Chapter 9
History, Challenges, and Opportunities in Tissue Engineering

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ABSTRACT

In the last several decades, the area of tissue engineering has experienced significant growth, bringing to the clinic treatments that were long thought to be the stuff of science fiction. Although the use of tissue engineering concepts in clinics is not particularly common, the field is predicted to have a very bright future as more tissues will be added to the list of "clinically applicable tissue engineered constructs." Future advancements are likely to make it feasible to combine immune-transparent cells with a commercially available scaffold and cultivate them in a sophisticated bioreactor to provide messages specifically designed for the target region. However, much basic and applied scientific study is still needed before off-the-shelf body parts become therapeutically useful. The development of innovative biomaterials for the various tissue engineering and regenerative medicine applications will be the main focus of future efforts. The biomaterials' structure and mechanical characteristics will be tailored to better fit the target tissue.

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INTRODUCTION

The multidisciplinary area of tissue engineering makes use of cells, biomaterials, biochemical (such as growth factors), physical (such as mechanical loading), and combinations of these signals to produce tissue-like structures (Berthiaume et al., 2011). Biological replacements that can preserve, repair, or enhance the function of injured tissues are what tissue engineering aims to produce (Langer & Vacanti, 1993). Despite the fact that the first tissue-engineered skin products were first made available in the late 1970s and early 1980s, giving rise to contemporary tissue engineering, the phrase "tissue engineering" was not created until 1987 (Bell et al., 1981; Green et al., 1979; Rheinwatd & Green, 1975; Viola et al., 2003).

In truth, the usage of prosthesis dates back to the ancient Egyptians, who used wooden limbs and toes in place of missing limbs and gold for the restoration of missing teeth. The nonliving materials used in all of these therapies, albeit they supplied some structure and function, were a very long way from the original tissue. Around the middle of the 20th century, medical advancements made it possible to replace a whole organ with an organ from a donor, a procedure now known as organ transplantation (Harrison, Merrill, & Murray, 1956). Although this is a common technique today and is recognised as the only effective treatment for organ failure, the demand for organs is always greater than the supply of donated organs (Vacanti & Vacanti, 2007). The notion of in vitro generated tissues was motivated by the limited availability of donors and the immune system's resistance of grafts. The success of skin transplant tissue engineering stimulated curiosity in using related ideas for other tissues and organs (Eberli et al., 2009). Yet, most tissues do not have the skin's relatively straightforward structure, minimal vascular requirements, or simplicity of in vitro keratinocyte growth. The fabrication of intricate, three-dimensional (3D), vascularized multicellular tissues presented significant challenges to the goal of regenerating tissues in vitro.

We give a quick overview of tissue engineering in this chapter. Brief discussions are made on the history, foundations, applications, and clinical requirements for tissue engineering. Tissue engineering's future is briefly reviewed, along with some of the significant obstacles and recent developments in the field.

CLINICAL NEED FOR TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Our desire to heal damaged tissues has led to a clinical demand for tissue engineering and regenerative medicine. Regardless of how these flaws developed (con- genital or acquired), conventional medical methods are still unable to effectively or totally correct them. In truth, there are many health issues for which traditional medicine is severely limited in its ability to provide a remedy. Pharmaceuticals are often used to treat illnesses and injuries, while prosthetic devices and organ transplants are utilised to treat more serious disorders. Pharmaceuticals may be helpful in treating a variety of ailments, but they are unable to treat some fatal illnesses (such as various cancers, strokes, diabetes, etc.) or illnesses that are in advanced stages (such as Alzheimer's, Parkinson's, osteoarthritis, etc.). Yet, prosthetic devices are unable to restore normal function, and there are always many fewer organ donors than needed. This kind of engineering can be utilised to heal illnesses that are resistant to conventional medicine and to produce real, functional organs, eliminating the need for organ donors and prostheses.

The creation of functional replacements for injured tissues is the primary objective of tissue engineering (Schachter, 2014). According to estimates, the majority of tissue engineering products are used to

repair birth abnormalities and injuries, whereas the usage of tissue engineering goods to treat illnesses is less prevalent. The market for tissue engineering and cell treatment was estimated to be worth $15 billion globally in 2014 and is projected to reach $32 billion by 2018. The orthopaedic, musculoskeletal, and spine sectors have the largest markets, followed by the skin, nervous tissues, and other organs (Finch, 2011). Because of its relatively straightforward structure (which can be generated using two-dimensional (2D) culture and has easy access to culturing media), skin was the first tissue to be designed. Because of the huge demand, particularly from battle burns, skin is a significant target for tissue engineering. Skin damage can result in disfigurement and incapacity, which can worsen existing infections and harm patients' psychological well-being. Skin became one of the first clinical tissue engineering targets as a result of all these variables. Every tissue can benefit from the use of tissue engineering and regenerative medicine solutions, albeit the complexity varies depending on the objective. The liver, intestines, pancreas, lungs, heart, kidneys, cornea, neurological system, bone, muscle, and so on are some examples. The ultimate objective is to one day eliminate the necessity for organ transplantation through the use of tissue engineering and regenerative medicine. The donor waiting list, which is always growing faster than the number of organ donors, highlights the urgent need for tissue engineering and regenerative medicine. A significant advance in the history of medical treatment would be made if it were possible to construct such organs or assist in their regeneration.

