Advances in tissue engineering*

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Abstract Tissue engineering is a newly developed specialty involved in the construction of tissues and organs either *in vitro* or *in vivo*. Tremendous progress has been achieved over the past decade in tisse construction as well as in other related areas, such as bone marrow stromal cells, embryonic stem cells and tissue progenitor cells. In our laboratory, tissues of full-thickness skin, bone, cartilage and tendon have been successfully engineered, and the engineered tissues have repaired full-thickness skin wound, cranial bone defects, articular cartilage defects and tendon defects in animals. In basic research areas, bone marrow stromal cells have been induced and transformed into osteoblasts and chondrocytes *in vitro*. Mouse embryo stem cell lines we established have differentiated into neuron precursor, cardiac muscle cells and epithelial cells. Genetic modifications of seed cells for promoting cell proliferation, delaying cell aging and inducing immune tolerance have also been investigated.

Keywords: **tissue engineering, marrow stromal cell, bone, cartilage, tendon.**

Since the early stage of modern surgery, the surgical replacement of one body part for another has been the technique to meet individual patient's need. Design of the replacement using synthetic materials to rebuild damaged, diseased, aged or genetically deficient parts of human body was gradually introduced into the surgical practice and has been nurtured by the discovery of the new synthetics since the early time of the last century. The concept of tissue engineering emerged when the focus of attention in designing body replacement shifted dramatically to the biological components of tissues from non-viable substance.

Tissue constitution using biological components had not been realized until the synthetic biomaterials became available in the last decade[1]. These synthetic materials are usually biocompatible, and biodegradable. When mixed with seed cells, these materials serve as a scaffold to provide implanted cells a three-dimensional environment. While undergoing a process of slow degradation, the materials also allow implanted cells to proliferate, differentiate, produce extracellular matrix within the scaffold and eventually to form a wanted tissue.

With the development of new synthetic materials and the advances in cell biology and molecular biology, tremendous progress has been achieved in the area of tissue engineering over the past decade. In this article, we attempt to review the new developments in tissue construction as well as in other related areas.

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1 Advances in tissue construction

In the early stage of tissue engineering, the aim of tissue constructions focused on exploring the possibility of forming a single cell-type tissue, such as bone or cartilage[2,3]. In these pioneering experiments, isolated osteoblasts or chondrocytes were mixed with the synthetic materials like polyglycolic acid (PGA) or pluronic and were implanted or injected into the subcutaneous tissue of nude mice[2,3]. The formed tissues were usually irregular in shapes. The formation of a tissue-engineered cartilage in the shape of a human ear is a milestone in the development of tissue engineering[4]. The success of constructing a cartilage tissue possessing a three-dimensional structure displays the tremendous potential of tissue engineering in the clinical repair of tissue damage and tissue loss.

Recently, the animal experiments of tissue engineering have been shifted from non-immunocompetent animals like nude mouse to immunocompetent, large mammalian animals such as sheep, pig and dog. In our laboratory, a full-thickness skin tissue was engineered in a porcine model by using cultured autologous keratinocytes and dermal fibroblasts, PGA and pluronic. The engineered skin tissue has a structure of epidermis and dermis, similar to that of native skin. An intact basement membrane was also demonstrated by histology and immunohistochemical staining, which shows the presence of type IV collagen and laminin[5]. In addition, composite grafting of cultured keratinocytes and acellular dermal matrix was used to successfully repair a full-thickness skin defect in a porcine model[6], suggesting that these approaches might possibly be used in burn treatment and reconstructive surgery.

More importantly, the results of several projects undertaken in our laboratory demonstrate that different tissues, such as cartilage, bone and tendon, can not only be engineered *in vivo* but also be used to repair tissue defects in a large mammalian animal model. For example, the articular cartilage defects in the weight-bearing areas of medial and lateral femoral condyles were created in porcine knee joints; a cell-scaffold construct was constituted using isolated autologous chondrocyte, PGA and pluronic to repair the articular cartilage defects[7] and twenty-four weeks post-repairing, gross examination demonstrated a complete repair of the defect by the engineered cartilage, shown by a smooth articular surface indistinguishable from nearby normal cartilage. A cross section demonstrated a nice interface healing to adjacent normal cartilage. Histology of the tissue harvested from the repaired defects further demonstrated a typical structure of cartilage lacuna and an ideal interface healing to adjacent normal cartilage as well as to underlying cancellous bone. Furthermore, the engineered cartilage exhibited enhanced extracellular matrix production and improved biomechanical properties, indicating that engineered cartilage resembles the native articular cartilage not only in morphology, histology, but also in biochemical components and biomechanical properties.

