Research Journal of Medical Sciences
[E-ISSN: 3078-2481 | P-ISSN: 3078-2473]
Available on: https://rjmsonline.com/
Volume: 05 | Issue: 01, Jan-Jun, 2023

Research Article *Open Access*

ARTICLE REVIEW ON REGENERATIVE MEDICINE

Nimesh Agrawal[*1], Saumya Srivastava[1], Sanch Srivastava[1], Navneet Kumar Verma[2]

[1] Students, Buddha Institute of Pharmacy, GIDA, Gorakhpur, UP, India-273209
[2] Associate Professor, Buddha Institute of Pharmacy, GIDA, Gorakhpur, UP, India-273209
Email: nimeshagrawal2735@gmail.com

Corresponding Author

Nimesh Agrawal
Students, Buddha Institute of Pharmacy, GIDA, Gorakhpur, UP, India-273209
Email: office.rjmsonline@gmail.com

Source of support: Nil.
Conflict of interest: None

Received: 2023-03-15
Accepted: 2023-04-10
Available online: 2023-05-21

Abstract

The replacement, restoration, or repair of damaged tissues is the focus of the multidisciplinary discipline of regenerative medicine. The necessity for reconstruction in patients with tissue damage from illnesses, trauma, and congenital abnormalities in both children and adults gave rise to this specialty. Through a range of techniques, including tissue engineering, cell-based therapies, and the creation of innovative medical devices, it seeks to restore the function of sick or injured tissues or organs. Because stem cells have a limitless capacity to divide, they are used by the body to regenerate and mend damaged tissue. A critical understanding of stem cell replacement is therefore necessary, as stem cell research is a promising topic with an enticing potential for therapeutic intervention. Knowing more about how an organism grows from a single cell and how healthy cells replace damaged cells in adult creatures is made possible by research on stem cells. Researchers looking into the potential of cell-based therapeutics to treat a range of chronic conditions are also being led by this exciting field of study. The facts regarding stem cells and their important significance in regenerative medicine is what we attempted to present in this review.

Key Words: *Regenerative Medicine, Tissue Engineering, Cell-Based Therapies, Innovative Medical Devices*

INTRODUCTION

All embryonic, foetal, and adult stem cells are undifferentiated cells that give rise to differentiated cells that form the structure of tissues and organs. Throughout the postnatal and adult phases of life, differentiated organs contain tissue-specific stem cells that play a crucial role in organ regeneration after injury. The primary features of stem cells are (a) clonality, which often originates from a single cell, (b) potency, which can differentiate into several cell types, and (c) self-renewal, which allows for significant proliferation. It's possible that different stem cells have different characteristics. Because they would not multiply widely and can only develop into tissue-specific cells, adult tissue stem cells have limited capacity for self-renewal, whereas embryonic stem cells (ESCs) generated from the blastocyst have more potency and capacity for self-renewal. Endoderm, Mesoderm, and Ectoderm are the germ layers from which ESCs are formed during the development of the human body, starting with the zygote and blastocyst. Particular organs emerge from the germ layers. Progenitor cells that have helped develop organs include those present in bone marrow, bone, blood, muscle, liver, brain, adipose tissue, skin, and the gastrointestinal tract. These cells do not eventually differentiate; instead, they remain tissue

stem cells [1, 2]. Given their ability to produce terminally differentiated and specialised cells within the tissue or organ, tissue stem cells may also be referred to as progenitor cells. These cells could be quiescent in the tissue, but in situations involving damage and healing, they would multiply [3-5]. The dynamics of tissue stem cells, also known as progenitor cells, differ depending on the tissue. For instance, stem cells regularly proliferate in the bone marrow, liver, lung, and gut to supplement cells during normal turnover or injury [6–9], whereas they do the same in the pancreas, heart, or nervous system to replace damaged cells after injury [10–14]. Ancient Greek mythology about the Greek titan Prometheus reflects the concept of organ regeneration and wound healing, which is as old as humanity

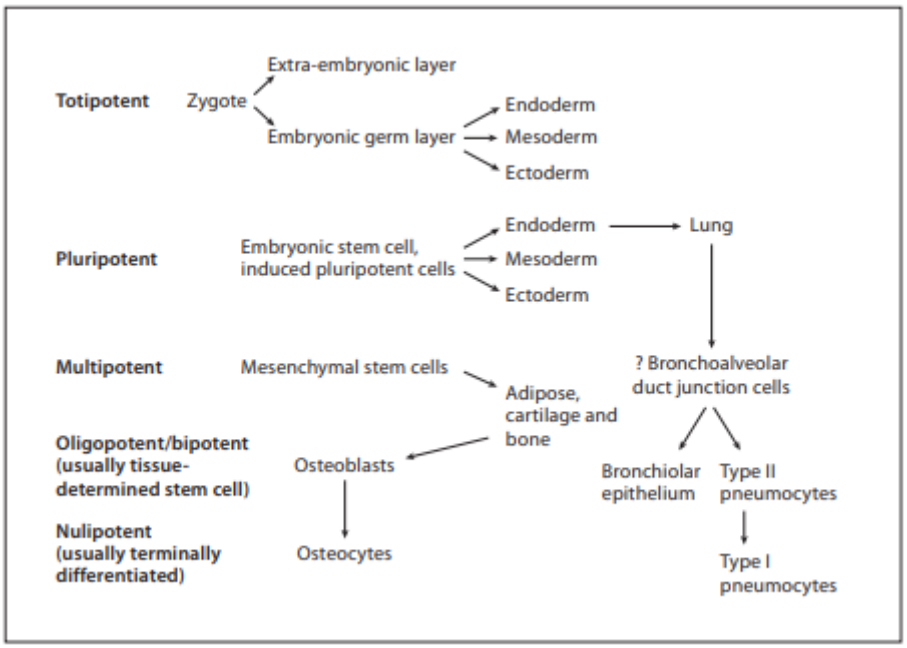

Fig. 1. The hierarchy of stem cells.Totipotent cells form embryonic and extra-embryonic tissue. Pluripotent cells form all 3 germ layers while multipotent cells generate cells limited to 1 germ layer. Bronchoalveolar duct junction cells in the lung may be multipotent while type II pneumocytes are oligopotent and differentiate into type Ipneumocytes of the alveoli.

Zeus chastised him for bringing fire and knowledge to humans in defiance of his commands. According to this tale, Prometheus is chained to a rock and his liver regenerates when an eagle consumes a portion of it every day. In contemporary medicine, the exploration of stem cells and organ regeneration began in the 1950s with the initial attempts at bone marrow transplantation in animal models. The treatment now commonly used for a variety of blood diseases [16], human bone marrow transplantation, was made possible by these groundbreaking investigations [15]. The discovery of adult tissue regeneration-promoting stem cells was made possible by this novel treatment approach [17]. Regenerative medicine is currently a prominent area of research attention, with the goal of understanding basic biology and the pathophysiology of disease in addition to developing therapeutics [18–20]. Recent developments in stem cell isolation and development have made it possible for scientists to identify and culture particular cell types for tissue regeneration in a variety of disorders, including Parkinson's [22], Alzheimer's [23], or diseases of the heart [24], muscles [25], lung [26, 27], liver [28], and other organs [14]. This is despite the fact that stem cell research has given rise to a number of ethical issues [21].

1. The basics of regenerative medicine

In order to create biological replacements that can preserve and restore the normal function of injured tissues and organs, tissue engineering makes use of elements of materials science, biomedical engineering, and cell biology and transplantation. The two most fundamental elements of tissue engineering strategies are cells and biomaterials. These approaches can involve the injection of viable cells into a nonfunctional region to promote regeneration and/or the use of biocompatible materials to produce new tissues and organs. The goal of introducing cells is to augment the synthesis of growth factors and hormones, encourage vascularization, and/or drive regeneration. Biomaterials, also referred to as "scaffolds," are crucial instruments in the field of regenerative medicine. They comprise both synthetic and natural matrices. Biomaterials have the ability to both control the creation of new tissue and provide the right spatial environment for the restoration of tissue structure and function. They can also be used to supply bioactive components [29,30] or draw cells and growth factors from the body after implantation [31, 32]. The goal of implanting a biomaterial devoid of cells is to promote the body's inherent capacity for self-healing.

1.1 Cell types used in regenerative medicines

1.1.1Native Targeted Progenitor Cells

The intrinsic difficulty of cultivating particular cell types in vast quantities has been one of the barriers to the application of cell-based approaches for organ replacement. Cell growth and expansion in vitro can be challenging, even in organs with great in vivo regenerative capacities, like the liver. Some of the barriers that formerly prevented cell multiplication in vitro have been removed by identifying progenitor cell locations within tissues and investigating the circumstances that encourage differentiation and/or self-renewal. Using native cells has the benefit of allowing for the autologous regeneration, expansion, and use of those cells in the same patient without fear of rejection.