HISTORY OF TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Since the beginning of time, the concepts of creating new tissues and replacing lost bodily parts or organs have been ingrained in human imagination. These hypothetical hypotheses were made possible by the human race's revolution, which led to their widespread application over time. According to spells known as the "Pyra- mid Texts" (2375 BC), the ancient Egyptians believed that rejoining and reassembling the body was crucial for enabling regeneration in the hereafter (Johnson, 1959). Around 2500 BC, it is thought that the first dental prosthesis was made in Egypt out of gold (Nerlich et al., 2000), (Finch et al., 2012). It's intriguing that this prosthesis has lately been shown to improve walking and function, suggesting that these designs may have had purposes other than the Afterlife (Zimbler, 2001).

The restoration of the structure, form, and function was made possible to some part by the employment of nonliving components. To accomplish a complete recovery, however, live tissues would be required. The amazing leg transplant is recorded throughout history. The first plastic surgeon to publish a book on the subject was Gaspaire Tagliacozzi of Bologna, Italy, who initially detailed the repair of the nose using a forearm flap. When Tagliacozzi was alive, changing one's physical appearance was against the law for religious reasons (Vacanti, 2010).

The nineteenth century saw fast improvement of surgical techniques thanks to advances in anaesthetic and infection control. This innovation made it possible for the first uses of live tissues and organs to repair damage (Webster, 1944). The first tissue-based treatments used skin transplants, and allograft skin banking was made possible by the development of ways to preserve cells and tissues (). The first successful full kidney transplantation between identical twins was accomplished shortly after (Harrison, Merrill, & Murray, 1956). The field of "tissue engineering" was founded on the idea of in vitro generated tissues due to a lack of available donors and immune system rejection of grafts.

Figure 1. Some random images showing the development of regenerative medicine throughout different eras in history (Nerlich, 2000 (Nerlich et al., 2000). Reproduced with permission of Elsevier.): (a) 2500 BC: false big toe developed in ancient Egypt; (b) 278 AD: Saints Cosmas and Damian performing a leg transplant from a deceased donor onto a patient with an amputated leg (Zimbler, 2001 (Zimbler, 2001). Wikipedia, public domain, https://commons.wikimedia.org/wiki/File: Fra_Angelico_064.jpg); (c) in 2013, Chinese doctors saved a man's severed hand by grafting it to his ankle before later reattaching it to the patient's arm (Gordon, 2006 (Gordon et al., 2006). Reproduced with permission of John Wiley and Sons.)

