Application and Development of Tissue Engineering Technology in the Fields of Cardiac Reconstruction, Nerve Regeneration and Bone Reconstruction

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Abstract. Tissue engineering technology has developed rapidly since its birth. In recent years, in the face of numerous challenges, scientists have developed many advanced technologies and applied them to the field of reconstructive surgery. However, most tissue engineering application technologies are still in the basic research or animal experiment stage, and there are relatively few clinical applications. This paper focuses on the development of tissue engineering technology in the fields of cardiac reconstruction, nerve regeneration and bone reconstruction. It focuses on the most promising technologies in the field discussing about their advantages, disadvantages and prospects.

1. Introduction
Stem cells are primitive cells that develop various tissues and organs of an adult body, and can not only be expanded in vitro but also maintain functional integrity. Under the induction of growth factors, stem cells can be differentiated into functional living organ tissues which are needed. Scientists have used synthetic or natural materials to prepare degradable stents for stem cell growth. Under the control of scientific technology, the stent can present specific tissue and organ morphology to help stem cells grow into three-dimensional tissue organs. This technique of cultivating and replicating tissue and organ with stem cells belongs to tissue engineering. The term tissue engineering was first proposed by the American Science Foundation at the Bioengineering Group meeting held in Washington in 1987. Since its inception, tissue engineering has continued to advance the development of surgical fields, so that patients with organ transplants no longer need to wait for proper donors, and are immune to rejection. In 2006, Professor Shinya Yamanaka invented induced pluripotent stem cells (iPSCs) similar to hard-to-obtain embryonic stem cells[1], which can use 3D Printing technology, decellularization, electrospinning and other techniques to produce scaffolds, which means that iPSC can be used as a pluripotent stem cell to differentiate target tissues and organs.

In the application of tissue engineering technology, scaffolds play an important role, and the production of non-discriminating and biologically active scaffolds has been the goal of scientists. At present, scientists have produced degradable cell scaffolds. There are three main ways to apply this scaffold: one is to inoculate stem cells in vitro and then culture them in a bioreactor, and then implant them into the human body through surgery. The specific part can continue to grow. The second is to use the human body as a bioreactor, the stem cells inoculated with stem cells are placed under the skin to be cultured into a fibrotic matrix, and the matrix is taken out and surgically implanted into the defect of the human body. The convenience of the ester cell scaffold is that it will be hydrolyzed by the action of water in the human body. The last one is implanted into a cell-free scaffold in the body,
which recruits endogenous cells and uses the body's regenerative capacity to grow new tissues in situ[2].

From the regeneration of skin tissue to the acquisition of immunocompatible organs or large volumes of tissue, such as limbs, face, etc., tissue engineering techniques have been applied in many ways. 3D printing technology in tissue engineering has been used to generate and transplant a variety of tissues, including skin, bone, cartilage, blood vessels, and tissues[3]. However, the progress of tissue engineering techniques in cardiac structural reconstruction and nerve regeneration in research has been slow. The internal structure of the heart is complex and closely related to blood vessels. It is very complicated to make a heart in vitro. Ten years ago, scientists succeeded in replicating the heart of an experimental mouse in vitro. They are also trying to replicate the heart of a pig. If successful, it will move toward the goal of replicating the human heart. In addition, the valve, as an important physiological structure of the heart, plays an important role in the blood circulation of the heart. There are countless patients who need to change their valves every year due to heart valve defects. Scientists have applied tissue engineering to valve remodeling. At present, they have successfully replicated valves with no immunogenic components and structural integrity, but they have not yet been applied to clinical practice[4]. The human brain is intricate, and spinal cord injury and mental illness have so far been a serious problem in medical interface. Scientists have been trying to apply tissue engineering methods for paralyzed patients and Patients with mental illness regenerate nerves and apply iPSC-related techniques to the study of neurological diseases. At present, iPSC technology has made great progress in regenerative medicine, but it is still necessary to establish a method for accurately evaluating each iPSC clone to ensure the safety of iPSC can be applied clinically[5].

