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Strategies for Regenerative Medicine

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Abstract

There is a wide gap between the number of patients requiring organ and tissue replacement and the organs available for transplantation. Regenerative Medicine holds the promise of narrowing this gap using cell therapy, bioengineering organs and harnessing the body's ability to self-heal. This review describes progress made in the basic components of Regenerative Medicine (collecting and expanding cells, selecting appropriate biomaterials for their scaffolding, and employing bioactive molecules to aid in cell migration differentiation and growth) and identifies both gaps in knowledge and challenges in execution requiring further research.

Keywords: Regenerative medicine; Cell therapy; Biomaterials; Bioactive molecules; Regenerative Pharmacology; Organ regeneration

Introduction

There is a wide gap between the number of patients suffering from diseased and injured organs that need to be replaced and the organs available for transplantation. The supply/demand ratio of organs is worsening yearly as the population ages and the number of new cases of organ failure increase [1]. In response to this need, Regenerative Medicine has been developed as a new science with the goal of constructing biological substitutes and harnessing the body's ability to self-heal in an attempt to restore and maintain normal function in diseased and injured tissues [1]. According to the US National Academy of Sciences report, approximately 128 million people in the United States alone might benefit from regenerative medicine approaches to their diseases [Organ Donation Opportunities for Action, Committee on Increasing Rates of Organ Donation, www.nap.edu]

In 1999, William Haseltine, the Scientific Founder and Chief Executive Officer of Human Genome Sciences, coined the term 'regenerative medicine' bringing cell transplantation, tissue engineering, stem cells, and nuclear transfer under one defining field [2]. National Institutes of Health (NIH) authors suggest that "the long-term promise of regenerative medicine is a world where there is no donor organ shortage, where victims of spinal cord injuries can walk, and where weakened hearts are replaced" [3]. The process of regenerating body parts may require cells, natural or artificial scaffolding materials, bioactive molecules such as growth factors, or a combination of all three elements [4]. In this review, we provide information about the components required for organ and tissue regeneration as well as the promise and complications associated with this new science.

Cell based Therapy

The use of cells in tissue bioengineering has become a reality with recent developments in stem cell biology [5]. There is a continuum of cells within the body with the ability to self-renew, differentiate and produce mature progeny consisting of both non renewing progenitors and terminally differentiated effector cells [6]. They can be classified according to their differentiation potential into pluripotent, multipotent, oligopotent and unipotent cells.

Embryonic stem cells: Embryonic stem (ES) cells are currently the only known natural pluripotent stem cell. Human embryonic stem cells have been shown to differentiate into cells representing all three embryonic germ layers. For example, ES cells have been differentiated *in vitro* into dermal and neuronal cells, indicating ectodermal differentiation potential [7-10]. Blood, cardiac, cartilage, endothelial, and muscle cells have been derived from ES cells, indicating mesodermal differentiation [11-13]. Pancreatic cells have been derived from ES cells, indicating endodermal differentiation [14]. As further evidence of their potential pluripotency, embryonic stem cells can form embryoid bodies, which are cell aggregations that contain all three embryonic germ layers while in culture, and can form teratomas *in vivo* [15].

New stem cell technologies such as somatic cell nuclear transfer and reprogramming are now available to convert partially or completely differentiated cells back to their embryonic routes. An advantage of these technologies is the potential of using autologous cells, thus overcoming the problem of immune rejection common with ES cells.