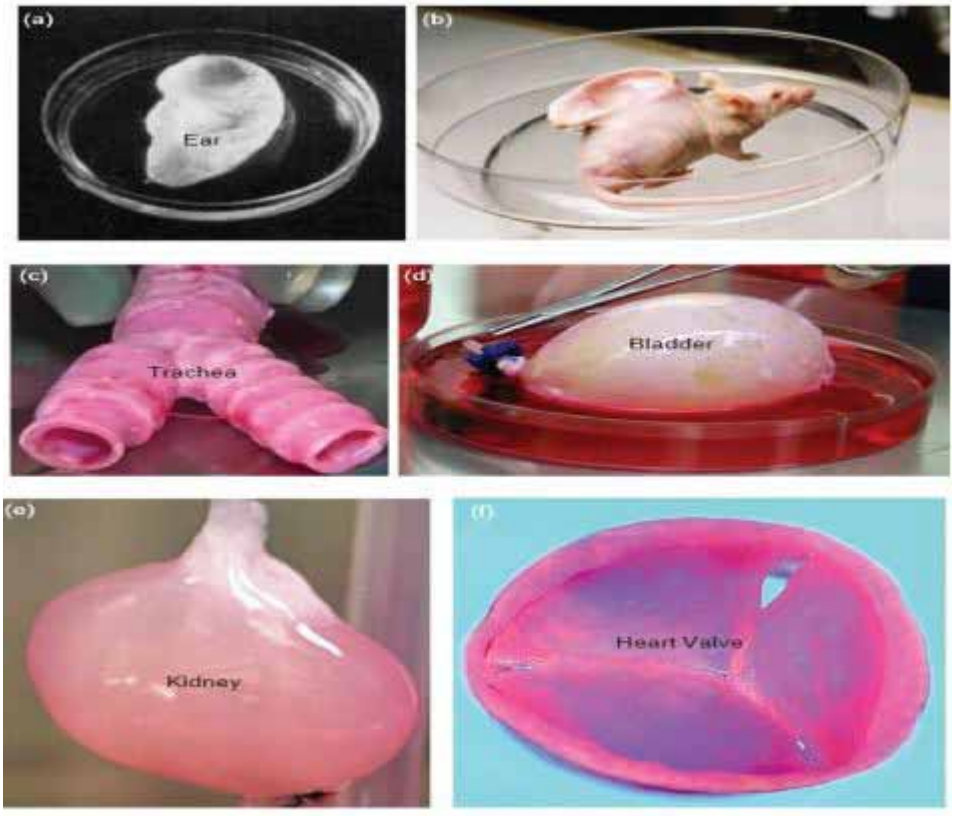
The success of creating artificial skin grafts sparked curiosity in using comparable ideas to create artificial tissues and organs. Skin's relatively straightforward structure, low vascular demand, and ease of in vitro keratinocyte growth, however, are not characteristics shared by most tissues. In 1997, the BBC published a report on the feasibility of engineering an ear (Figure 1) (Cao et al., 1997), which greatly increased public awareness of and media interest in tissue engineering. The Vacanti mouse served as a symbol of the potential that tissue engineering provides for tissue recovery, which is now being understood by millions of people all over the world. The Vacanti experiment was novel and thrilling, but it was simply the beginning of the tissue engineering adventure and the organ engineering "proof of concept." The Vacanti experiment's synthetic tissue had a number of drawbacks that made it challenging, if not impossible, to use the technology in a clinical setting without significant adjustments. The modified ear was created using a polyglycolic acid (PLA) scaffold seeded with bovine chondrocytes and implanted in an immunodeficient mouse for culture. It was designed for a three-year-old boy. Such a tissue would trigger a potent immunological reaction if implanted in a human, both because of the mouse where it was produced and because of the cultivated bovine cells. To completely decrease immunological rejection, a human, namely the ear recipient, would be the perfect alternative for the mouse. On the other side, autologous chondrocytes, which are in extremely short supply, would be used to replace the bovine cells. Other cell sources can also be employed, each with its own benefits and drawbacks that are covered in greater detail in the sections that follow. A second drawback is the engineered tissue's skin covering, which would either be absent if the scaffold/cell structure were to be removed alone or would have immunological and structural restrictions if it were removed together with the mouse skin. Skin grafting may be used to overcome the skin covering restriction, but this would greatly increase the system's complexity. The ability to regulate ear development both throughout the mouse culture phase and after transplantation is a third restriction. Tissue engineering still has limitations regarding the mechanical and chemical characteristics of the produced tissue deriving from the scaffold and culture conditions. The fabrication of complicated three-dimensional (3D) vascularized multicellular tissues presented, and continues to present, significant challenges to the dream of regenerating tissues in vitro.

1.4 Fundamentals of Tissue Engineering and Regenerative Medicine

1.4.1 Tissue Engineering vs. Regenerative Medicine

Regenerative medicine and tissue engineering are frequently used synonymously. Regenerative medicine, on the other hand, refers to methods for assisting a patient's body in healing a damaged tissue in vivo, as opposed to tissue engineering, which often entails creating a tissue in vitro. One of the main roadblocks to the development of the area of tissue engineering was the requirement for cell sources. The use of renewable cell sources, such stem cells and progenitors, was sparked by this lack of cell sources, giving rise to the phrase "regenerative medicine." Regenerative medicine often uses stem cells and progenitors since it is largely founded on a knowledge of morphogenesis and natural, innate self-repair processes. The terms "Tissue Engineering and Regenerative Medicine," or "TERM," are now interchangeable. A shorter waiting period and reduced costs are the effects of rising interest in the utilization of diverse stem cell sources and the requirement to shorten culture durations for created tissues. As a result, tissue engineering and regenerative medicine will eventually become more closely related and maybe even inseparable.

