited supply of human tissue could be generated from ES cells and that these tissues could be used to replace and repair damaged tissue in any organ system. It should be noted that although these human cells are referred to as ES cells, they cannot be qualified as a true "ES cell" which is defined by the ability to contribute to the germ line during embryonic development. ES cells from other species are tested in this manner, however it is ethically unfeasible with human ES cells. Having qualified the definition of a human ES cell, several studies have demonstrated that human ES cells retain the capacity to differentiate into a variety of tissues, including neuronal cells, myocytes, adipocytes and hematopoietic cells [71-77]. The challenge now is to be able to direct differentiation or select for the desired phenotype and to develop these therapies in the absence of immunosuppression, similar to the strategies taken by the field of xenotransplantation. The ability to genetically modify ES cells is a great advantage and could be used to overcome both the directed differentiation/selection hurdle as well as the immune response hurdle.

Xenotransplantation

In the ongoing search for a reliable source of tissue to replace lost cells, tissues and organs, research in the area of xenotransplantation (cross species transplantation) has grown tremendously in the last 20 years. Overcoming the immunologic hurdle of cross species transplantation as well as the problem of cross-species pathogen infectivity, i.e., xenozoonosis, are the scientific challenges facing the field [78,79]. The ability to genetically modify species such as the pig through transgenesis and nuclear transfer, to express human genes and to mutate detrimental genes expressed in pig cells [80-86] still holds promise for engineered tissues and organs for human transplantation. The production of gal α 1,3gal transferase null transgenic pigs [84] represents a significant development towards eliminating both hyperacute and acute vascular rejection and

may lead to extended survival of pig organs in old world primates, including humans, in combination with standard triple drug immunosuppressive therapy.

Interestingly, there have been a series of pig-to-human xenotransplantation clinical experiments for the treatment of diabetes [87,88] and FDA approved clinical trials for the treatment of neurologic disorders using outbred pig tissue [89-91]. Although there was some evidence of cell engraftment in both indications [87,90], no efficacy was established due to the transplant [87,89]. To date, a Phase I clinical trial was completed using transgenically engineered pig livers to detoxify the blood of fulminant hepatic failure (FHF) patients via extracorporeal perfusion [92], however there is yet to be an FDA approved transgenic animal tissue for use in human transplantation.

Although the theoretical risk of xenozoonosis is a risk and represents a significant psychosocial issue, several studies investigating the possibility of cross species infectivity, including a retrospective analysis of 160 human transplant recipients exposed to porcine tissues have yet to reveal transmission of porcine viruses to humans or primates in vivo [93-96]. The prospect of xenotransplantation is still relevant to solid organ and islet transplantation and with FDA oversight, animal as well as patient monitoring, the risks associated with xenozoonosis will be overcome.

The Future

The challenges associated with stem cell differentiation, tissue engineering, and xenotransplantation are manifold in each of the respective fields, however one of the biggest challenges beyond the science and biology is the development of an FDA approved product from any of the developmental areas discussed above. The regulatory issues and points to consider documents are in the early stages of development by the FDA in collaboration with expert review panels and will continue to change as the technology advances into clinical applications. The experiences of Genzyme Biosurgery, Organogenesis, Diacrin, Layton Biosciences, Titan Pharmaceuticals and other tissue engineering companies will benefit the field as a whole.

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