Somatic Cell Nuclear Transfer (SCNT): includes removal of an oocyte nucleus in culture, followed by its replacement with a nucleus derived from a somatic cell of a patient resulting in Embryonic Stem cells that are genetically identical to the source [16,17]. Nuclear transfer was used to clone a sheep named Dolly in 1997 [18]. This type of cloning is known as reproductive cloning where the blastocyst is implanted into the uterus of a female to produce an infant that identical to the donor. However its use for human applications is prohibited in most countries. Another type of cloning is therapeutic cloning which is used to generate only Embryonic Stem (ES) cell lines genetically similar to their sources. This technique doesn't involve implantation of the blastocyst into a uterus. Instead blastocysts are allowed to grow till 100 cell-stages to obtain ES cells [19-21]. While potentially useful, SCNT technology still must overcome major hurdles to become clinically relevant. These include induced chromosomal abnormalities [22], inadequate supply of human oocytes, remaining ethical issues and low efficiency(0.7%) [23]. The low efficiency of SCNT is because the majority of embryos

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derived from cloning don't survive after implantation [24]. In other words, multiple nuclear transfers must be performed to produce one live offspring. To improve cloning efficiency, modifications are required in the steps of nuclear transfer such as enucleation, activation of oocytes, and cell cycle synchronization between donor and recipient cells [25].

Reprogramming: involves production of induced pluripotent stem (IPS) cells from adult somatic cells without the use of embryos. The major advantage of this approach is that generated cells would be genetically identical to the somatic cells and would avoid the complex ethical issues of manipulating embryos. This technique was first described by Takahashi and Yamanaka in 2006, who reported that the expression of a set of four genes (Klf4, Sox-2, Oct 3/4, and c-Myc) transformed mouse somatic (fibroblast) cells back into a pluripotent-like state [26]. Okita then showed that retrovirus-mediated transfection of the same four genes could generate human IPS cells that are similar to human ES cells in morphology, proliferation potential, gene expression, and surface markers [27]. Of the four genes transferred during IPS cells initialization, the concern was with the existence of c-Myc specifically which is an oncogene, would lead to carcinogenesis. There was additional concern that using a retrovirus as the vector for gene transfer may increase the possibility of tumor formation after its insertion into a genome [28]. Thus, there remain obstacles facing IPS technology before it can be translated into clinical use [29].

To address some of these obstacles, researchers are working on a technology called direct conversion to differentiated cells. Using this technology, IPS cells are produced using transfer of 4 genes in one batch. A first report described differentiation of pancreatic exocrine cells into β-like cells secreting insulin by transfer of three genes (Ngn3/Pdx1/MafA) [30]. Later reports showed that transferring Asc11/Brn2/Myt11 into fibroblasts can induce functional neurons [31] and myocardial cells can be induced by transferring Gata4/Tbx5/Baf60c into mouse mesodermal cells [32].

Fetal-derived stem cells: A potential alternate source of stem cells is from fetal tissues. Cells derived from umbilical cord have been used as an alternative source of stem cell since 1988 [33]. The blood remaining in the umbilical vein following birth contains a rich source of hematopoietic stem and progenitor cells that have been used successfully as allogeneic donor sources to treat a variety of pediatric genetic, hematologic, immunologic, and oncologic disorders [34-36]. Fresh cord blood is also a promising source of non-hematopoietic stem cells. Among others, it contains endothelial cells, MSCs and unrestricted somatic stem cells [37-39]. Primitive stromal cells can be isolated from umbilical cord Wharton's jelly and can be differentiated into different cells like osteoblasts, chondrocytes, adipocyte, and neurocytes [40, 41].

Amniotic fluid and the placenta are known to contain multiple partially differentiated cell types derived from the developing fetus. Cells derived from amniotic fluid and placenta have shown many advantages such as quick availability, less risk of immunogenicity, higher compatibility rates, less risk of infections and tumor formation. They do not require the destruction of human embryos for their isolation and thus avoid the controversies associated with the use of human embryonic stem cells. Therefore, this source of stem cells has been proposed as a good candidate to be used in cellular therapy and regenerative medicine. [42]. In an initial study, De Coppi et al. isolated stem cell populations from these sources, called amniotic fluid stem cells (AFS) [42] that express embryonic and adult stem cell markers. The undifferentiated stem cells expand extensively without feeders and double every 36 hours. Unlike human ES cells, the AFS cells did not form tumors *in vivo*. Lines maintained for over 250 population doublings, retained long telomeres and a normal karyotype. AFS cells were broadly multipotent. Clonal human lines verified by retroviral marking were induced to differentiate into cell types representing each embryonic germ layer, including cells of adipogenic, osteogenic, myogenic, endothelial, neuronal, and hepatic lineages. In this respect, they meet a commonly accepted criterion for pluripotent stem cells, without implying that they can generate every adult tissue. Examples of differentiated cells derived from AFS cells and displaying specialized functions include neuronal lineage (secreting the neurotransmitter L-glutamate or expressing G-protein-gated potassium (GIRK) channels), hepatic lineage cells (producing urea), and osteogenic lineage cells (forming tissue-engineered bone) [43]. It is estimated that a bank of 100, 000 specimens could potentially supply 99% of the US population with near perfect genetic match for transplantation. This represents approximately 1% of the amniocentesis samples collected each year. As such, a bank may be easier to create than with other cell sources, since there are approximately 4.5 million births per year in the USA [42].