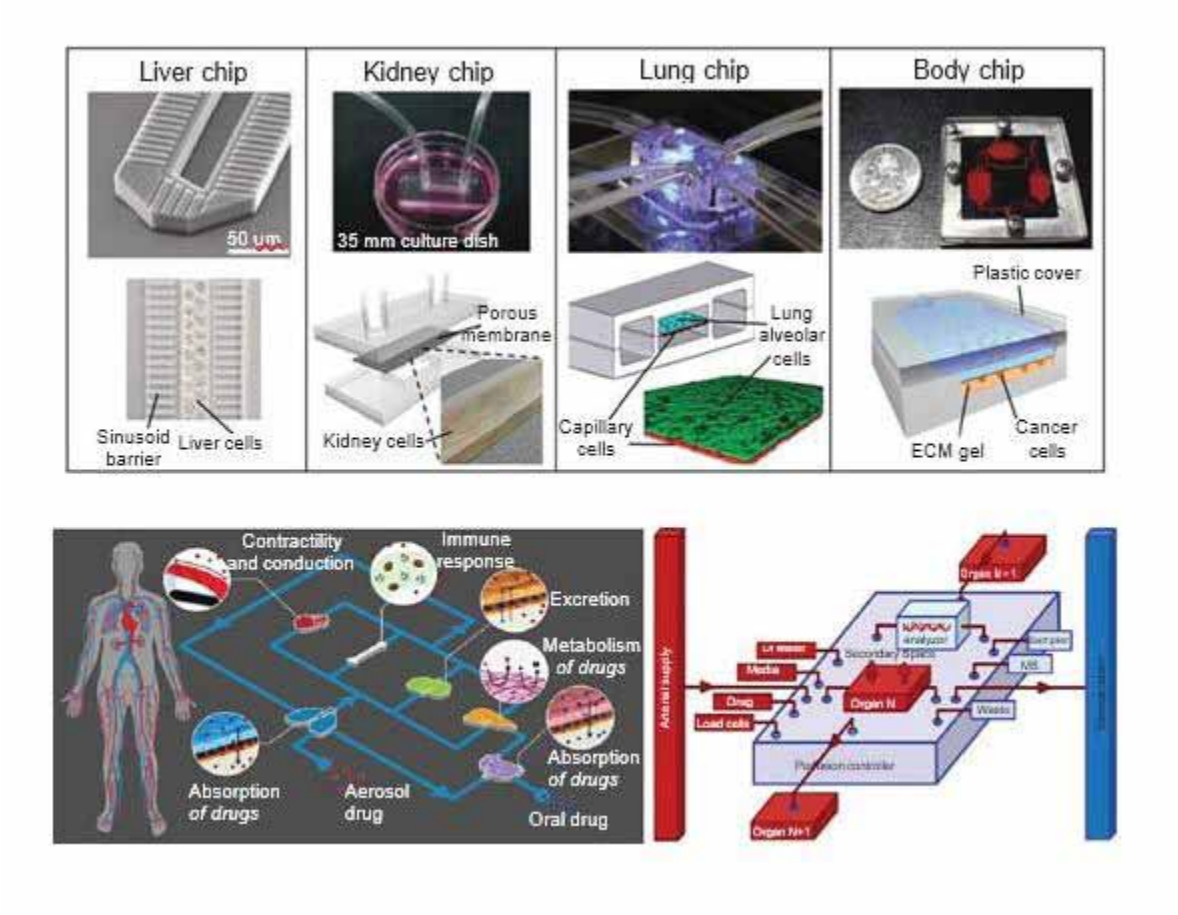
Figure 2. Different tissue-engineered organs: (a) scaffold prepared from synthetic biodegradable polyglycolic acid (PLA) in the shape of a 3-year-old auricle; (b) scaffolds implanted subcutaneously on the back of an immunodeðcient mouse (reproduced with permission from (Polge et al., 1949)); (c) first trachea organ transplant using human's bone marrow stem cells; (d) constructed artiðcial bladder seeded with human bladder cells and dipped in a growth solution; (e) bioengineered kidney that mimics the function of a normal kidney concerning the control of the urinary system and blood ðltration; (f) tissue-engineered heart valve using human marrow stromal cells



1.5 Uses for Tissue Engineering

In the relatively new subject of tissue engineering, tissues are created by the use of cells, biomaterials, physical signals (such as mechanical stimulation), biochemical signals (such as growth factors and cytokines), and combinations of these. The most typical purpose of tissue engineering is to produce tissues that may be utilized to repair or replace bodily tissues that have lost all or part of their functionality. The creation of extracorporeal life support systems (such the bioartificial liver and kidney), in vitro disease models, tissues for drug screening, smart diagnostics, and customized medicine are just a few of the novel uses for tissue engineering that have recently emerged. The next sections will go into further depth about these applications. In Figure 3, a few of these uses are shown.

Figure 3. Recent advances in the applications of microñuidics in developing "organs-on-chips" models for in vitro investigations of engineered tissues and organs



1.5.1 Implantable Tissues and Organs

The development of tissue engineering was a response to the partial or total loss of organ functions brought on by congenital failure, illness, or accident. Connective (such as bone, cartilage, and blood), muscle (such as cardiac and skeletal), epithelium (such as skin, linings of the digestive system), and neurological tissues (such as central and peripheral neural tissues) are among the several tissues that may be restored via tissue engineering.