In another study, we also used isolated autologous fibrocartilage cells harvested from contralateral meniscus to engineer a meniscus and to bridge the meniscus defect on the experimental side[8]. Grossly, the engineered tissue resembled native meniscus in morphology, color and texture 25 weeks post-transplantation of cell-scaffold construct. Histologically, the engineered meniscus displayed a typical structure of fibrocartilage tissue, similar to that of native meniscus. The tendon defect is a major challenge to plastic surgeons because of the limited donor site for harvesting autologous graft. In a

a hen model, a tenocyte-PGA construct was used to successfully engineer a tendon tissue[9]. This tendon tissue appears similar to the native tissue both grossly and histologically. In addition, the tensile strength of the engineered tendon reached 70% of native tendon's strength. Furthermore, the engineered tendon was used to bridge the defects of flexor digitorum profundus tendons in the same model.

2 Advances in other areas related to tissue engineering

2.1 Bone marrow stromal cells (BMSCs)

The potential of using BMSCs as seed cells for tissue construction has been actively investigated worldwide. BMSCs have the capability for multi-lineage differentiation. Therefore, these cells, theoretically, can be induced into different cell types, such as osteoblasts, chondrocytes, dermal fibroblasts, myoblasts, tenocytes and adipose cells[10]. Currently, most studies in this area focus on the *in vitro* induction of BMSCs into different cell types. In our laboratory, we have successfully induced cultured BMSCs into osteoblasts and chondrocytes. The induced osteoblasts demonstrated an enhanced production of alkaline phosphatase and osteocalcin. Calcium deposition was also observed in BMSC culture dishes. The induced chondrocytes exhibited increased production of type II collagen. In addition to *in vitro* studies, BMSCs were also used as seed cells for *in vivo* tissue engineering and repairing tissue defects. In a sheep model, bilateral cranial bone defects with a size of one tenth of skull were created and were repaired with a bone graft constituted with the *in vitro* cultured autologous BMSCs and calcium alginate[11]. Eighteen weeks after transplantation, the bone defects were almost completely repaired by the engineered bone tissue. Histology study demonstrated the presence of mature bone tissue at the experimental defects, while the control defects, which were left unrepaired or repaired with calcium alginate without BMSCs, were not healed and were filled with fibrous tissues. A French group recently reported that a long bone was successfully engineered in a rabbit model using *in vitro* expanded autologous BMSCs and coral scaffold, the tissue-engineered artificial bone underwent morphogenesis *in vivo*, leading to complete recorticalization and the formation of a medullary canal with mature lamellar cortical bone[12]. All these findings indicate that autologous BMSCs are very likely to become an important source of seed cells for tissue constitution.

2.2 Embryonic stem (ES) cells

ES cells are highly undifferentiated cells, which can be isolated from the inner cell mass of preimplantation embryo. Because of the pluripotency and the capability of being cultured *in vitro* for a long term, ES cells are expected to play a key role in the induction of organogenesis.

In the area of tissue engineering, ES cells also have huge potential of being induced into desired cell types, thus serving as seed cells for engineering different tissues. In our laboratory, we have successfully established mouse ES cell lines. Moreover, these cell lines have been differentiated into neuron precursors, cardiac muscle cells, and epithelial-like cells. Chong et al. successfully induced mouse ES cells into endothelial cells using recombinant human TGF-β1[13]. Xu et al. reported that mouse ES cells could be induced into myoblasts by transfecting *myoD* gene[14]. It is expected that the advances in ES cell research will eventually provide another important source of seed cells for engi-

neering tissues of different types, such as blood vessels, muscles and skin.