Placental tissue itself also contains stem/progenitor cell populations. Mesenchymal stem cells from the placenta have been considered for use in autologous grafts for fetuses and newborns. Additionally, MSCs may be useful for *in utero* transplantation in case of genetic disorders without immunologic rejection by the recipient [44, 45].

Adult stem cells: Adult stem cells, especially hematopoietic stem cells, are some of the most widely studied cell types in Regenerative Medicine [46]. Adult bone marrow include two well defined populations of stem cells: hematopoietic stem cells (HSCs) that give rise to all mature lineages of blood; and mesenchymal stem cells (MSCs) which differentiate into bone, cartilage, muscle and fat [47]. HSCs are already used to restore hematopoietic function after chemotherapy, and cancer irradiation, as well as treatment of certain hematologic and autoimmune diseases [48]. MSCs are currently used in clinical trials for treatment of bone fractures and arthritis [49, 50]. MSCs were originally described by Friedentstein and colleagues 40 years ago as adherent cells with a fibroblast-like appearance capable of differentiating into osteocytes, chondrocytes, adipocytes and myocytes [51, 52]. Although they were initially defined by their ability to differentiate into cells of mesodermal origin, recent studies revealed that they can differentiate into cells of all three germ layers [53]. They are described as MHC II negative cells, lacking co stimulatory molecules as CD40, CD80 and CD86, which allow their allogenic transplantation with no immune rejection [54]. MSCs have been successfully isolated from different tissues including: periosteum, bone marrow, liver, skeletal muscle, hair follicle and amniotic fluid [55-58]. To date, no cell receptors or markers have been found to be specific for MSCs [59]. The International Society of Cryotherapy has put three criteria for MSCs: (1) plastic adherence of isolated cells in culture, (2) expression of cluster differentiation (CD) markers such as CD105, CD90 and CD73 in >95% of culture with absence of markers including CD34, CD19, CD14, CD45 and human leukocyte antigen-DR (HLA-DR) in >95% of culture, and (3) ability to differentiate into adipocytes, chondrocytes and osteocytes [60]. MSCs need to be expanded *ex vivo* to obtain the sufficient numbers required for use in different clinical applications [61]. Numerous factors affect MSCs behavior including culture parameters such as cell confluence, nutrition level, oxygen level, number of passages and plastic surface quality [62].

Within the past decade, adult stem cell populations have been found in many adult tissues other than the bone marrow and the

gastrointestinal tract, including the brain [63, 64], skin [65], and muscle [66]. These cells are thought to serve as the primary repair entities for their corresponding organs [67]. The advantage of adult stem cells resides in the fact that they can be obtained in an autologous manner and used in the patient without rejection. In one such use, Atala et al. [68] engineered human bladders for patients with neurogenic bladder disease requiring cystoplasty. Urothelial and muscle cells obtained from bladder biopsies were grown and expanded in culture. Cells were then seeded on a biodegradable bladder-shaped scaffold. After 46-month follow up, the new bladders showed improved function, compliance, and capacity. In a recent study, it was found that these urothelial cells may be derived from the urine [69], thus simplifying collection techniques.