The first tissue to be studied for the goal of replacing skin was skin epidermal tissue (Chan & Leong, 2008). Explant or organ cultures were the basis for the early attempts to generate Keratinocytes in vitro, but these cultures quickly looked to be overrun with fibroblasts and showed no proliferative ability (Cheng et al., 2014; Nicodemus & Bryant, 2008). The use of lethally irradiated epithelial cells as a feeder layer for a cocultured cell layer was made possible by Puck et al.'s (Yamato & Okano, 2004) 1956 finding that these cells can give mitogens without proliferating. The first product of tissue engineering was created in 1975 when Green and Rheinwatd employed the feeder layer idea to generate human epithelial cells (Jaklenec et al., 2012; Zhang et al., 2012). In their research, Green and colleagues outline

techniques for growing skin epidermis using a patient's skin sample. To get appropriate cell numbers, autologous keratinocytes from the collected biopsy are cocultured for many weeks with a feeder layer of mouse mesenchymal stem cells. This method was subsequently marketed under the name "Epicel" to create autologous keratinocyte sheets used to treat burn victims. The focus of subsequent research was on enhancing the culture medium by including calcium and hormones, which allowed the formation of stratified keratinocyte sheets without the need for feeder layers. However, from the laboratory to the patient, these sheets are exceedingly delicate and prone to breakage. The use of polyurethane backing materials and, more recently, the use of aerosol devices to spray keratinocytes directly into the wound have both been used extensively to address stability difficulties (Badylak, 2014; Wystrychowski et al., 2014). The study of cartilage in tissue engineering was another area of interest. Peterson and colleagues conducted the initial attempts to employ cell-based methods to repair cartilage abnormalities in the late 1980s (Nie et al., 2012). Human trials of the procedure afterwards known as autologous chondrocyte transplantation (ACT) were first reported in 1994 (Takahashi & Yamanaka, 2006) by Brittberg et al. In ACT, chondrocytes are purified from a biopsy of sound autologous cartilage and multiplied in vitro for a period of weeks that is sufficient. The proximal medial tibia is used to harvest a periosteal flap that is large enough to cover the lesion and is then sutured to the cartilage surface, leaving a tiny space for cell injection. To stop cell leakage, fibrin glue is used in the gaps between the sutures. Even though ACT represented a significant leap in cartilage treatment, periosteal hypertrophy sparked research into more effective repair methods (Vacanti et al., 2014; Murphy & Atala, 2014). A membrane made of type I/III porcine collagen has been created in order to solve the aforementioned issue and is utilized in place of the periosteal flap in ACT operations (Mironov, Reis, and Derby, 2006; Nakamura et al., 2005; Melchels et al., 2012). The ChondroGide® membrane features a porous surface on the side that confronts the defect, which a smooth, compact surface that discourages cell leakage and permits cell adhesion. Although ACT and its variations marked a significant advancement in cartilage treatment, they nevertheless confront a number of difficulties. The biggest problem is the chondrocyte dedifferentiation process, which occurs when the cartilage phenotype is lost during in vitro monolayer growth (Bajaj et al., 2014). As a result of morphological and gene expression alterations, chondrocyte dedifferentiation causes cells to act more like fibroblasts (Denk et al., 1990; Luo & Shoichet, 2004). Cells implanted in ACT operations frequently result in fibrous tissue rather than hyaline cartilage because dedifferentiated chondrocytes create type I collagen rather than type II collagen (Kloxin et al., 2009). The current emphasis of study is on the creation of a variety of strategies to either stop dedifferentiation during serial growth or to restore the cartilage phenotype of cells before or after they are delivered to the defect location (Mhanna, Öztürk, Vallmajo-Martin et al, 2014; Salinas & Anseth, 2008). Replicating the stratified cartilage structure, which is made up of layers that are physically and chemically unique, at the level of cells and matrix, is another issue. A very critical component in the engineering of cartilage tissue is to select the proper scaffold, which can be made of synthetic or natural materials and may have different mechanical, chemical, and physical properties. Another important component of tissue engineering is signals (mechanical or chemical). Currently available tissue engineering strate- gies for cartilage repair do not yet fully recapitulate the cartilage ultrastructure or the cartilage microenvironment that provides a multitude of cues necessary for cartilage homeostasis (Liu, Tian, Wang et al, 2010). In its original form, ACT does not use all ele- ments of tissue engineering, especially the scaffold and signaling (chemical and physical). So far, numerous scaffolds have been proposed to be used as chon- drocyte carriers in ACT-like procedures. Additionally, either before to or following tissue transplantation, chemical and mechanical signalling was employed. In addition, a number of cell sources, including chondrocytes, mesenchymal

stem cells, adipose-derived stem cells, and, most recently, iPSCs, have been studied. There isn't yet agreement on which of these techniques is the best for cartilage tissue engineering, despite the fact that each has its merits. In order to create the ideal engineered cartilage, current research on cartilage tissue engineering is concentrated on addressing fundamental questions regarding cells, their interaction with their matrix, and their response to mechanical stimulation in health and disease. The development of chemically and mechanically functioning cartilage will be made possible by the information gathered in the tissue engineering trio during the next few years.