1.1.1. Stem cells and other pluripotent cell types

Not all human cells can be produced or expanded in vitro, despite the great advancements in primary cell culture techniques. Human organs like the pancreas, liver, and nerves are examples where technology has not yet progressed to the point where these cells can be extensively cultured and expanded outside of the body. In this instance, it is anticipated that stem cells and other pluripotent cell types will replace native cells in the near future. Two characteristics of human embryonic stem cells are their capacity to divide into numerous specialised cell types and their capacity to proliferate in an undifferentiated but pluripotent state (self-renewal) [33]. They can be separated from the embryo by aspirating the inner cell mass while it is in the blastocyst stage, which occurs five days after fertilisation. In vitro, it has been demonstrated that they can develop into cells from all three embryonic germ layers. Ectodermal differentiation is indicated by the formation of skin and neurones [34–37]. Mesodermal differentiation is indicated by the formation of muscle, blood, endothelial cells, cartilage, and heart [38–40]. Additionally, pancreatic cells have developed, demonstrating endodermal differentiation [41]. They can create teratomas and are an allogenic resource, which means they have the potential to trigger an immune response, which limits their therapeutic applicability. Cloning and reprogramming are two examples of new stem cell technologies that promise to get over the obstacle of rejection.Early stage embryos are created using therapeutic cloning, also known as somatic cell nuclear transfer, or SCNT, using an egg and a somatic cell's nucleus. To create embryonic stem cell lines with genetic material identical to its source, these are explanted in culture. Because these autologous stem cells can differentiate into nearly any type of adult cell, they could be helpful for replacing organs and tissues in the body [42]. The method of developing tissues and organs by combining tissue engineering and therapeutic cloning is illustrated in Fig. 1.

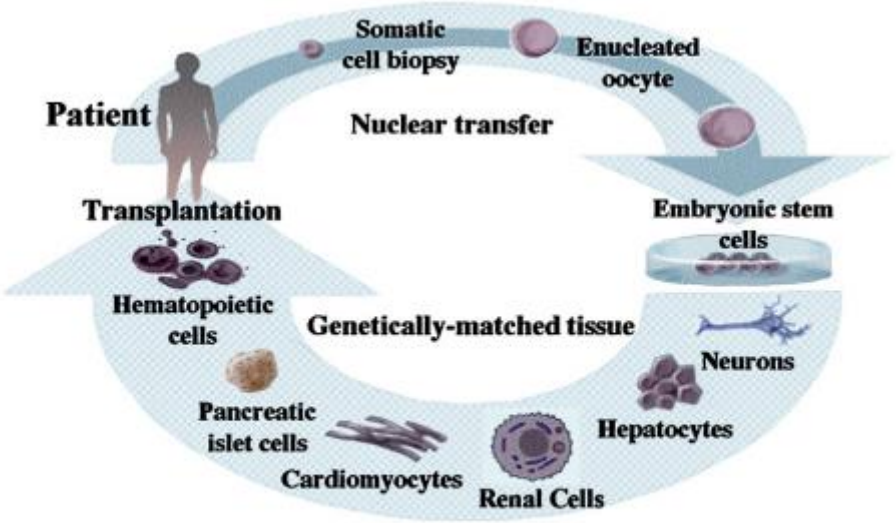

Fig. 1 Strategy for therapeutic cloning and tissue engineering.

Though promising, somatic cell nuclear transfer technology still has to be improved upon in order to overcome several obstacles before therapeutic cloning may be extensively used in replacement therapy. It is also feasible for adult cells to be successfully "reprogrammed" genetically to become pluripotent stem cells. Reprogramming is a non-embryonic method of producing patient-specific pluripotent stem cells by dedifferentiating adult somatic cells. Reprogrammed cells would not be rejected since they share the same genetic makeup as the somatic cells and, consequently, the patient who gave them. The ability to reprogramme mouse fibroblasts into a "induced pluripotent state" was initially discovered by Yamanaka [43]. The resulting induced pluripotent state cells expressed genes unique to embryonic stem cells, produced teratomas in vivo and embryoid bodies in vitro, and had the immortal growth properties of self-renewing embryonic stem cells. Human cell reprogramming has recently been demonstrated to be feasible [44, 45], indicating that this technology may prove helpful for regenerative medicine efforts in the future. The placenta and amniotic fluid are additional sources of stem cells. It is known that the placenta and amniotic fluid both contain a variety of partially differentiated cell types that are derived from the growing foetus. Amniotic fluid and placental stem cells, which express markers for both embryonic and adult stem cells, are the stem cell populations that we recovered from these sources [46]. It has been demonstrated that AFS cells

are extensively multipotent and can develop into cells from all three lineages. These cells can be extracted via amniocentesis, chorionic villous sampling throughout foetal development, or from the placenta at the moment of birth. They can then be banked or maintained for future use without hindrance.

2.1 Biomaterials

Cells are seeded onto a scaffold made of a suitable biomaterial in cell-based tissue engineering. In tissue engineering, biomaterials act as an artificial extracellular matrix (ECM), mimicking the physiological and mechanical properties of the natural ECM present in bodily tissues. Biomaterials can facilitate the transfer of cells and suitable bioactive substances (such as growth factors and adhesion peptides) to specific locations inside the body by offering a three-dimensional environment in which the cells can proliferate and develop into new tissues with the proper structure and function [47]. Biomaterials also offer a cell-adhesion substrate because most mammalian cell types are anchorage-dependent and would perish in the absence of one. Biomaterials can additionally offer mechanical support against forces generated in vivo so that the predetermined. Generally speaking, three classes of biomaterials have been used to engineer tissues and organs: acellular tissue matrices, like bladder and small-intestinal submucosa; naturally derived materials, like collagen and alginate [48–53]; and synthetic polymers, like polyglycolic acid (PGA), polylactic acid (PLA), and poly(lactic-coglycolic acid) (PLGA). Biologic recognition may be an advantage of naturally generated materials and acellular tissue matrices, however synthetic polymers can be mass-produced in a reliable manner with regulated strength, degradation rate, and microstructure characteristics.Studies in regenerative medicine have lately concentrated on the application of synthetic materials and acellular matrices. Collagen-rich matrices known as acellular tissue matrices are created by extracting tissues' cellular constituents. A tissue segment is frequently mechanically and chemically manipulated to create the matrices [54–57]. After implantation, the matrices gradually deteriorate and are replaced and remodelled by extracellular matrix (ECM) proteins produced and synthesised by ingrowing or transplanted cells. It has been demonstrated that acellular tissue matrices can promote the regrowth and ingrowth of genitourinary tissues, such as the bladder and urethra, without causing immunogenic rejection [57,58]. In regenerative medicine, polyesters containing naturally occurring α-hydroxy acids, including as PGA, PLA, and PLGA, are frequently utilised. The Food and Drug Administration has approved these polymers for use in human medicine for a number of uses, including sutures [34]. Natural, harmless metabolites of PGA, PLA, and PLGA break down into carbon dioxide and water, which are eventually expelled from the body [59]. These polymers are easily made into a three-dimensional scaffold with the necessary microstructure, gross shape, and dimension using a variety of processes because they are thermoplastics [60–62]. Rapidly producing extremely porous scaffolds in different conformations has been accomplished using electrospinning [63–66]. Lack of biologic recognition is one disadvantage of synthetic polymers, however several teams are working to create synthetic scaffolds that include proteins or other molecules to help with recognition [67–69].

Current therapies and future directions for regenerative medicines

Regenerative medicine has promise for normalising congenital abnormalities and healing or replacing tissues and organs damaged by illness, ageing, or trauma. As of now, promising preclinical and clinical data point to the potential for regenerative medicine to help treat a wide range of organ systems and contexts, including wounds on the skin, traumas and cardiovascular diseases, some cancer treatments, and more. It may also be able to treat both acute insults and chronic diseases (70, 71, 72). Regenerative medicine techniques may be able to get beyond the existing approach of transplanting intact organs and tissues to cure organ and tissue failures and loss, which is hindered by a shortage of donors and frequently serious immunological problems (73). Regenerative medicine is a broad field that uses a variety of approaches, such as materials and de novo generated cells, in different combinations, to replace missing tissue in a structurally and functionally effective manner or to aid in tissue healing (74). Although mature humans have a reduced ability for regeneration than other vertebrates, the body's natural healing response can also be used to encourage regeneration (75).