Limitations to adult stem cells may reduce their clinical utility. In some cases, it is difficult to isolate cells because of their low numbers in the adult tissue. Such cells are often selected by Fluorescent Activated Cell Sorting (FACS) or Magnetic Activated Cell Sorting (MACS) against surface markers specific to the stem cell of interest [70]. However, sometimes there is no known marker [23] specific for a type of stem cell, so these methods cannot be used. Also, not all human cells can be isolated or grown easily *in vitro,* including those from ectodermal or endodermal sources. Importantly, stem cell populations also tend to decrease with age and disease – just when they are needed most. Before stem cells can be used as any type of clinical therapy, strict guidelines must be established to ensure the quality of the cells, the specificity of differentiation, and the assessment of mixed phenotypes [23].

Biomaterials

Biological tissues are composed not only of cells, but also extracellular matrix - which is known to have a dynamic and functional role in providing cell growth factors and producing chemokines that attract cells to the site of regeneration [29]. They also provide a three-dimensional space for either seeded or native cells to incorporate into new tissues [71].

Biomaterials can be defined as any natural or synthetic substance that incorporates or integrates into a patient's tissues during the treatment. The ideal compound should be inert, sterile, non carcinogenic, mechanically durable, should cause no inflammatory or immune reaction, be inexpensive, easy to use and withstand modification by body tissues. The purpose of a biomaterial is to perform, supplement, or replace a natural function that is attenuated or lost [72]. Thus, the rate of degradation of scaffolds used for tissue engineering is an important factor for timely maintenance of biomechanical properties, while allowing resorption of the material when a new matrix has developed [73].

Classification of biomaterials

A summary of classification of biomaterials can be found in Table 1. Generally, biomaterials used for engineering tissues and organs are classified into three classes: naturally derived materials, such as collagen [74] and alginate [75]; acellular tissue matrices, such as bladder submucosa and small-intestinal submucosa (SIS) [76-78]; and synthetic polymers, such as polyglycolic acid (PGA), poly-lactic acid (PLA), and poly (- lactic-co-glycolic acid) (PLGA) [79,80].

Collagen is the most abundant structural protein in the body and can be purified from both animal and human tissue following enzymatic treatment and salt/acid extraction and salt/acid extraction [74]. Collagen evokes minimal and antigenic responses [74], and has been approved by the U.S. Food and Drug Administration (FDA) for many types of clinical applications [81]. Alginate is a polysaccharide isolated from seaweed and has been used as a vehicle for injected cells and as a matrix for cell immobilization [75]. Recently these natural materials have been used as "bio-inks" in a newly developed bio-printing technique based on inkjet technology. Using this technology these scaffold materials can be printed into a desirable scaffold shape that can provide three dimensional construct [82,83]. Inkjet printing has been used to print living cells in a defined pattern [83] and also to construct a desired scaffold shape using a specific arrangement of cells, growth factors and extracellular matrix [84,85]. These constructs can be implanted in a host to provide a structural backbone for a new tissue or organ [1]. Organ printing using the inkjet technology has been used to form tubular vascular like structures in a process referred to as "directed tissue self-assembly" where the individual tissue spheroids are bioprinted in a pre-designed pattern and upon their fusion, a tubular structure is formed [86].

Another approach to bioengineering is the use of decellularized tissue matrices. They are collagen rich matrices prepared by removing cellular components from tissues. They possess the required biocompatibility [87,88] and are known to degrade slowly upon implantation where they are usually replaced and remodeled by ECM proteins synthetized and secreted by transplanted or ingrowing cells [89,90]. Acellular tissue matrices have been used to support cell