Tissue engineering and regenerative medicine have achieved substantial advancements in bone healing, in addition to skin and cartilage. When a minor fracture occurs, bone tissue spontaneously remodels and sometimes heals itself. However, when considerable bone removal is required due to cancer, infection, or reconstructive surgery, or when there have been serious bone injuries such non-union fractures, natural healing does not always take place. In these circumstances, bone grafting is often carried out using autologous grafts, however allograft and xenograft may also be used. Bone extracts have been suggested, such as the use of demineralized bone matrix (DBM), to prevent potential immunogenicity from nonhuman graft usage (Liu, Tian, Hedrick et al, 2010). Despite the several processes used in the nonhuman graft preparation, immunogenicity still poses a problem. Collagen and porous ceramics, such as those made of phosphate and calcium, have also been used in addition to bone tissue and DBM (Kraehenbuehl et al., 2008). The best repair is often achieved using autografts, but they have several serious disadvantages, including limited availability, surgical problems, donor site morbidity, and discomfort. The drawbacks of using autografts spur the search for substitutes, among which tissue engineering may be the most suitable.

The combination of scaffolds, cells, and signals is employed in tissue engineering and regenerative medicine techniques for bone healing because they are based on the fundamental principles of tissue engineering. The engineered implant should support one or more of the following characteristics: osteoconduction (implant allows good integration with host tissue and bone spreading), osteoinduction (implant encourages bone formation by inducing cell differentiation toward the bone lineage, e.g., DBM and uroepithelium), or osteogenesis (specific osteoprogenitors form bone) (Silva et al., 2004). Scaffolds primarily function as a mechanical support that structurally fills the deficient bone region in bone tissue engineering and regenerative medicine. By using biologically/chemically active components that further assist or expedite bone regeneration (such as cells, growth hormones, enzymes, and attaching moieties), this quiet mechanical role can be enhanced. The scaffold's effectiveness depends on a number of factors, such as its structural and mechanical qualities (such as compressive modulus), biocompatibility (the scaffold doesn't cause toxic or inflammatory reactions), and biodegradability (the scaffold slowly breaks down to be replaced by newly formed bone). Tricalcium phosphate (TCP) and hydroxyapatite (HA) are biocompatible scaffolds that resemble the inorganic bone component; the former is extremely biodegradable and the latter is nondegradable. Other biodegradable scaffolds, such as poly(l-lactic acid), poly(glycolic acid), poly(dl-lactic-co-glycolic acid), and poly(-caprolactone), have also been studied for bone tissue engineering. Additionally, in animal models, polymers including poly(propylene fumarate) (PPF), poly(orthoester), and polyanhydrides have demonstrated strong biocompatibility (Benoit et al., 2008; Mhanna, Kashyap, Palazzolo et al, 2014). PEG can be used to modify bone grafts to make them more biocompatible, and MMP-sensitive peptides can promote biodegradability. Enhancing osteoconductivity by scaffold modification with ECM molecules (such as collagen) or peptides (such as Arg-Gly-Asp (RGD) and, more recently, Gly-Phe-Hyp-Gly-Glu-Arg (GFOGER)) improves cell attachment. Growth factors, which are essential for bone development and repair, are a second crucial tenet of bone

tissue engineering and regenerative medicine. Over 20 different BMP isoforms, including BMP-2 and BMP-7, have been demonstrated to promote bone formation (Kamiya et al., 2010; Mhanna et al., 2013). In addition to BMPs, additional growth factors are also involved in bone production and repair, including TGF- (Grad et al., 2005), VEGF (Davisson et al., 2002), fibroblastic growth factor (FGF), insulin-like growth factor (IGF), and platelet-derived growth factor (PDGF) (Smith et al., 1996). Finally, cells can be included into bone tissue engineering techniques to speed up implant healing or to start bone production in vitro before implantation. stromal bone marrow

The most popular cell type that may develop into the osteogenic lineage is BMSCs (Smith et al., 2000; Lee et al., 2005; Scherberich et al., 2007). But other cells, including adipose-derived stem cells (Altman et al., 2002), muscle-derived stem cells (Juncosa-Melvin et al., 2007), dermal-derived stem cells (Chen et al., 2008), placenta-derived stem cells (Wang et al., 2005), embryonic stem cells (Hirano et al., 2008), and recently induced pluripotent stem cells, have also been demonstrated to have the capacity to generate bone-like tissue (Sumanasinghe et al., 2006). The effective production of bone may result from the combination of some, all, or none of the tissue engineering pillars mentioned above.