Therapies in the Market

Many treatments that have been approved or cleared by the Food and Drug Administration (FDA) and made commercially available since tissue engineering and regenerative medicine became a business roughly 20 years ago (Table 1). One of the main tenets of regenerative medicine up to this point has been the release of therapeutic cells that directly influence the composition and functionality of new tissues (76, 77). These treatments employ either autologous or allogeneic cells, which are normally differentiated cells with the ability to proliferate. For instance, autologous chondrocytes are used in Carticel, the first biologic product approved by the FDA in the orthopaedics area, to treat focal articular cartilage abnormalities. Here, autologous chondrocytes are expanded ex vivo from articular cartilage, collected, and implanted at the site of injury. The healing that is achieved is similar to what has been seen with procedures such as mosaicplasty and microfracture (78). the use of autologous fibroblast injections to treat wrinkles in the nasolabial folds; A medical instrument called a celution is used to remove cells from adipose tissue obtained after liposuction; Epicel, autologous keratinocytes for severe burn injuries; cord blood collection for haematopoietic stem and progenitor cells. When using autologous cells, a patient's tissue must be harvested in order to create a new wound site. Additionally, because the cells must be culture-expanded before being used, treatment is frequently delayed. In addition to reducing the possibility of an unfavourable immune response, allogeneic cell sources with minimal antigenicity—such as human foreskin fibroblasts utilised in the creation of wound-

healing grafts (GINTUIT, Apligraf) (79)—allow the bulk production of readily available tissues. Because materials can replicate the natural extracellular matrix (ECM) of tissues and control cell behaviour, help shape and function new tissue, and locally provide growth hormones, they are frequently a key part of modern regenerative medicine techniques (80). To encourage chondrocyte expansion during cartilage repair, for instance, 3D polymer scaffolds are utilised in matrix-induced autologous chondrocyte implantation.

Table 1. Regenerative medicine FDA-approved products

Category	Name	Biological agent	Approved use
Biologics	laViv	Autologous fibroblasts	Improving nasolabial fold appearance
	Carticel	Autologous chondrocytes	Cartilage defects from acute or repetitive trauma
	Apligraf, GINTUIT	Allogeneic cultured keratinocytes and fibroblasts in bovine collagen	Topical mucogingival conditions, leg and diabetic foot ulcers
	Cord blood	Hematopoietic stem and progenitor cells	Hematopoietic and immunological reconstitution after myeloablative treatment
Cell-based medical devices	Dermagraft	Allogenic fibroblasts	Diabetic foot ulcer
	Celution	Cell extraction	Transfer of autologous adipose stem cells
Biopharmaceuticals	GEM 125	PDGF-BB, tricalcium phosphate	Periodontal defects
	Regranex	PDGF-BB	Lower extremity diabetic ulcers
	Infuse, Infuse bone graft, Inductos	BMP-2	Tibia fracture and nonunion, and lower spine fusion
	Osteogenic protein-1	BMP-7	Tibia nonunion

(MACI)] and give fibroblasts a scaffold to work on for treating venous ulcers (Dermagraft) (81). Decellularized donor tissues are also employed as tissue substitutes (CryoLife and Toronto's heart valve replacements and cardiac patches) (83), or to encourage wound healing (Dermapure, a range of exclusive bone allografts) (82). As in the instance of bioglass-based grafts that allow fusion with bone, a substance by itself can occasionally give cues for regeneration and graft or implant integration (84).

Biomaterials that incorporate growth factors for healing or regeneration can present these factors locally and sustainably. This strategy has been used to promote bone formation through the delivery of bone morphogenic proteins 2 and 7 (Infuse, Stryker's OP-1) and wound healing through the delivery of platelet derived growth factor (PDGF) (Regranex) (85). However, issues can occur with these approaches (Regranex black box warning, Infuse) (86,87), most likely because factor release kinetics are not well controlled with the materials that are now in use. The FDA has cleared or approved a variety of regenerative medicine products, some of which are more effective than others or at least similar to those that were previously on the market (78).
They provide benefit in terms of healing and regeneration but are unable to fully resolve injuries or diseases (88-90).

Therapies at the Preclinical Stage and in Clinical Testing
Numerous approaches are presently being investigated at the preclinical and clinical stages of research. The following subsections will provide an overview of these various approaches, which can be divided into three main categories: (i) recreating organ and tissue structure using scaffolds, 3D bioprinting, and self assembly; (ii) vascularization and innervation to integrate grafts with the host; and (iii) modifying the host environment to trigger therapeutic responses, especially by infusing cells and regulating the immune system. Lastly, strategies for taking advantage of newly discovered and developing cell sources for regenerative medicine will be discussed.

A: Recapitulating Tissue and Organ Structure
Since the design of tissues and organs is intimately linked to their functions, it is generally accepted that successful recapitulation of healthy tissue requires the ability to reconstruct structure (91). Recellularizing organs after they have been decellularized is one method of capturing organ structure and material composition in artificial tissues in preparation for transplantation. Decellularization eliminates immunogenic cells and molecules while, in theory, maintaining the native extracellular matrix's structure, mechanical characteristics, and material composition (92,93). This method has been applied in animal models of disease affecting the lungs, kidneys, liver, pancreas, and heart in conjunction with bioreactors (91, 94–97).

As previously mentioned, decellularized tissues—which do not require the recellularization step—have also been commercialized as medical devices and have been applied to the healing of significant muscle abnormalities in human patients (98). An alternative method to this one is to create blood vessels in vitro, decellularize them afterwards, and then implant them in kidney dialysis patients (99). Many obstacles still exist in spite of these achievements. Decellularization may remove different kinds and quantities of ECM-associated signaling molecules, which may alter the mechanical properties of tissues and organs. Additionally, processed tissue may deteriorate over time following transplantation without being matched by host cell replacement (100, 101).

Using this method, tissue-engineered vascular grafts (TEVGs), for the treatment of congenital heart abnormalities in adult and pediatric patients, have entered clinical trials (102) (Fig. 2 A and B). Using animal models, it was discovered that the seeded cells in TEVGs controlled the inflammatory response that helped host vascular cells fill the graft and construct the new blood artery, rather than providing structural support to the graft once it was within the host (103, 104). In the setting of end-stage renal failure (Humacyte), biodegradable vascular grafts seeded with cells, cultivated so the cells created extracellular matrix, and then decellularized are undergoing clinical trials (99).

Instructive signals for adhering cells and bulk mechanical support for the developing tissue are two purposes for which scaffolds with a broad range of mechanical properties have been designed (80). Soft fibrin-collagen hydrogels, for instance, have been investigated as lymph node substitutes (105), whereas alginate hydrogels that degrade more quickly have been shown to enhance bone healing in critical defects (106). In certain instances, it is thought that the mechanical characteristics of the polymer alone have a therapeutic impact.

For instance, in models of dilated cardiomyopathy, injection of alginate hydrogels to the left ventricle slowed the course of heart failure (107) and is presently undergoing clinical trials (Algisyl). As demonstrated by composite polyglycolide and collagen scaffolds that were seeded with cells and used as bladder replacements for human patients, combining materials with various characteristics can improve scaffold performance (108). In a different instance, bone morphogenic protein 2 was loaded onto an electrospun nanofiber mesh that was electrospun and electrospun with peptide-modified alginate hydrogel to enhance bone formation in severely large defects (109).

3D images of replacement tissues can be produced using medical imaging technologies like computed tomography (CT) and magnetic resonance imaging (MRI), sometimes using the patient's own body as a model (110, 111). (Fig. 2C). After that, these 3D pictures can be utilized as molds to create scaffolds that are specially made for each patient. For instance, patient CT scans were used to create synthetic trachea made of polyurethane and polyethylene that were subsequently seeded with cells (112). Using a range of recently discovered techniques, tiny building blocks, which are typically composed of cells implanted in a small volume of hydrogel, can also be constructed into tissuelike structures with defined topologies and cell patterning (113, 114) (Fig2D)