2.3 Tissue specific progenitor cells

Tissue progenitor cells or tissue stem cells are believed to exist in each of different kind tissues. A typical example is epidermal stem cells. When a wound created, these stem cells proliferate and migrate to the wound area to regenerate epidermis. Thanks to the discovery of a specific marker, keratin 19, isolation and *in vitro* expansion of epidermal stem cells became possible[15]. The use of keratin-19 positive cells for skin engineering is currently being actively investigated either in China or abroad. Recently, neuron stem cells[16] and lung tissue stem cells[17] have been isolated from the spinal cord or the lung tissue. Upon *in vivo* transplantation, these stem cells can promote nerve regeneration or form lung-like tissues[16,17]. Thus, the discovery of more tissue-specific progenitor cells and the defining of their specific markers will lead to new approaches to tissue engineering and organogenesis.

2.4 Use of allogeneic cells as seed cells for tissue engineering

Because of limited source of autologous seed cells, the use of allogeneic cells to engineer tissues becomes an attractive area worthy of further exploration. In our laboratory, we used allogeneic chondrocytes harvested from fetal lamb to engineer cartilage tissue in adult recipients. The result demonstrated that the fetal allo-chondrocyte (less immunogenic than adult cells) engineered cartilage possessed normal cartilage structure and survived longer than adult allo-chondrocyte engineered cartilage[18]. This finding suggests that genetic modification of allogeneic cells to make them less immunogenic or tolerant to immune system may help apply allogeneic seed cells to tissue engineering. We recently transfected the allo-chondrocytes with the cDNA of Fas ligand to induce a localized immune tolerance and therefore to dampen the immune rejection (unpublished data). The potential of engineering cartilage using these genetically modified allo-chondrocytes is currently investigated.

2.5 Genetic modification of seed cells

Availability of a reliable source of seed cells remains a major challenge to the researchers. The ultimate goal of tissue engineering is to constitute tissues with biomaterials and with small amounts of harvested autologous cells that can be *in vitro* expanded. However, cell aging and phenotype changes often occur during the *in vitro* expansion, which prevents the use of the expanded seed cells from engineering tissues. In our laboratory, application of recombinant basic fibroblast growth factors (bFGF) and transforming growth factor β1 (TGF-β1) in cell culture has been shown to promote chondrocyte proliferation and maintain the cell phenotype during *in vitro* culture. Gene transfer instead of recombinant molecules enables us to avoid using expensive recombinant growth factor, which is critical in the large scale tissue engineering. More importantly, when genetically modified seed cells are implanted *in vivo*, the carried genes can further play their important role in the process of tissue formation. In our laboratory, the recombinant retroviral vectors containing the full-length cDNA of growth factors have been constructed and will be used to modify chondrocytes for *in vivo* cartilage engineering.

Among different cell types, aging process of chondrocyte is more prominent than other cell types during *in vitro* cell expansion. Intervention of aging process will enable us to achieve the ultimate goal

mentioned above. It has been shown that fibroblasts over-expressing teloramase gene has a lower apoptosis rate, and thus may delay the cell aging process[19]. Using telomerase recombinant retrovirus we tried to genetically modify isolated chondrocytes in the laboratories in order to observe its effect on the quality of *in vivo* engineered cartilage. Humanized xenograft containing specific human genes has been attempted for clinical transplantation[20]. Similarly, genetic modification of xenogeneic cells might be valuable for tissue engineering.

2.6 Modification of synthetic biomaterials

Biomaterial is one of the two essentials of tissue engineering. An ideal scaffold material should be biocompatible to both seed cells and to recipient's tissues. It should also be biodegradably controlled at a desired rate. Using new technologies of material engineering, most synthetic materials have met these two criteria. However, the adhesion and interaction of seed cells to these materials remain less satisfactory than natural extracellular environment. Recently, the modification of synthetic biomaterials using natural extracellular matrix molecules received a great deal of attention. Because cell-matrix interaction plays an important role in cell growth, differentiation and matrix production, improvement of the adhesion and interaction between the seed cells and the synthetic materials is expected to facilitate tissue formation. Coating scaffold materials with natural extracellular matrix molecules, such as arginine-glycine-aspartic acid (RGD) peptide, collagen and fibrin, were experimented to mimic the natural cell-matrix interaction. Integration of growth factors into synthetic materials is another important development, which is believed to enhance the process of engineering different types of tissues[21].