Nearly all human tissues are now capable of being repaired because to TERM. Using silicon- or collagen-based nerve guides (hollow tubes), which can be paired with matrices, scaffolds, growth factors, and/or cells to promote healing, it is possible to treat spinal cord injuries as well as damage to peripheral nerves. By planting urothelial cells and smooth muscle cells on acellu- lar matrices, it has been possible to successfully construct the bladder in canine uro- genital tissues (Illi et al., 2005).

By seeding autologous epithelial and smooth muscle cells on biodegradable collagen or collagen/PGA bladder-shaped scaffolds and implanting them in myelomeningocele patients with high pressure or imperfectly compliant bladders, Atala and colleagues advanced the process (Kwang et al., 2008). Similar to the above approach, urethral tissue has been created and clinically evaluated in animal models utilizing autologous epithelial and smooth muscle cells isolated from a bladder biopsy that were planted in a collagen matrix's lumen and outer surface, respectively (Angele et al., 2003). Similar techniques were employed by Atala and colleagues to construct uterine and vaginal tissues as well (Angele et al., 2004). Despite the intricacy of the tissue, attempts to build a kidney—the first organ to be transplanted—have also been made. Renal tissue may be created by culturing renal cells on tubular polycarbonate membranes, which can then be implanted to replace damaged kidneys or utilized as extracorporeal dialysis machines (Wagner et al., 2008). Leydig cells were encased in alginate-poly-l-lysine spheres in an effort at testicle tissue engineering, and it was demonstrated that this maintained normal testosterone levels (Tsutsumi et al., 2001). Using autologous rabbit collagen matrices seeded with autologous smooth muscle cells and endothelial cells, penile tissues, including corporal tissue, have been created. Experimental rabbits were given the modified corporal tissue, which allowed for normal erection, mating, and conception (Awad et al., 2003). Hepatocytes and PGA/PLGA scaffolds have been used to create engineered liver tumours, and either a vascular bed or porous scaffolds that promote angiogenesis were used to provide vascularization (Groeneveld & Burger, 2000). By planting autologous muscle cells and fibroblast cells on porcine collagen matrices, bioartificial patches for tracheal replacement have been created. The patch was coated with live ciliated respiratory epithelium and was able to seal up a tracheal opening, promoting neovascularization (Matsuda et al., 1988; Wobus et al., 1987). In order to protect against immunosuppression, calcium alginate/poly-l-lysine/alginate (APA) beads are commonly used in pancreatic tissue engineering to deliver islet -cells or insulin-producing cells, such as differentiated stem cells, progenitors, or genetically engineered somatic cells, either alone or in a matrix (Singh & Schwarzbauer, 2012). Finally, there have been significant improvements in the TERM of digestive tissues, particularly the

intestines and the stomach. In 2003, Vacanti and colleagues created artificial intestinal tissue by seeding intestinal organoid-derived cells onto a PGA scaffold with a tubular collagen coating (Plaas et al., 1998; Rodgers et al., 2008). The designed Structures were implanted in the omenta of animals and showed strong angiogenesis from omental vessels in addition to various features of normal intestine, such as epithelial submucosa and muscle layers. For uses in gut tissue engineering, a variety of biomaterials have been employed, such as acellular scaffolds like small intestine submucosa, natural materials like collagen, and synthetic materials like PGA, PLGA, and PLA. It is possible to build scaffolds to release certain growth factors important for intestinal development. Additionally, various cell sources, such as stem cells and genetically altered cells, have been researched.

Furthermore, heart tissue engineering is the subject of substantial study. It attempts to develop functional tissue constructions that can restore the myocardium's structure and functionality.

1.6 Challenges in Tissue Engineering

Despite all the developments in tissue engineering, a number of problems still exist that are connected to the three components of cells, scaffolds, and signals. There are several places to obtain cells, which may then be seeded in the scaffold. In actuality, allogeneic, xenogeneic, and autologous cells are all possible sources, and each of them may be further classified into differentiated or adult or embryonic stem cells. The selection of the best source for the cells and their culture is a difficulty in and of itself (Higginson et al., 2012) since they all have benefits and drawbacks of their own (immune reaction, differentiation, etc.). The selection of scaffold biomaterials is a difficult challenge as well. The scaffolds must truly satisfy the body's structural and functional needs. It needs to be mechanically supportive, biocompatible, and able to communicate with the ECM at the same time (Black et al., 1998). While synthetic materials often have superior mechanical qualities, natural ones typically have higher biocompatibility and biodegradability. Because of this, the usage of composite materials is occasionally necessary, allowing the scaffold to have the necessary porosity structure (Raya-Rivera et al., 2011). The movement of nutrients and waste excretion inside the created tissue is another significant barrier in tissue engineering (Jungebluth et al., 2011). The 3D manufactured tissue must be vascularized using a vascular capillary network since the bulk of tissues depend on blood vessels to carry oxygen and nutrients (Hasan et al., 2014). This is not a simple operation; when the scaffold is implanted within the body, the available oxygen is immediately depleted, and new blood vessels only grow after many days (Matsumura et al., 2003). The need for alternatives to angiogenesis has led to the development of several strategies for prevascularizing engineered tissues based on subtractive, additive, and hybrid methodologies (L'heureux et al., 1998).