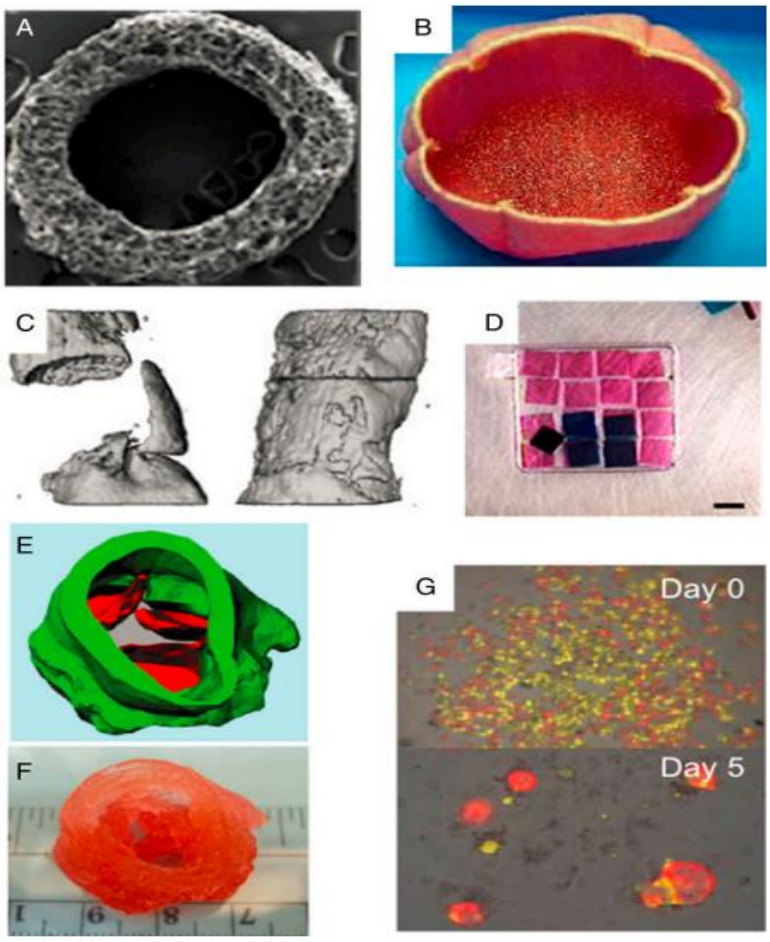

FIG.2.:Regenerative medicine strategies that recapitulate tissue and organ structure. (A) Scanning electron microscopy image of a TEVG cross-section. Reproduced with permission from ref. 41. (B) Engineered bladder

consisting of a polyglycolide and collagen composite scaffold, fabricated based on CT image of patient and seeded with cells. Reproduced with permission from ref. 46. (C) CT image of bone regeneration in critically sized defects without (Left) and with (Right) nanofiber mesh and alginate scaffold loaded with growth factor. Reproduced with permission from ref. 47. (D) Small hydrogel building blocks are assembled into tissue-like structures with microrobots. Reproduced from ref. 52, with permission from Nature Communications. (E) Blueprint for 3D bioprinting of a heart valve using microextrusion printing, with different colors representing different cell types. (F) Printed product. Reproduced with permission from ref. 59. (G) Intestinal crypt stem cells seeded with supporting Paneth cells self-assemble into organoids in culture. Reproduced from ref. 67, with permission from Nature.

3D bioprinting can produce structures that combine high resolution control over material and cell placement within designed constructs, despite the fact that cell placement within scaffolds is typically poorly managed (115). Inkjet and microextrusion are two of the most popular bioprinting techniques (116). Using pressure pulses produced by short electrical heating or acoustic waves, inkjet bioprinting produces ink droplets with cells at the nozzle (117, 118). A continuous stream of ink is dispensed onto a stage using microextrusionbioprinting (119). Both are actively being utilized to create a variety of tissues. For instance, chondrocytes suspended in a fibrin–collagen matrix and electrospunpolycaprolactone fibers have been deposited one layer at a time using inkjet bioprinting to construct cartilage.

Following implantation, it was discovered that the cells injected in this manner produced collagen II and glycosaminoglycans (120). Aortic valve replacements have been created by microextrusion printing by employing cells encapsulated in a gelatin/alginate hydrogel. The valve root and leaflets, respectively, were printed with two different cell types—smooth muscle cells and interstitial cells—in two different areas (121) (Fig. 2 E and F). Complex 3D tubular networks have been printed via microextrusion printing using inks with varying gelation temperatures. The printed tubes were subsequently seeded with endothelial cells to replicate the vasculature (122). There are numerous commercially accessible 3D bioprinting equipment that provide various bioprinting methodologies and capabilities (115).

The development of bioprinting technologies that excel in all three areas is an important focus of research in this field. Bioprinting tactics, although very promising, sometimes suffer trade-offs in terms of feature resolution, cell survival, and printing resolution (115). It might be feasible to create new tissues using scaffold-free techniques in specific circumstances. Cell-cell adhesion and signaling molecules, as well as extracellular matrix molecules (ECM) produced by the cells themselves, can be preserved when a confluent sheet of cells is extracted from a temperature-responsive substrate, which is the basis for cell sheet technology (123, 124). It is possible to layer successive sheets to create thicker structures (125). This method has been investigated in many different settings, such as corneal reconstruction (126). Reepithelialization of human corneas has been achieved with the growth, harvesting, and implantation of autologous oral mucosal cells into sheets (126). Moreover, tissues can be produced via autonomous cellular self-assembly, which can be utilized in conjunction with bioprinting. For instance, agarose rods and vascular cells aggregated into multicellular spheroids were printed layer by layer using microextrusion; once the cells formed a continuous structure, the agarose was physically removed to produce hollow and branching structures that resembled a vascular network (127). Even sophisticated structures can emerge on their own when the right stimuli and starting cell composition are present (128).

For instance, using increased Wnt signaling in conjunction with a single crypt base columnar stem cell, intestinal cryptlike structures can be produced in 3D culture (129) (Fig. 2G). A comprehensive grasp of the biological mechanisms propelling and guiding self-assembly is necessary to optimize this strategy. For the building of complex organs, the capacity to induce autonomous self-assembly of modular organ components, such as intestine crypts, kidney nephrons, and lung alveoli, may be particularly useful.

B: Integrating Graft Tissue by Inducing Vascularization and Innervation

Implanted grafts must correctly integrate with the body in order to contribute both structurally and functionally. Integration with the host vasculature is crucial for the success of grafts using cell-based implants (Fig. 3A) (130). The majority of body cells are situated at a distance of no more than 100 μm from the closest capillary, which is the minimum distance necessary for efficient exchange of nutrients and diffusion of oxygen from the bloodstream (130). By presenting angiogenic growth factors, one can take advantage of the body's natural angiogenic response to vascularize manmade tissues (131). Numerous growth factors, such as platelet-derived growth factor (PDGF), angiopoietin (Ang), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF), have been linked to angiogenesis (132, 133).

But because growth factors have a short half-life in vivo and bolus treatment might be toxic and have systemic effects, using them might not be effective without the right delivery method (107). Strong angiogenic responses are produced by the prolonged production of VEGF, bFGF, Ang, and PDGF, which can prevent necrosis in ischemia limbs (107, 134, 135). More functional networks can be produced by supplying an angiogenic factor sequence that first initiates and then promotes the maturation of newly formed vessels (136) (Fig. 3 B and C). Similarly, by simulating development through the delivery

of both angiogenesis promoters and inhibitors from distinct spatial locations, tightly defined angiogenic zones can be created (137).

Prevascularizing the graft or target site before to implantation is another strategy to encourage graft vascularization at the target location. When placed on the proper substrate, endothelial cells and their progenitors can selforganize into vascular networks (138–141). Tissues with improved vascularization and tissue-specific activity can be obtained by combining endothelial cells with tissue-specific cells on a scaffold prior to transplantation (142).

Alternatively, a scaffold can be first placed around a large host vessel or on richly vascularized tissue, and the engineered tissue can then be moved to its final anatomic location once it becomes vascularized at the original site (143–145). This approach has been demonstrated in the context of both bone and cardiac patches. The creation of a vascular pedicle for the engineered tissue can also facilitate subsequent transplantation. (Fig. 3D).An whole mandibular replacement that was subsequently engrafted in a human patient was vascularized using this technique with success (146). Vascular networks that can be anastomosed to the femoral artery are presently being engineered through the investigation of microfluidic and micropatterning techniques (147, 148) (Fig. 3E).

As demonstrated in a recent report, the placement of a catheter device allowed the site to become vascularized due to the host foreign body response to the material; this device significantly improved the efficacy of pancreatic cells subsequently injected into the device (149). Alternatively, the site for cell delivery could be prevascularized to enhance cell survival and function. Many tissues must also be innervated by the host in order for them to operate properly and integrate fully (150, 151). This is especially true for tissues that depend on motor control—like skeletal tissue—or sensation—like the epidermis—for essential functions (152, 153).

development factors have the ability to generate innervation of designed tissues, as demonstrated by the in vitro model where nerve development was stimulated from mouse embryonic dorsal root ganglia to epithelial tissue (154). This strategy has been utilized to enhance neuron regeneration following damage. Hydrogels patterned with channels that are then loaded with the proper extracellular matrices and growth hormones can direct nerve growth upon implantation (155, 156). Axon renewal in regenerating skeletal muscle has been promoted by the regulated supply of VEGF employing biomaterials, as angiogenesis and nerve growth are known to share specific signaling pathways (157, 158, 159) (Fig. 3 F and G).