Category		Polymer	Application
Natural	Proteins	Collagen	collagen-scaffolds seeded with myoblasts used to repair skeletal muscle defects [156], and collagen hydrogel used in cartilage tissue engineering [157]
		Fibronectin	Fibronectin scaffolds used to enhance neural stem cell transplantation into the injured brain [158]
		Fibrin glue	Used in skin tissue engineering [159] and as scaffolds for neuronal differentiation of adult stem cells derived from adipose tissue and skin [160]
	Polysaccharides	Chitosan	Used for dental pulp regeneration [161] and cartilage tissue engineering [162]
		Alginate	Hydrogel used as synthetic extracellular matrix materials [163]
		Hyaluronic acid	Used in bone regeneration [164]
Acellular tissue matrices		Bladder submucosa	Used in urological tissue engineering [165]
		Small intestinal submucosa (SIS)	Used in urological tissue engineering [166]
Synthetic polymers		Polyglycolic acid (PGA)	Used in vascular tissue engineering [167] and neo cartilage formation [168]
		poly-lactic acid (PLA)	Used in bone regeneration [169]
		poly-lactic-co-glycolic acid) (PLGA)	Used in bone regeneration [170]

Table 1: Classification of biomaterials.

ingrowth and regeneration of genitourinary tissues including urethra and bladder [76,91]. However, despite many advantages, there are concerns about the use of decellularized materials, including the possible presence of infectious agents, variability among preparations, and the inability to completely specify and characterize the bioactive components of the material [92].

Synthetic polymers can be manufactured with controlled properties of strength, ultrastructure and degradation rate [1] in a way that meet demands of the host tissue. However they can lead to inflammatory reaction depending on the chemical and physical structure of the implant, amount of material and surface of the contact-area with the host [93,94]. Simple polymers, while providing architectural support for neo-tissue development, do not adequately mimic the complex interactions between adult stem and progenitor cells and the ECM that promotes functional tissue regeneration. New applications in regenerative medicine will benefit from interactive "smart" biomaterials that serve to orchestrate cell attachment and growth, as well as tissue morphogenesis [92].

Smart biomaterials are semi-synthetic biomaterials that can combine the advantages of both the synthetic materials (mechanical strength, controlled degradation rate) and that of natural materials (ability to provide proliferation signals, cellular invasion and specific cell recognition) [95]. Often, these scaffolds incorporate bioactive [96] and signaling molecules as cells adhesion peptides [92], growth factors [97,98] and cytokines [99] that can modulate and control cell behavior. Sahni and colleagues investigated the natural binding affinity of fibroblast growth factor-2 (FGF-2), a fibrous protein formed during wound healing processes. Their results showed that FGF-2 stimulated the growth and proliferation of endothelial cells [100]. West and colleagues studied the effect of b-FGF loaded into hydrogel scaffolds on smooth muscle cell (SMC) behavior. The b-FGF gradient hydrogels increased smooth muscle cell proliferation by approximately 41% and migration by approximately 15% [101]. Vascular endothelial growth factor (VEGF) is an angiogenic protein capable of regulating new blood vessel formation [102]. The influence of VEGF on cell behavior was studied by Zisch et al. [102] in a study that showed increased migration and cell survival of endothelial cells within polyethylene glycol (PEG)-peptide hydrogels. Fan and coworkers proposed the incorporation of epidermal growth factor (EGF) into a synthetic polymer matrix material to increase cell survival and found that [98] surface-exposed EGF promoted cell attachment to the matrix material and increased mesenchymal stem cell spreading and survival when compared with media containing solubilized EGF. While potential very useful, the clinical application of these smart biomaterials is still limited because few were approved for human use and this delayed their translation into clinical practice [92].

Organ-Regeneration / Regenerative Pharmacology

Cell therapy and organ/tissue bioengineering offer the promise of cures for a multitude of diseases and disorders while helping solve the organ transplantation shortage. However, *ex vivo* expansion of cells and engineered tissues and organs are limited by the length of preparation, the cost, the availability of high quality cell sources, immunosuppression complications, ethical and religious considerations and complex regulatory approval. Increasing knowledge of how cells are attracted to damaged tissues and acknowledgement of the limitations of cell-based therapies has led to increasing interest in the concept of organ-regeneration as an approach for tissue repair. Organ-regeneration includes a variety of technologies for enhancing the body's intrinsic capacity for self-healing [103]. Development of smart biomaterials is, in fact, an example of developing a scaffold that stimulates the body's ability to self-heal using the biomaterial as the signaling mechanism.