The mass manufacture and commercialization of the altered tissues remain a significant obstacle. It is necessary to guarantee specific production conditions and quality control methods. Furthermore, it's crucial to meet patients' precise demands (demand) and offer long-term storage and transportation facilities while maintaining the tissues' structural and functional integrity (L'Heureux et al., 2006).

1.7 The Future of Tissue Engineering

Major advancements in healthcare over the past few decades have resulted in better surgical techniques and illness treatment. Overall, health care advancements have increased life expectancy while also increasing illness and organ failure vulnerability. As a result of the aforementioned developments, there is now more demand for tissues and organs. Producing whole organs is the ultimate goal of tissue en-

gineering, which aims to close the ever-widening gap between organ demand and supply (Macchiarini et al., 2008). It is anticipated that this field will be used as a reliable clinical remedy more frequently.

Future research will focus on the differentiation capacity of stem cells and expand the range of possible uses. Whether induced, embryonic, or adult stem cells, the main difficulty is getting them committed to the targeted lineages. More stem cell-based applications are anticipated to enter clinical trials in the near future. Additionally, it is anticipated that medication delivery and gene therapy (the silencing and activation of target genes) would be employed to support the preservation of the desired cell phenotype. The ultimate objective is to create immune-transparent stem-like cells using well-defined methods, allowing for committed differentiation of these cells into desired tissues. Future research will focus heavily on advances in basic and applied science linked to the creation of tissue engineering scaffolds. To ascertain how different cell types are affected by combinations of chemicals and materials, high-throughput screening approaches may be helpful. Given their large availability and the proper chemical and structural composition of decellu- larized tissues, these tissues are also anticipated to continue serving as a significant supply of scaffolds. The lack of supply (for allogeneic scaffolds, for example), possible immunoreactions, and ethical concerns (for xenogeneic scaffolds, for example) will always be potential drawbacks of such scaffolds. In order to treat tar- geted tissues, it is anticipated that novel biomaterials will be created in the future that incorporate certain chemicals. The effects of chemicals on cells will also be investigated, as well as the ideal degradation rate and material characteristics (porosity, mechanical properties, and structural qualities) appropriate for each tissue engineering application. A long-term objective would be to combine scaffolds and cells to create tissues in vitro that can be decellularized to provide customisable off-the-shelf tissue supplies for different engineering applications. Future studies will further clarify the functions of ECM components in order to provide the best formulas for creating constructions that most nearly mimic real tissues. Stretch-activated ion channels and integrins are two of the ways by which cells assess load and respond to their environment, and these systems are still being fully understood (Atala et al., 2006; Novosel et al., 2011). Understanding these mechanisms will provide the groundwork for creating new bioreactors and tools for tissue engineering, as well as perhaps leading to the discovery of novel compounds that may be used to treat diseased organs and tissues. Future bioreactors will be able to carry out challenging combinatorial operations to design whole organs. For instance, bioreactors may be made to give different amounts of oxygen to different areas of the created tissue, different types of mechanical stimulation, or growth hormones and chemicals.

1.8 Conclusion

It can be examined whether to use covalent bonding based on naturally occurring tissue residues and designed residues on the scaffold, or whether to use biomaterials with muscle-adhesive proteins and other glueing surfaces. Future studies will concentrate on cell silencing and transfection to promote better repair and regeneration. It will be necessary to have a deeper fundamental scientific knowledge of cell behaviour in tissue engineering systems, including cell-cell interactions and cell-scaffold interactions, both in vitro and in vivo. For the many tissue engineering applications, it is also important to establish the impact of various growth factors as well as the best dosage and timing of supplementation. Techniques used in in vitro culture need also be updated, notably the transition from 2D to 3D systems and oxygen levels to reflect the environment of thick tissues in vivo. The biological implications of the currently utilized culturing procedures may be better understood by systematic investigations that contrast the in vitro culture systems now used in tissue engineering with the in vivo environment. With the use of this

understanding, current cell culture methods can be enhanced to provide better tissue healing. Last but not least, efforts should be made to improve present ethical and regulatory concerns, which would make it possible to more easily and safely introduce tissue engineering and regenerative medicine solutions into the clinic.

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