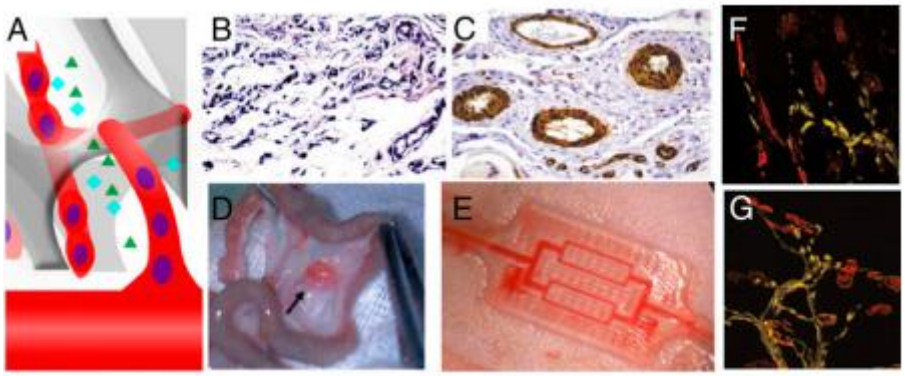

FIG.3.: Strategies for vascularizing and innervating tissue-engineered graft. (A) Tissue-engineered graft may be vascularized before implantation: for example, by self-assembly of seeded endothelial cells or by host blood vessels in a process mediated by growth factor release. Compared with bolus injection of VEGF and PDGF (B), sustained release of the same growth factors from a polymeric scaffold (C) led to a higher density of vessels and formation of larger and thicker vessels. Reproduced from ref. 74, with permission from Nature Biotechnology. (D) Scaffold vascularized by being implanted in the omentum before implantation at the injury site. Reproduced with permission from ref. 83. (E) Biodegradable microfluidic device surgically connected to vasculature. Reproduced with permission from ref. 85. Compared with blank scaffold (F), scaffolds delivering VEGF (G) increase innervation of injured skeletal muscle. Reproduced from ref. 97, with permission from Molecular Therapy.

Feasibility of Building Capacity in Regenerative Medicine in Developing Countries

Developing nations' decisions to develop their own regenerative medicine capabilities or to wait to adopt treatments created elsewhere may be influenced by their economic status as well as the amount of research focus industrialized nations place on a given regenerative medicine application. [161]

However, domestic innovation by developing countries is significant since it is more likely to be oriented towards local health needs and can contribute to both economic and health development, as noted in the 2005 report of the United Nations Task Force on Science, Technology, and Innovation. [162] Furthermore, prior research on the health biotechnology

industries in developing nations has demonstrated that scientific and technological innovation at the local level can result in more reasonably priced medicines for the populations of these nations.163 In contrast to imported vaccines that retail for $US8–$US10 each dosage, India's Shantha Biotechnics has created a recombinant hepatitis B vaccine that costs about $US0.40 per dose. [164]

Regarding the viability of implementation, it should be mentioned that organ transplantation is already practiced in nearly all Asian, Latin American, and some African nations. Additionally, some regenerative medicine treatments, like cell therapy, might be considerably easier to implement technically than traditional transplantation.

Current Regulations [160,165]

Even though laws differ from nation to nation, nearly all jurisdictions have the same characteristics: The FDA categorizes therapies as either a medication, a device, or a biologic. Every categorization has a different set of rules and a review center made up of subject matter experts. Some or all of these categories apply to products used in regenerative medicine, thus regulators must properly combine various laws to guarantee sufficient product efficacy and safety.

Good Manufacturing Practice (GMP) and Good Tissue Practice (GTP) regulations must be followed by producers of these goods. Regenerative medicine is governed by the FDA in the United States. By combining several regulatory initiatives into the Office of Cellular, Tissue, and Gene Therapies (OCTGT), the FDA has been creating a more cohesive approach to the regulation of novel products related to regenerative medicine. Tissue, cellular therapy, gene therapy, cellular plus gene therapies, and tissue engineering are under the purview of this office.

. Furthermore, on December 24, 2002, the FDA established the Office of Combination Products (OCP) in accordance with The Medical Device User Fee and Modernization Act of 2002 in order to expedite the regulatory review process for products that combine two or more regulated components. Examples of these include drugs and devices (e.g., drug-eluting stents), biologics and devices (e.g., bioartificial organs), drugs and devices (e.g., recombinant proteins), and orthopedic implants with growth factors and anti-inflammatory drugs that are combined or mixed in any other way and produced as a single entity. With the establishment of these new regulatory offices, the FDA has started to transform into a more agile approval body that can handle the technological advancements of the twenty-first century.

In an effort to bring regenerative medicine to their own citizens, some nations have also launched national projects. In an effort to achieve their own breakthroughs in regenerative medical technology, a number of EU members—including Sweden, Germany, Great Britain—as well as China, Japan, and Australia—have started putting significant national commitments into place.

Conclusion

New therapeutic treatments and the creation of reasonably priced, efficient medications are necessary for the country's health to continue improving. With treatments that promote in vivo regeneration and the in vitro production of healthy tissue for implantation, regenerative medicine holds the promise of curing a plethora of deadly and crippling illnesses. In the next 20 years, the next phase of medical technology development could materialize. It is currently in sight. Regenerative medicine is a field that science will inevitably advance, but it will take too long for the technology to catch up without direction. To understand regenerative medicine and bring this technology to market within the next 20 years, Initiative for Regenerative Medicine is needed.

REFERENCES:

1- Denham M, Conley B, Olsson F, Cole TJ, Mollard R: Stem cells: an overview. CurrProtoc Cell Biol 2005;23:23.1.

2- Vats A, Bielby RC, Tolley NS, Nerem R, PolakJM: Stem cells. Lancet 2005;366:592–602.

3 -Lajtha LG: Stem cells and their properties. Proc Can Cancer Conf 1967;7:31–39.

4 -He S, Nakada D, Morrison SJ: Mechanisms of stem cell self-renewal. Annu Rev Cell Dev Biol 2009;25:377–406.

5 -Falanga V: Stem cells in tissue repair andregeneration. J Invest Dermatol 2012;132: 1538–1541.

6 -Lane S, Rippon HJ, Bishop AE: Stem cells in lung repair and regeneration. Regen Med 2007;2:407–415.

7 -Fausto N: Liver regeneration and repair: he-patocytes, progenitor cells, and stem cells. Hepatology 2004;39:1477–1487.

8 -Shaker A, Rubin DC: Stem cells: one step clos-er to gut repair. Nature 2012;485:181–182.

9 -Han W, Yu Y, Liu XY: Local signals in stem cell-based bone marrow regeneration. Cell Res 2006;16:189–195.

10 -Angelini A, Castellani C, Vescovo G, ThieneG: Pathological evidence of stem cell regen-eration in the heart. Int J Cardiol 2004;96: 499–504.

11 -Mirotsou M, Jayawardena TM, Schmeck-peper J, Gnecchi M, Dzau VJ: Paracrinemechanismsof stem cell reparative and re-generative actions in the heart. J Mol Cell Cardiol 2011;50:280–289.

12 -Mansergh FC, Wride MA, Rancourt DE: Neurons from stem cells: implications for understanding nervous system development and repair.Biochem Cell Biol 2000;78:613–628.