This paper mainly summarizes the application, research progress and prospects of tissue engineering technology in three fields of cardiac reconstruction, nerve regeneration and bone reconstruction.

2. Application of Tissue Engineering Technology in the Field of Cardiac Reconstruction

Patients with valvular heart disease require valve replacement surgery. Heart valve replacement is usually a mechanical valve made of synthetic material or a biological valve made of biological material. However, both mechanical and biological valves have limitations. Mechanical valve endurance is strong and can be used by patients for a long time, but patients who use mechanical valves need to take anticoagulants for life and face the threat of thromboembolism and bleeding. The biological valve has better blood phase fusion, does not require lifelong anticoagulant, and often uses the hearts of pigs to extract the valves. The structure, size and function of the hearts of pigs are close to the human's, and its physiologically active gene is 85%-95% similar to humans. Therefore, the valve replacement of the pig can be naturally blended into the human body. However, the use of biological valves has a longevity problem, and most patients have to face a second operation.

The study of replacement heart valves has progressed in reducing immune rejection from allogeneic, heterologous donors to degradable natural scaffolds, but it is difficult to completely avoid rejection and allow cells to infiltrate effectively. For a long time, scaffolds can be produced on a large scale by decellularization of allogeneic tissues. However, such scaffolds are non-vascularized and have a limited thickness, so that it is impossible to repair a valve with a clinically large volume defect. With the development of tissue engineering technology, perfusion decellularization technology has begun to be applied in this field. Endogenous cells are recruited by in situ methods, injected back into the corresponding position of the cardiovascular system, and new tissue is grown by utilizing the body's ability to regenerate. Scientists perfuse allogeneic flaps, ex vivo and decellularize them, remove antigenic components and cells with special functions, and retain only the basic tissue structure to form compatible flaps, which can effectively avoid immune rejection. A framework can be formed for the growth of recipient cells. After obtaining a compatible flap, the recipient cells are reperfused and cultured into a new valve and implanted into the body. At present, scientists have successfully perfused decellularized human large-volume fat flaps, and proved that the immunogenic cell components of the valve are completely removed, while retaining the structural components and vascular network[6].
In addition, patients with heart failure require heart transplant surgeries, but the problem of rejection of transplanted organs and donor receptor selection has not been well resolved. There are also many problems with installing an artificial heart. Accepting artificial heart standards is strict. To avoid wound infections, regular cleaning of wounds is required. Patients need to carry artificial heart devices and charge them daily. For a serious heart disease, only replacing the entire heart can save the patient's life. Ten years ago, the Reproductive Heart Laboratory at the Massachusetts General Hospital combined perfusion and decellularization technology to create a complex, biocompatible cardiac scaffold with a perfusion vascular tree that successfully replicated the heart of an experimental mouse[7]. Transplantation of acellular heart can be achieved by intramural injection of cardiac-derived cells and by perfusion of endothelial cells into the vascular catheter, enabling re-implantation of its intrinsic vascular structures and endothelial cells. This approach can bring hope to the replication of virtually any solid organ, suggesting that the technology can be extended to the heart of human size and complexity. In 2016, Japanese researchers made significant progress in achieving organ regeneration: Regeneration of 5 macaques with myocardial infarction using stem cells produced by monkey skin cells[8]. Experiments have shown that induced pluripotent stem cell derived cardiomyocytes(iPSC-CMs) has a certain effect on the treatment of myocardial infarction in primates. iPSC-CM can improve systolic function until the end of the study, the diseased macaques do not have any abnormal behavior. Only transient non-fatal arrhythmias occurred after transplantation. However, there are some limitations in the study design. This study only tested one iPSC line, so a variety of cell lines are needed for further research. Furthermore, in view of the relatively small infarct size of young adolescent monkeys, there are some differences between clinical and human myocardial infarction diseases, and more efforts are needed to study how to control arrhythmia before clinical application. Finally, the 12-week observation period after cell transplantation does not lead to definitive conclusions about graft survival without chronic rejection, requiring longer follow-up to investigate whether iPSC-CM is at risk of chronic rejection. Now scientists are working to replicate the heart of the pig and further optimize the technology. If successful, the scientists will further move toward the goal of replicating the human heart, aiming to cultivate the heart of the implantable patient's chest without reflexes in vitro. Applying the theoretical approach of tissue engineering, regenerative surgery will hopefully bring dawn to patients who have heart disease.