Regenerative Pharmacology is the application [104] of pharmacological sciences to accelerate, optimize and characterize these processes. The grand challenge for regenerative pharmacology is: 1) to utilize integrative pharmacology (studies done on whole animals in vivo and ex vivo) to obtain better knowledge about mechanisms of tissue regeneration and repair [105], 2) to improve localized delivery of therapeutic drug concentrations to the specific tissue [106], and 3) to combine the formal studies to create a new generation of drug therapy that does not have a symptomatic treatment of disease only, but extend to be a curative therapy [104].

The general approach to organ-regeneration is to stimulate the exit of reparative cells from their nitche to enter the blood stream and travel, or move by ameboid action, to a site of injury. Once at the injury site, they then differentiate into the cells needed to repair the tissue [107]. The navigational cues can include chemokines such as monocyte chemotactic protein-1 (MCP-1), stromal-derived factor-1α (SDF-1α), angiogenic molecules such as vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), and interleukin-8 (IL-8). Once at the site, smart biomaterials can be used to further help engraft and differentiate the cells [107]. This approach has been used for tooth and periodontal regeneration [108-110]; musculoskeletal regeneration [111,112]; and cardiovascular disease [113-116].

While this approach has exciting possibilities, it also has its limitations. For this approach to work there is a need to have a keen appreciation of the cell trafficking mechanisms of the cells needed for repair. Cell sources needed for repair may decline with age and disease. A multi-cue approach may be needed to stimulate cells to leave their nitche, travel, migrate, differentiate and engraft in the damaged tissue.

An example of multiple signaling strategy is the use of both SDF-1α and substance P. SDF-1α has been shown to recruit mesenchymal (MSCs) [117] and hematopoietic (HSCs) stem cells to injured tissues through CXCR4 (SDF-1 receptor) expression [118]. A new and innovative delivery method, in which combined systemic and local delivery of multiple factors (substance P and SDF-1α) was used to enhance recruitment of endogenous stem cells into implanted scaffolds by inducing balanced cell infiltration. Substance P injection increases the number of host stem cells in the body's stem cell pool and SDF-1α enhances the recruitment of these host stem cells into the implanted scaffolds via local release from the scaffolds [96]. Results showed that delivery of both factors from PLLA/gelatin scaffolds increased recruitment of stem cell-like cells to help in situ tissue regeneration [96].

Another aspect of regenerative pharmacology is preconditioning of stem cells prior to transplantation in order to augment their defense mechanisms against oxidative stress/membrane damage and further to encounter the inflammatory reaction caused by the host environment [119]. Preconditioning of stem cells could enhance the expression of survival signaling molecules, microRNAs, and trophic factors for intracrine, autocrine, and paracrine effects on cytoprotection [120]. For example, Atorvastatin, a commonly used drug in lowering the level of cholesterol in blood has been shown to improve cardiac micro-environments created by acute myocardial infarction (AMI) and reperfusion, thus facilitating the survival and differentiation of in vivo implanted mesenchymal stem cells (MSCs) [121]. Lipopolysaccharide (LPS) enhanced survival of engrafted MSCs and their efficacy of

transplantation in a rat model of acute myocardial infarction [122]. Yao et al. reported that LPS preconditioning improved the survival of MSCs by promoting expression of vascular endothelial growth factor (VEGF) and Phosphoinositide 3-kinase (PI3K) and its downstream target serine/threonine kinase (Akt) in infarcted myocardium [122]. VEGF has a potent angiogenic function, stimulates endothelial cell proliferation, delays cell senescence, suppresses apoptosis and promotes survival of various cells [123, 124]. Also, activation of PI3K/Akt-dependent signaling has been shown to prevent cardiac myocyte apoptosis and protect the myocardium from acute myocardial infarction [125, 126].