13 -Bouwens L: Transdifferentiation versus stem cell hypothesis for the regeneration of islet beta-cells in the pancreas. Microsc Res Tech 1998;43:332–336.
14 -Lodi D, Iannitti T, Palmieri B: Stem cells in clinical practice: applications and warnings. J ExpClin Cancer Res 2011;30:9.
15 -Dameshek W: Bone marrow transplantation; a present-day challenge. Blood 1957;12:321–323.
16 -de la Morena MT, Gatti RA: A history of bone marrow transplantation. Immunol Allergy Clin North Am 2010;30:1–15.
17 -Le BK, Ringden O: Mesenchymal stem cells: properties and role in clinical bone marrow transplantation. CurrOpinImmunol 2006; 18:586–591.
18 -Chien KR: Regenerative medicine andhu-man models of human disease. Nature 2008; 453:302–305.
19 -Inoue H, Yamanaka S: The use of induced pluripotent stem cells in drug development. ClinPharmacolTher 2011;89:655–661.
20 -Fahey MC, Wallace EM: Stem cells: research tools and clinical treatments. J PaediatrChild Health 2011;47:672–675.
21 -McCormick JB, Huso HA: Stem cells and ethics: current issues. J CardiovascTranslRes 2010;3:122–127.
22 -Xi J, Zhang SC: Stem cells in development of therapeutics for Parkinson's disease: a per-spective. J Cell Biochem 2008;105:1153–1160.
23 -Magga J, Savchenko E, Malm T, Rolova T, Pollari E, Valonen P, Lehtonen S, Jantunen E, Aarnio J, Lehenkari P, Koistinaho M, MuonaA, Koistinaho J: Production of monocyticcells from bone marrow stem cells: therapeu-tic usage in Alzheimer's disease. J Cell MolMed 2012;16:1060–1073.
24 -Perin EC, Silva GV, Zheng Y, GahremanpourA, Canales J, Patel D, Fernandes MR, Keller LH, Quan X, Coulter SA, Moore WH, Herlihy JP, Willerson JT: Randomized, double-blind pilot study of transendocardial injection ofautologous aldehyde dehydrogenase-bright stem cells in patients with ischemic heart fail-ure. Am Heart J 2012;163:415–421, 421.e1.
25 -CerlettiM,Jurga S, Witczak CA, HirshmanMF, Shadrach JL, Goodyear LJ, Wagers AJ: Highly efficient, functional engraftment of skeletal muscle stem cells in dystrophic mus-cles. Cell 2008;134:37–47.
26 -Tzouvelekis A, Koliakos G, Ntolios P, Baira I, BourosE,Oikonomou A, Zissimopoulos A, Kolios G, Kakagia D, Paspaliaris V, Kotsian-idis I, Froudarakis M, Bouros D: Stem cell therapy for idiopathic pulmonary fibrosis: a protocol proposal. J Transl Med 2011;9:182.
27 -Weiss DJ, Bertoncello I, Borok Z, Kim C, Panoskaltsis-Mortari A, Reynolds S, Rojas M, Stripp B, Warburton D, Prockop DJ: Stem cells and cell therapies in lung biology and lung diseases. Proc Am ThoracSoc 2011;8: 223–272.
28 -Rashid ST, Corbineau S, Hannan N, Marci-niak SJ, Miranda E, Alexander G, Huang-Doran I, Griffin J, Ahrlund-Richter L, Skep-per J, Semple R, Weber A, Lomas DA, VallierL: Modeling inherited metabolic disorders of the liver using human induced pluripotent stem cells. J Clin Invest 2010;120:3127–3136.
29- Chun SY, Lim GJ, Kwon TG, et al. Identification and characterization of bioactive factors in bladder submucosa matrix. Biomaterials 2007;28:4251-6.
30- Sharon JL, Puleo DA. The use of N-terminal immobilization of PTH(1- 34) on PLGA to enhance bioactivity. Biomaterials 2008;29:3137-42.
31-Kim BS, Baez CE, Atala A. Biomaterials for tissue engineering. World J Urol 2000;18:2-9.
32-Tabata Y. Current status of regenerative medical therapy based on drug delivery technology.Reprod Biomed Online 2008;16:70-80.
33-Brivanlou AH, Gage FH, Jaenisch R, et al. Stem cells. Setting standards for human embryonic stem cells. [see comment]. Science 2003;300:913-6.
34-Reubinoff BE, Itsykson P, Turetsky T, et al. Neural progenitors from human embryonic stem cells. [see comment]Nat Biotechnol 2001;19: 1134-40.
35-Schuldiner M, Eiges R, Eden A, et al. Induced neuronal differentiation of human embryonic stem cells. Brain Res 2001;913:201-5.
36-Schuldiner M, Yanuka O, Itskovitz-Eldor J, et al. Effects of eight growth factors on the differentiation of cells derived from human embryonic stem cells. Proc Natl AcadSci U S A 2000;97:11307-12.
37- Zhang SC, Wernig M, Duncan ID, et al. In vitro differentiation of transplantable neural precursors from human embryonic stem cells. [see comment]Nat Biotechnol 2001;19:1129-33.
38-Kaufman DS, Hanson ET, Lewis RL, et al. Hematopoietic colonyforming cells derived from human embryonic stem cells. Proc Natl AcadSci U S A 2001;98:10716-21.
39-Kehat I, Kenyagin-Karsenti D, Snir M, et al. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. [see comment]J Clin Invest 2001;108: 407-14.
40-Levenberg S, Golub JS, Amit M, et al. Endothelial cells derived from human embryonic stem cells. Proc Natl AcadSci U S A 2002;99: 4391-6.
41-Assady S, Maor G, Amit M, et al. Insulin production by human embryonic stem cells. Diabetes 2001;50:1691-7.
42-Hochedlinger K, Rideout WM, Kyba M, et al. Nuclear transplantation, embryonic stem cells and the potential for cell therapy.Hematol J 2004;5(Suppl 3):S114-7.
43- Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663-76.

44- Takahashi K, Tanabe K, Ohnuki M, et al. Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. Cell 2007;131:861-72.
45- Yu J, Vodyanik MA, Smuga-Otto K, et al. Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells. Science 2007:1151526.
46- De Coppi P, Bartsch Jr G, Siddiqui MM, et al. Isolation of amniotic stem cell lines with potential for therapy. [see comment]. Nat Biotechnol 2007;25:100-6.
47- Kim BS, Mooney DJ. Development of biocompatible synthetic extracellular matrices for tissue engineering. Trends Biotechnol 1998;16:224-30.
48- Li ST. Biologic biomaterials: tissue derived biomaterials (collagen). In: Bronzino JD, editor. The Biomedical Engineering Handbook. Boca Raton (Fla): CRS Press; 1995. p. 627-47.
49-Furthmayr H, Timpl R. Immunochemistry of collagens and procollagens.Int Rev Connect Tissue Res 1976;7:61-99. \
50- Cen L, Liu W, Cui L, et al. Collagen tissue engineering: development of novel biomaterials and applications. Pediatr Res 2008;63:492-6.
51-Yannas IV, Burke JF, Gordon PL, et al. Design of an artificial skin. II. Control of chemical composition. J Biomed Mater Res 1980;14: 107-32.
52-Yannas IV, Burke JF.Design of an artificial skin. I. Basic design principles. J Biomed Mater Res 1980;14:65-81.
53-Cavallaro JF, Kemp PD, Kraus KH. Collagen fabrics as biomaterials.BiotechnolBioeng 1994;43:781-91.
54-Dahms SE, Piechota HJ, Dahiya R, et al. Composition and biomechanical properties of the bladder acellular matrix graft: comparative analysis in rat, pig and human. Br J Urol 1998;82:411-9.
55-Piechota HJ, Dahms SE, Nunes LS, et al.In vitro functional properties of the rat bladder regenerated by the bladder acellular matrix graft. J Urol 1998;159:1717-24.
56-Yoo JJ, Meng J, Oberpenning F, et al. Bladder augmentation using allogenic bladder submucosa seeded with cells. Urology 1998;51: 221-5.
57- Chen F, Yoo JJ, Atala A. Acellular collagen matrix as a possible "off the shelf" biomaterial for urethral repair. Urology 1999;54:407-10.
58- Probst M, Dahiya R, Carrier S, et al. Reproduction of functional smooth muscle tissue and partial bladder replacement. Br J Urol 1997;79:505-15.
59- Gilding D. Biodegradable Polymers. In: Williams D, editor. Biocompatibility of clinical implant materials. Boca Raton (Fla): CRC Press; 1981. p. 209-32.
60- Freed LE, Vunjak-Novakovic G, Biron RJ, et al. Biodegradable polymer scaffolds for tissue engineering. Biotechnology (N Y) 1994; 12:689-93.
61-Mikos AG, Lyman MD, Freed LE, et al. Wetting of poly(L-lactic acid) and poly(DL-lactic-co-glycolic acid) foams for tissue culture. Biomaterials 1994;15:55-8.
62- Harris LD, Kim BS, Mooney DJ. Open pore biodegradable matrices formed with gas foaming. J Biomed Mater Res 1998;42:396-402.
63- Han D, Gouma PI. Electrospunbioscaffolds that mimic the topology of extracellular matrix. Nanomedicine 2006;2:37-41.
64- Choi JS, Lee SJ, Christ GJ, et al. The influence of electrospun aligned poly(epsilon-caprolactone)/collagen nanofiber meshes on the formation of self-aligned skeletal muscle myotubes. Biomaterials 2008;29: 2899-906.
65- Lee SJ, Liu J, Oh SH, et al. Development of a composite vascular scaffolding system that withstands physiological vascular conditions. Biomaterials 2008;29:2891-8.
66- Lee SJ, Oh SH, Liu J, et al. The use of thermal treatments to enhance the mechanical properties of electrospunpoly(epsilon-caprolactone) scaffolds. Biomaterials 2008;29:1422-30.
67- Barrera DA, Zylstra E, Lansbury PT, et al. Synthesis and RGD peptide modification of a new biodegradable copolymer poly (lactic acid-colysine). J Am ChemSoc 1993;115:11010-1.
68- Cook AD, Hrkach JS, Gao NN, et al. Characterization and development of RGD-peptide-modified poly(lactic acid-co-lysine) as an interactive, resorbable biomaterial. J Biomed Mater Res 1997;35: 513-23.
69-Intveld PJA, Shen ZR, Takens GAJ. Glycine glycolic acid based copolymers. J PolymSciPolymChem 1994;32:1063-9.
70- Jaklenec A, Stamp A, Deweerd E, Sherwin A, Langer R (2012) Progress in the tissue engineering and stem cell industry “are we there yet?”. Tissue Eng Part B Rev 18(3):155–166.
71- Bailey AM, Mendicino M, Au P (2014) An FDA perspective on preclinical development of cell-based regenerative medicine products. Nat Biotechnol 32(8):721–723.
72-Mendelson A, Frenette PS (2014) Hematopoietic stem cell niche maintenance during homeostasis and regeneration. Nat Med 20(8): 833–846.
73- Vacanti JP, Otte J-B, Wertheim JA (2014) Introduction: Regenerative medicine and solid organ transplantation from a historical perspective. Regenerative Medicine Applications in Organ Transplantation, eds Orlando G, Lerut J, Soker S, Stratta RJ (Elsevier, London), pp 1–15.
74- Bajaj P, Schweller RM, Khademhosseini A, West JL, Bashir R (2014) 3D biofabrication strategies for tissue engineering and regenerative medicine. Annu Rev Biomed Eng 16:247–276.