3. Application of Tissue Engineering Technology in the Field of Nerve Regeneration

As an important system of the human body, the nervous system has complex physiological structure and limited regenerative capacity, which has always caused great challenges in restoring damaged nerves. At present, tissue engineering technology has been used to regenerate nerve tissue.

It is well known that structural abnormalities or loss of function of neurons and death of neurons lead to cognitive and dysfunction, which is a neurodegenerative disease caused by degenerative degeneration of central nervous tissue. Pathologically, neuronal degeneration, degeneration, and loss occur in the brain and spinal cord. The mechanisms and causes of neurodegenerative diseases are complex and have not yet been elucidated. The number of people with neurodegenerative diseases increases with age. Therefore, it is important to study its mechanism and medication. Inducing iPSC mimic disease models brings new ideas for studying neurological diseases. Scientists have successfully established several models of neurodegenerative diseases using this method.

Alzheimer’s disease (AD) is a type of neurodegenerative disease. Typical pathological features of the brains of patients are amyloid plaque deposition and neuronal and synaptic loss in specific regions of the brains. Exploring the formation process of pathological features, tracking the source, and manufacturing the AD model, can make more discoveries about the mechanism of Alzheimer's disease, and also provide a new idea for drug development, such as manufacturing can block neurons and Drugs in the process of synaptic loss. The study found that presenilin (PS) mutations in presenilin ps1 and ps2 are involved in the formation of autosomal-dominant early-onset familial Alzheimer's disease (FAD). And β-amyloid has been considered to be the starting factor for the occurrence of AD in the amyloid polar junction hypothesis. The fibroblasts of FAD patients induce iPSCs of the presenilin
gene variant, and then these iPSCs are differentiated into neurons. It is demonstrated that iPSC-differentiated neurons increase the secretion of β-amyloid and reproduce PS1 and PS2 mutations lead to the mechanism of FAD. Therefore, FAD-iPSC-derived differentiated neurons can be used as an effective model for studying AD[9].

Another type of neurodegenerative disease Parkinson's disease (PD), pathological features of the brain dopaminergic neurons progressive death, regulation of exercise, mood and other important functions of the neurotransmitter dopamine secretion, leading to substantia nigra par compacta with dopamine neurons death, α-synuclein misfolds to form a Lewy body. It is still unknown why the patients who suffer Parkinson have such pathological features in their brains. The research obstacles are mainly difficult to obtain diseased tissues. When PD clinical manifestations appear, most of the cells have died. The use of iPSC technology to create a Parkinson disease neuron model will provide clues for studying the mechanism of the disease. However, due to the long latency of the disease and the elimination of epigenetic factor in the process of cloning iPSC[10], the application of iPSC technology in the PD disease model has certain challenges which need to be explored further.

Mesenchymal stem cells have the ability to differentiate into nerve cells, which can be used to regenerate nerve cells. According to reports, in May 2019, Sapporo Medical University of Japan first attempted to use regenerative medicine as a new clinical trial therapy to treat spinal cord injury. The scientists extracted the mesenchymal stem cells from the patient's bone marrow and cultured them in vitro, and then returned them to the patient's blood by drip. Mesenchymal stem cells attach to damaged nerve cells, causing the death of nerve cells to be activated. Scientists believe that some of the special substances released by mesenchymal stem cells play a role, while mesenchymal stem cells gradually differentiate into new nerve cells. A total of 3 subjects participated in the experiment, 12 of which had a significant improvement in the degree of spinal cord injury, and the remaining 1 subject had a significant improvement in respiratory capacity. There are no side effects having been found with this new treatment yet.