Clinical applications of regenerative medicine in organ engineering

A summary of clinical applications of Regenerative Medicine are shown in Table 2. Some of these studies have already been reviewed. However, studies are being performed all over the world to develop cell types, tissues and organs for clinical applications [68,127-129]. In the following section we provide a brief overview [1] of the progress in tissue engineering of some structures. Tissues include, but are not limited to:

Bladder and urethra

The first successful tissue engineered bladder was transplanted by Atala et al. [68] in 1999 for patients with neurogenic bladder. Eight weeks after initial bladder biopsy, urothelial and muscle cells obtained from the biopsy were cultured and seeded on a biodegradable bladder shaped scaffold. The scaffold was anastomosed to the stump of the native bladders with omental coverage to enhance angiogenesis. This study included seven patients and showed [68] increased bladder compliance and longer dry periods. Urethral regeneration has been used successfully in both animal models and recently in human beings. Woven meshes of PGA without cells [79, 130] or with cells [131] have been used to regenerate urethras in various animal models. Non seeded scaffolds are able to replace short segments (less than 1 cm) of urethral defects while very large defects, up to 30 cm, can be successfully treated using cell seeded scaffolds to avoid risk of stenosis and urethral stricture formation [1]. In 2004 bioengineered urethras were successfully implanted in patients having severe urethral stenosis [127].

Organ	Advances of tissue engineering
Bladder	First successful tissue engineered bladder transplanted by Atala et al. [68]
Urethra	First successful bioengineered urethra by Atala [127]
Blood vessels	First implantation of bioengineered vessel (pulmonary artery) by Shinoka et al. [128]
Heart	Ott et al. [135] constructed novel heart *in vitro* using decellularized cadaveric hearts seeded with cells
Bone	Marcacci et al. [147] used 100% hydroxyapatite porous ceramic scaffolds seeded with MSCs to treat fracture non-union in patients with good outcome. Horwitz et al [150] used gene marked donor marrow-derived MSCc to treat 6 children with severe osteogenesis imperfecta with good results in 5 patients.
Cartilage tissues	Yoshikawa et al. [155] reported two case studies of intervertebral disc regeneration therapy using marrow mesenchymal cell transplantation with good results after 2 years follow up. Autologous chondrocyte implantation (ACI) were used for treatment of knee articular cartilage defects with numerous successful case report studies [152,153]
Liver	Tissue engineering of liver organoids *in vitro* by groups of Uygun et al. [171], and Baptista et al. [172]

Table 2: Recent advances of tissue engineering in different organs.

Kidney

Although the kidney was the first successful transplanted organ [132], current modalities to replicate kidney in laboratory is not satisfactory because the renal tissue is very complex with unique structural and cellular heterogeneity. Isolated renal cells may not result in structural remodeling and can't be implanted in large volumes due to limited oxygen and nutrient supply [1]. The feasibility of achieving renal cell growth and in vivo reconstitution using regenerative medicine techniques is under investigation and need continued research and studies before being applied in human beings.

Blood Vessels

Tissue engineered vascular grafts have been constructed using biodegradable scaffolds seeded with autologous cells in dog and sheep models [133,134]. The clinical application of this approach has been initiated with autologous constructs used to replace stenosed pulmonary artery which showed no evidence of graft obstruction seven months post-transplantation [128].

Heart

Various types of stem cells have been investigated for possibility of injection into a small damaged area of [129] a patient's heart instead of performing invasive surgical procedure. However the injectable therapies are inefficient due to cell loss and lack of cell engraftment. Newer methods involve the use of engineered patches seeded with cells to replace the damaged areas [129]. These techniques might be promising but still need further research. In cases of heart failure [1] bioengineered hearts could be ideal. Recently Ott et al. [135] constructed novel heart using decellularized cadaveric hearts. After seeding heart cells in a bioreactor system [135] that mimics physiologic conditions, the construct was able to perform pumping action of a normal heart.