75- Kami D, Gojo S (2014) Tuning cell fate: From insights to vertebrate regeneration. Organogenesis 10(2):231–240
76- Buckler L (2011) Opportunities in regenerative medicine. Bioprocess Int 2011(March):14–18.
77- Fisher MB, Mauck RL (2013) Tissue engineering and regenerative medicine: Recent innovations and the transition to translation. Tissue Eng Part B Rev 19(1):1–13.
78- Dewan AK, Gibson MA, Elisseeff JH, Trice ME (2014) Evolution of autologous chondrocyte repair and comparison to other cartilage repair techniques. BioMed Res Int 2014:272481.
79-Falanga V, Sabolinski M (1999) Abilayered living skin construct (APLIGRAF) accelerates complete closure of hard-to-heal venous ulcers. Wound Repair Regen 7(4):201–207.
80- Huebsch N, Mooney DJ (2009) Inspiration and application in the evolution of biomaterials. Nature 462(7272):426–432.
81- Harding K, Sumner M, Cardinal M (2013) A prospective, multicentre, randomised controlled study of human fibroblast-derived dermal substitute (Dermagraft) in patients with venous leg ulcers. Int Wound J 10(2):132–137.
82- Song JJ, Ott HC (2011) Organ engineering based on decellularized matrix scaffolds. Trends Mol Med 17(8):424–432.
83- Chambers JB, Rimington HM, Rajani R, Hodson F, Shabbo F (2007) A randomized comparison of the Cryolife O'Brien and Toronto stentless replacement aortic valves. J ThoracCardiovascSurg 133(4): 1045–1050.
84- Oryan A, Alidadi S, Moshiri A, Maffulli N (2014) Bone regenerative medicine: Classic options, novel strategies, and future directions. J OrthopSurg 9(1):18.
85- Walsh G (2010) Biopharmaceutical benchmarks 2010. Nat Biotechnol 28(9):917–924.
86-Papanas N, Maltezos E (2008) Becaplermin gel in the treatment of diabetic neuropathic foot ulcers. ClinInterv Aging 3(2):233–240.
87- Epstein NE (2014) Basic science and spine literature document bone morphogenetic protein increases cancer risk. SurgNeurolInt 5(Suppl 15):S552–S560.
88- Kim PJ, Dybowski KS, Steinberg JS (2006) A closer look at bioengineered alternative tissues. Podiatry Today 19(7):1–9.
89- Supp DM, Boyce ST (2005) Engineered skin substitutes: Practices and potentials. ClinDermatol 23(4):403–412.
90- O'Brien T, Barry FP (2009) Stem cell therapy and regenerative medicine. Mayo Clin Proc 84(10):859–861.
91- Nelson CM, Bissell MJ (2006) Of extracellular matrix, scaffolds, and signaling: Tissue architecture regulates development, homeostasis, and cancer. Annu Rev Cell Dev Biol 22:287–309.
92- Crapo PM, Gilbert TW, Badylak SF (2011) An overview of tissue and whole organ decellularization processes. Biomaterials 32(12): 3233–3243.
93- Macchiarini P, et al. (2008) Clinical transplantation of a tissueengineered airway. Lancet 372(9655):2023–2030.
94- Petersen TH, et al. (2010) Tissue-engineered lungs for in vivo implantation. Science 329(5991):538–541.
95- Uygun BE, et al. (2010) Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. Nat Med 16(7):814–820.
96- Nakayama KH, Batchelder CA, Lee CI, Tarantal AF (2010) Decellularized rhesus monkey kidney as a three-dimensional scaffold for renal tissue engineering. Tissue Eng Part A 16(7):2207–2216.
97- Goh SK, et al. (2013) Perfusion-decellularized pancreas as a natural 3D scaffold for pancreatic tissue and whole organ engineering. Biomaterials 34(28):6760–6772.
98- Mase VJ, Jr, et al. (2010) Clinical application of an acellular biologic scaffold for surgical repair of a large, traumatic quadriceps femoris muscle defect. Orthopedics 33(7):511.
99- Dahl SL, et al. (2011) Readily available tissue-engineered vascular grafts. SciTransl Med 3(68):68ra9.
100- Fishman JM, et al. (2014) Airway tissue engineering: An update. Expert OpinBiolTher 14(10):1477–1491.
101- Badylak SF, Taylor D, Uygun K (2011) Whole-organ tissue engineering: Decellularization and recellularization of threedimensional matrix scaffolds. Annu Rev Biomed Eng 13:27–53
102- Patterson JT, et al. (2012) Tissue-engineered vascular grafts for use in the treatment of congenital heart disease: From the bench to the clinic and back again. Regen Med 7(3):409–419.
103-Roh JD, et al. (2010) Tissue-engineered vascular grafts transform into mature blood vessels via an inflammation-mediated process of vascular remodeling. Proc Natl AcadSci USA 107(10):4669–4674.
104- Hibino N, et al. (2011) Tissue-engineered vascular grafts form neovessels that arise from regeneration of the adjacent blood vessel. FASEB J 25(8):2731–2739.
105-Cupedo T, Stroock A, Coles M (2012) Application of tissue engineering to the immune system: Development of artificial lymph nodes. Front Immunol 3:343.
106- Simmons CA, Alsberg E, Hsiong S, Kim WJ, Mooney DJ (2004) Dual growth factor delivery and controlled scaffold degradation enhance in vivo bone formation by transplanted bone marrow stromal cells. Bone 35(2):562–569.
107- Lee K, Silva EA, Mooney DJ (2011) Growth factor delivery-based tissue engineering: General approaches and a review of recent developments. J R Soc Interface 8(55):153–170.
108- Atala A, Bauer SB, Soker S, Yoo JJ, Retik AB (2006) Tissueengineered autologous bladders for patients needing cystoplasty. Lancet 367(9518):1241–1246.
109-Kolambkar YM, et al. (2011) An alginate-based hybrid system for growth factor delivery in the functional repair of large bone defects. Biomaterials 32(1):65–74.