In addition, polymers have their own advantages in shaping and biocompatibility compared to other biomaterials. Therefore, the manufacture of polymer catheters as a culture environment for healthy nerve tissue is an important direction for the development of neural tissue engineering[11].

4. Application of Tissue Engineering Technology in the Field of Bone Reconstruction

The bone tissue has a strong self-repairing ability and can be repaired by the dynamic process of osteoclasts and osteoblasts. However, when the bone injury is serious and the bone defect area is large, external intervention treatment is needed to repair it. Autologous or allogeneic transplantation is generally chosen. At present, autologous bone transplantation is the gold standard treatment for filling defects. However, there are many problems of autologous transplantation, such as lesions at the donor site or recipient site, which result that bone resorption is difficult to heal. There are also problems with allogeneic transplantation, including risks of transmitting donor diseases, an immune rejection reaction occurs, and the like. The birth of tissue engineering provides new ideas for bone reconstruction and is growing rapidly. Bone tissue engineering has become an important research area in orthopedics, and mature technology has been applied to treat small bone defects clinically. Articular cartilage has poor self-healing ability, so it is necessary to develop a corresponding tissue engineering technology strategy for repair[12].

At present, bone tissue engineering often uses 3D printing stents. One reason why a large range of bone defects are difficult to regenerate is that it is difficult for blood vessels to provide nutrition for newly formed bone tissue. In order to solve the problem that bone regeneration is difficult to vascularize, scientists have used 3D printing technology to manufacture hollow-pipe-packed silicate bioceramic (BRT-H) stents[13]. BRT-H scaffold can release biologically active ions to induce endothelial cell migration to promote angiogenesis in the affected area into the hollow canal, and also promote stem cells and growth. The transmission of factors promotes bone regeneration. In addition to silicon salts, scientists have also successfully produced magnesium and calcium salt scaffolds. These stents have been used in the regeneration of rabbit humeral segmental defects and have been shown to
be effective in promoting bone regeneration and remodeling, forming new connections in the medullary cavity and the formation of bone marrow. Therefore, these stents have great prospects in the regeneration of large segment.

The periosteum is rich in blood vessels, lymphatic vessels and nerves. There are a large number of osteoblasts and osteoclasts on the inner surface, which plays an important role in vegetative bone growth and repair damage. Autologous transplantation of periosteum shows its potential to promote bone remodeling, but it is difficult to obtain a sufficient amount of autologous healthy periosteum. Scientists have now studied many biomaterials that mimic periosteum. Decellularized periosteum is a very advantageous scaffold, which is characterized by complete removal of cellular components and retention of biological structure. However, decellularized periosteum also lacks certain mechanical stability. In order to provide an excellent growth environment for bone cells, bone substitutes need to be biocompatible, biodegradable, osteoconductive and mechanically stable. Chitosan, a natural polymer, has many excellent properties, including biofunctionality and compatibility, blood compatibility, safety, microbial degradation, etc., therefore chitosan has become a promising bone. Alternative, however, it also has the drawback of high mechanical strength and fragility. To compensate for the shortcomings of both decellularized periosteum and chitosan, scientists have combined acellular periosteal membranes with chitosan to take advantage of each other[14]. Through preliminary tests, the scientists found that the biological characteristics and mechanical properties of the acellular periosteal chitosan complex were good, and there was no immunological rejection or toxicity in rabbit bone repair. In the future, scientists will experiment on other animals to identify other properties of the acellular periosteal chitosan complex, such as vascularization, cell recruitment and so on.