References

1 Langer, R. et al. Tissue Engineering. Science, 1993, 260: 920.
2 Vacanti, C. A. et al. Synthetic polymers seeded with chondrocytes provide a template for new cartilage formation. Plast. Reconstr. Surg., 1991, 88(5): 753.
3 Vacanti, C. A. et al. Tissue engineered growth of bone and cartilage. Transplantation Proceedings, 1993, 25(1): 1019.
4 Cao, Y. L. et al. Transplantation of chondrocytes utilizing a polymer-cell construct to produce tissue-engineered cartilage in the shape of a human ear. Plast. Reconstr. Surg., 1997, 100: 297.
5 Cai, X. et al. Repair of full-thickness skin defect with tissue engineering approach. In: Proceedings of Second National Symposium of Tissue Engineering (in Chinese), Guangzhou, 2000.
6 Yang, G. H. et al. Experimental study of repairing full-thickness skin defect using transplanted composite graft constituted with cultured epidermal cells and acellular dermal matrix. In: Proceedings of Second National Symposium of Tissue Engineering (in Chinese), Guangzhou, 2000.
7 Liu, Y. C. et al. The repair of large full-thickness auricular defects using tissue-engineered autograft on porcine. Tissue Engineering, 2000, 6(6): 678.
8 Cui, Y. M. et al. Proliferation and ECM molecule production by fibrochondrocytes isolated from meniscus. In: Proceedings of Second National Symposium of Tissue Engineering (in Chinese), Guangzhou, 2000.
9 Liu, Y. T. et al. An experimental study of repairing tendon defect with engineered tendon. In: Proceedings of Second National Symposium of Tissue Engineering (in Chinese), Guangzhou, 2000.
10 Caplan, A. I. Mesenchymal stem cells. J. Orthop. Res., 1991, 9(5): 641.
11 Wang, Z. et al. Tissue engineered bone repair of ram cranial defects with autologous cultured bone marrow stromal cells. Tissue Engineering, 2000, 6(6): 655.
12 Petite, H. et al. Tissue-engineered bone regeneration. Nature Biotechnology, 2000, 18: 959.
13 Chong, X. Q. et al. The mechanism of inducing ES cells into endothelial cells——The role of TGF-β1 in blood vessel genesis during embryo development. Acta Biologiae Experimentalis Sinica (in Chinese), 1996, 29(3): 273.
14 Xu, P. et al. Experimental study of *MyoD* gene in regulating myoblast differentiation of mouse ES cells. In: Proceedings of

Second National Symposium of Tissue Engineering (in Chinese), Guangzhou, 2000.

15 Michel, M. et al. Keratin 19 as a biochemical marker of skin stem cells *in vivo* and *in vitro*: keratin 19 expressing cells are differentially localized in function of anatomic sites, and their number varies with donor age and culture stage. J. Cell. Sci., 1996, 109: 1017.

16 Vacanti, M. P. et al. Tissue engineered spinal cord to reverse paralysis in rats. Tissue Engineering, 2000, 6(6): 661.

17 Cortiella, J. et al. Tissue engineered lung. Tissue Engineering, 2000, 6(6): 661.

18 Wang, J. et al. Preliminary study of the use of allogeneic chondrocyte for cartilage engineering. In: Proceedings of Second National Symposium of Tissue Engineering (in Chinese), Guangzhou, 2000.

19 Xie, H. Q. et al. Growth kinetic study of dermal fibroblasts transfected with human telomerase cDNA. In: Proceedings of Second National Symposium of Tissue Engineering (in Chinese), Guangzhou, 2000.

20 Levy, M. F. et al. Liver allotransplantation after extracorporeal hepatic support with transgenic (hCD55/hCD59) porcine livers: clinical results and lack of pig-to-human transmission of the porcine endogenous retrovirus. Transplantation, 2000, 69(2): 272.

21 Wang, S. G. et al. Tissue engineering scaffold material and related researches. In: Proceedings of Second National Symposium of Tissue Engineering (in Chinese), Guangzhou, 2000.