110-Ballyns JJ, et al. (2008) Image-guided tissue engineering of anatomically shaped implants via MRI and micro-CT using injection molding. Tissue Eng Part A 14(7):1195–1202.
111- Appel AA, Anastasio MA, Larson JC, Brey EM (2013) Imaging challenges in biomaterials and tissue engineering. Biomaterials 34(28):6615–6630.
112-Ajalloueian F, et al. (2014) Biomechanical and biocompatibility characteristics of electrospun polymeric tracheal scaffolds. Biomaterials 35(20):5307–5315.
113-Guven S, et al. (2015) Multiscale assembly for tissue engineering and regenerative medicine. Trends Biotechnol 33(5):269–279.
114-Tasoglu S, Diller E, Guven S, Sitti M, Demirci U (2014) Untethered micro-robotic coding of three-dimensional material composition. Nat Commun 5:3124.
115-Ozbolat IT (2015) Bioprinting scale-up tissue and organ constructs for transplantation. Trends Biotechnol 33(7):395–400.
116- Murphy SV, Atala A (2014) 3D bioprinting of tissues and organs. Nat Biotechnol 32(8):773–785.
117-Tekin E, Smith PJ, Schubert US (2008) Inkjet printing as a deposition and patterning tool for polymers and inorganic particles. Soft Matter 4(4):703.
118- Cui X, Boland T, D'Lima DD, Lotz MK (2012) Thermal inkjet printing in tissue engineering and regenerative medicine. Recent Pat Drug DelivFormul 6(2):149–155.
119- Derby B (2012) Printing and prototyping of tissues and scaffolds. Science 338(6109):921–926.
120- Xu T, et al. (2013) Hybrid printing of mechanically and biologically improved constructs for cartilage tissue engineering applications. Biofabrication 5(1):015001.
121-Duan B, Hockaday LA, Kang KH, Butcher JT (2013) 3D bioprinting of heterogeneous aortic valve conduits with alginate/ gelatin hydrogels. J Biomed Mater Res A 101(5):1255–1264.
122- Kolesky DB, et al. (2014) 3D bioprinting of vascularized, heterogeneous cell-laden tissue constructs. Adv Mater 26(19): 3124–3130.
123- Okano T, Yamada N, Sakai H, Sakurai Y (1993) A novel recovery system for cultured cells using plasma-treated polystyrene dishes grafted with poly(N-isopropylacrylamide). J Biomed Mater Res 27(10):1243–1251.
124- Nakajima K, et al. (2001) Intact microglia are cultured and noninvasively harvested without pathological activation using a novel cultured cell recovery method. Biomaterials 22(11):1213–1223.
125- Yamato M, Okano T (2004) Cell sheet engineering. Mater Today 7(5):42–47.
126- Nishida K, et al. (2004) Corneal reconstruction with tissueengineered cell sheets composed of autologous oral mucosal epithelium. N Engl J Med 351:1187–1196.
127-Norotte C, Marga FS, Niklason LE, Forgacs G (2009) Scaffold-free vascular tissue engineering using bioprinting. Biomaterials 30(30): 5910–5917.
128-Sasai Y (2013) Next-generation regenerative medicine: Organogenesis from stem cells in 3D culture. Cell Stem Cell 12(5): 520–530.
129- Sato T, et al. (2011) Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. Nature 469(7330):415–418.
130- Lovett M, Lee K, Edwards A, Kaplan DL (2009) Vascularization strategies for tissue engineering. Tissue Eng Part B Rev 15(3): 353–370.
131- Battler A, et al. (1993) Intracoronary injection of basic fibroblast growth factor enhances angiogenesis in infarcted swine myocardium. J Am CollCardiol 22(7):2001–2006.
132-Darland DC, D'Amore PA (1999) Blood vessel maturation: Vascular development comes of age. J Clin Invest 103(2):157–158.
133- Conway EM, Collen D, Carmeliet P (2001) Molecular mechanisms of blood vessel growth. Cardiovasc Res 49(3):507–521.
134- Silva EA, Mooney DJ (2007) Spatiotemporal control of vascular endothelial growth factor delivery from injectable hydrogels enhances angiogenesis. J ThrombHaemost 5(3):590–598.
135-Hea L (2007) The effect of the controlled release of basic fibroblast growth factor from ionic gelatin-based hydrogels on angiogenesis in a murine critical limb ischemia model. Biomaterials 28(16):8.
136- Richardson TP, Peters MC, Ennett AB, Mooney DJ (2001) Polymeric system for dual growth factor delivery. Nat Biotechnol 19(11):1029–1034.
137- Yuen WW, Du NR, Chan CH, Silva EA, Mooney DJ (2010) Mimicking nature by codelivery of stimulant and inhibitor to create temporally stable and spatially restricted angiogenic zones. Proc Natl AcadSci USA 107(42):17933–17938.
138-Nör JE, et al. (2001) Engineering and characterization of functional human microvessels in immunodeficient mice. Lab Invest 81(4):453–463.
139- Chen X, et al. (2009) Prevascularization of a fibrin-based tissue construct accelerates the formation of functional anastomosis with host vasculature. Tissue Eng Part A 15(6):1363–1371.
140-Fedorovich NE, Haverslag RT, Dhert WJ, Alblas J (2010) The role of endothelial progenitor cells in prevascularized bone tissue engineering: Development of heterogeneous constructs. Tissue Eng Part A 16(7):2355–2367.

141-Montaño I, et al. (2010) Formation of human capillaries in vitro: The engineering of prevascularized matrices. Tissue Eng Part A 16(1): 269–282.
142-Lesman A, et al. (2011) Engineering vessel-like networks within multicellular fibrin-based constructs. Biomaterials 32(31):7856–7869.
143-Mikos AG, et al. (1993) Prevascularization of porous biodegradable polymers. BiotechnolBioeng 42(6):716–723.
144-Levenberg S, et al. (2005) Engineering vascularized skeletal muscle tissue. Nat Biotechnol 23(7):879–884.
145-Dvir T, et al. (2009) Prevascularization of cardiac patch on the omentum improves its therapeutic outcome. Proc Natl AcadSci USA 106(35):14990–14995.
146-Warnke PH, et al. (2004) Growth and transplantation of a custom vascularised bone graft in a man. Lancet 364(9436): 766–770.
147- Zhang B, et al. (2013) Microfluidic tissue: A biodegradable scaffold with built-in vasculature for cardiac tissue vascularization and surgical vascular anastomosis. Proceedings of the Seventeenth International Conference on Miniaturized Systems for Chemistry and Life Sciences (Chemical and Biological Microsystems Society, San Diego), pp 2019–2021.
148- Hasan A, et al. (2014) Microfluidic techniques for development of 3D vascularized tissue. Biomaterials 35(26):7308–7325.
149- Pepper AR, et al. (2015) Aprevascularized subcutaneous deviceless site for islet and cellular transplantation. Nat Biotechnol 33(5): 518–523.
150-Anand P, et al. (1996) The role of endogenous nerve growth factor in human diabetic neuropathy. Nat Med 2(6):703–707.
151-Tuszynski MH, Steward O (2012) Concepts and methods for the study of axonal regeneration in the CNS. Neuron 74(5):777–791.
152- Griffith M, et al. (2009) Artificial corneas: A regenerative medicine approach. Eye (Lond) 23(10):1985–1989.
153- Cezar CA, Mooney DJ (2015) Biomaterial-based delivery for skeletal muscle repair. Adv Drug Deliv Rev 84:188–197.
154-Suuronen EJ, et al. (2004) Functional innervation in tissue engineered models for in vitro study and testing purposes. ToxicolSci 82(2):525–533.
155-Midha R, Munro CA, Dalton PD, Tator CH, Shoichet MS (2003) Growth factor enhancement of peripheral nerve regeneration through a novel synthetic hydrogel tube. J Neurosurg 99(3):555–565.
156- Tsai EC, Dalton PD, Shoichet MS, Tator CH (2006) Matrix inclusion within synthetic hydrogel guidance channels improves specific supraspinal and local axonal regeneration after complete spinal cord transection. Biomaterials 27(3):519–533.
157- Carmeliet P (2005) Angiogenesis in life, disease and medicine. Nature 438(7070):932–936.
158-Borselli C, et al. (2010) Functional muscle regeneration with combined delivery of angiogenesis and myogenesis factors. Proc Natl AcadSci USA 107(8):3287–3292.
159-Shvartsman D, et al. (2014) Sustained delivery of VEGF maintains innervation and promotes reperfusion in ischemic skeletal muscles via NGF/GDNF signaling. MolTher 22(7):1243–1253.
160- Regenerative Medicine 2009. Industry Briefing Commercial opportunities and Ontario's strengths.MaRS Advisory Services, Canada.
161- Greenwood HL, Singer PA, Downey GP, Martin DK, Thorsteinsdóttir H, Daar AS. Regenerative Medicine and the Developing World.PLoS Medicine. 2006;3(9):1496-1500.
162- UN Millennium Project 2005 Task Force on Science, Technology, and Innovation (2005) Innovation: Applying knowledge in development. Available: http://www.unmillenniumproject.org/reports/tf_science.htm.
163-Thorsteinsdottir H, Quach U, Daar AS, Singer PA. Conclusions: Promoting biotechnology innovation in developing countries. Nature Biotechnology. 2004;22 (Suppl): DC48–DC52.
164-Juma C, Yee-Cheong L. Reinventing global health: The role of science, technology, and innovation. Lancet. 2005;365:1105–1107.
165-FDA Establishes Office of Combination Products. Food and Drug Administration. http://www.fda.gov/bbs/topics/NEWS/2002/NEW00862.html.