In addition, articular cartilage damage is also one of the most common types of bone damage. The general treatment is to relieve the pain of patients through joint replacement surgery and bone marrow stimulation. However, the defects of these treatments can’t solve the problem of cartilage defects fundamentally. Cartilage tissue engineering has now become the most promising approach to repair cartilage defects. In the course of surgery, implants are often difficult to integrate well with surrounding tissues, causing cartilage regeneration to fail, therefore scientists have invented hydrogels. The hydrogel fills the bone defect by minimally invasive injection, which can stimulate the patient's bone marrow to produce stem cells, grow new cartilage, and also promote the integration of new cartilage with natural cartilage and surrounding tissues. As a scaffold, hydrogels not only have good biodegradability, but also have tough mechanical properties. At present, scientists have developed a double network hydrogel (M-O-G) similar to natural cartilage extracellular matrix (ECM) composed of gelatin methacyrlylated (GelMA), oxidized dextran and gelatin, which not only have superior mechanical properties, but also have Good tissue integration ability can significantly enhance the formation of hyaline cartilage tissue and enhance integration with surrounding tissues. Therefore, the hydrogel is a promising biological material that exhibits good cartilage repair ability and can be applied to the treatment of other articular cartilage diseases in the future. However, before applying it to human clinical research, further research is needed. In the initial safety test, the scientists only verified the safety of subcutaneous implantation and could not rule out that it was completely non-toxic. Furthermore, the hydrogel exhibits good results in short-term bone repair experiments in rabbits and does not represent the same good effect in humans, therefore long-term experiments in dogs or pigs are required before application to the clinic[15].

5. Summary
Tissue engineering has developed rapidly since its birth, making striking contributions in areas such as cardiac reconstruction, nerve regeneration, and bone remodeling. However, many techniques have only achieved good results in short-term experiments in animals. The animal tissues and organs have a simple physiological structure compared with humans. More long-term experiments are needed if the technology is to be applied to the human body. For tissue engineering in which stem cells are cultured using autologous cells, a large number of seed cells need to be obtained from a certain tissue. However,
due to the existence of allogeneic rejection and the limited number of common cell passages, it is challenging to culture a large number of cells. Adult stem cells, such as mesenchymal stem cells in bone marrow, can differentiate into a variety of cellular components under certain regulation, and the differentiation of seed cells into stem cells by stem cells requires a large number of basic experiments to study the regulatory mechanism. The iPSC technology has enabled people to have a breakthrough understanding of the pluripotency regulation mechanism, which has narrowed the distance between stem cells and clinical disease treatment. However, the application is often time consuming, laborious and expensive, and needs further optimization. Furthermore, in order to ensure the safety of iPSCs, it is necessary to study a better re-encoding method to avoid the serious consequences of iPSCs being difficult to differentiate due to abnormal re-encoding. Aiming at the scaffolds used in tissue engineering, many conditions need to be met at the same time. The decellularized scaffolds have great development prospects. The application of decellularization related technology has reached the production of biocompatible and mechanically good scaffolds, but further enhancement is needed. And assess the vascularization capacity of the scaffold and the growth state of cells and factors on the scaffold. The 3D printing technology in tissue engineering has developed rapidly and has been applied to the reconstruction of many organizations. At present, 3D printing is not only used for making stents but also for drug discovery, reagent analysis, basic research and so on. Although 3D printing has the potential to transform regenerative medicine, from skin tissue to blood vessels, to hollow organs and substantial organs, vascularization and innervation become more and more complex, and the number of cells required is getting larger and larger. The potential also requires a lot of research. For chemical biomaterials such as hydrogels that replace tissue, focus on assessing their safety before application. In addition, it is difficult to regulate the process of tissue regeneration by seed cells, and it is difficult to secrete extracellular matrix with various components. It is also necessary to do a lot of basic experiments to study the mechanism of normal tissue differentiation and synthesis and secretion. For a complex organ like the heart, how to regulate the correct growth and arrangement of its valves, blood vessels, nerves and tissues is still very difficult. In addition, implants implanted into the human body through tissue engineering need to be nutritious and establish blood circulation as soon as possible, so the bio-melting and vascularization ability of the implant is a goal that needs to be improved. Tissue engineering technology has found solutions for most of the most important and difficult problems. In the future, more experiments and evaluations will be used to further improve the technology. After successful in animal experiments, it will be applied to the treatment of human-related diseases.

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