Liver

Cell transplantation has been suggested as alternative therapy to liver transplantation in cases of liver failure. This is based on the fact that liver cells have high great regenerative capacity *in vivo*. Different techniques have been proposed to expand liver cells including identification of growth factors to help cell proliferation and the use of specialized media and culture on scaffolds within special bioreactors [136]. Perfusion decellularization technique [137] has been recently used to seed stem cells and mature hepatocytes on decellularized liver in various animal models [138,139]. Because of the high demand and low availability of livers for transplantation, this area of research will continue to be of high importance in the field of Regenerative Medicine.

Skin

Skin regeneration remains a complex challenge, but offers the possibility of much needed treatment for injured and burned patients [140]. Normal regeneration of the skin is achieved through stem cell differentiation within the epidermis and the hair follicle. Stem cells have capacity to differentiate into keratinocytes and open a new perspective on the healing of different types of skin disorder including severe burns, chronic leg ulcers, skin cancer, alopecia, and acne [142,143]. The continued advancement of iPS cell reprogramming technology offers a promising approach to replace large amounts of damaged skin with autologous cells. Conversely, the efficiency of iPS generation was recently found to be markedly improved with the use of keratinocytes compared to fibroblasts [144].

Bone

Recent advances in stem cell research have prompted development of cell based therapies for bone repair and treatment of metabolic bone diseases [145]. Despite the advances in orthopedic surgery, some fractures don't heal properly resulting in either delayed union or nonunion, prolonged hospital stay and increased cost. Cell based therapy for fracture repair in cases of nonunion are currently receiving considerable attention [145]. Autologous bone marrow derived pluripotent MSCs have been seeded onto 100% hydroxyapatite macroporous ceramic scaffolds and used in treatment of four patients with diaphyseal segmental defects in a tibia, humerus and ulnar fractures [146,147]. In all patients follow up 6-7 years after surgery showed good integration of the implants and recovery of limb function.

Osteogenesis imperfecta (OI) is a group of inherited disorders of connective tissue characterized by bone fragility where there is a genetic defect resulting in abnormal type I collagen production and leading to osteopenia, multiple fractures and severe bone deformities [145]. An initial clinical trial was conducted in three infants with severe forms of OI. They were transplanted with whole marrow from normal matched individuals. Results demonstrated that two patients had an increase in total body mineral content, increase in growth and reduction in fracture rates 3 months after cell transplantation [148,149]. Later, Horwitz et al. [150] used gene marked donor marrow-derived MSCc to treat 6 children with severe OI. Each child received 2 infusions of allogenic cells. Five patients out of six showed engraftment in bone, marrow stroma and increase in growth velocity during 6 months after infusion.

Cartilage tissues

Clinical results of autologous chondrocyte implantation (ACI) for treatment of knee articular cartilage defects have been encouraging. Numerous case-series reports show positive effectiveness with follow up for more than 10 years postoperatively [151,152]. By 2003, more than 15,000 patients had undergone ACI worldwide [153] and is now considered the frontline treatment for defects larger than 2 cm^2 [154].

Studies have also been done to regenerate damaged intervertebral discs using cultured marrow mesenchymal cells. Yoshikawa et al. [155] reported two case studies of intervertebral disc regeneration therapy using marrow mesenchymal cell transplantation. MSCs were collected from the ilium of each patient and cultured using medium containing autogenous serum. Then pieces of collagen sponge containing autologous MSCs were grafted percutaneously to degenerated intervertebral discs. Two years after surgery radiograph and computed tomography showed improvements in both patients and symptoms were relieved.

Conclusion

Regenerative medicine holds the promise of regenerating and engineering body organs in the future where there will be no sufficient organs available for transplantation. The process of regenerating body parts may require cells, scaffolding materials, bioactive molecules such as growth factors, or a combination of all three elements. Although advances have been made in the clinical applications of regenerative medicine, translation of tissue engineering to the patient in need of organ replacement requires more effort. None-the-less, progress will continue to be made and someday Regenerative Medicine may provide the answer to all those needing replacement tissues and organs.

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