# Recent progress in tissue engineering

Yasuhiko Tabata

**Tissue engineering is a newly emerging biomedical technique that involves the artificial manipulation of cells to promote tissue and organ regeneration. Its medical significance will undoubtedly increase in the 21st century. This review summarizes recent progress that has been made in tissue engineering as well as its future implications.**

**Yasuhiko Tabata**
Dept of Biomaterials
Field of Tissue Engineering
Institute for Frontier Medical Sciences
Kyoto University
53 Kawara-cho Shogoin
Sakyo-ku, Kyoto 606 8507
Japan
tel: +81 75 751 4121
fax: +81 75 751 4144
e-mail:
yasuhiko@froniter.kyoto-u.ac.jp

▼ Recently, tissue engineering has become a recognized technique, and many researchers with different areas of expertise are entering this medical field, thereby increasing its scientific implications. The objective of tissue engineering is to regenerate natural tissues from living cells to replace defective or lost tissues and organs. This can be seen as the third medical therapy in regenerative medicine following the two major clinical therapies, organ transplantation and reconstructive surgery. As a regenerative therapy, tissue engineering avoids the problems associated with organ transplantation, such as donor shortages and permanent immunosuppresive medication, and it does not require the implantation of artificial biomaterials, which might have poor biocompatibility. Tissue engineering with these advantages gives tissue regeneration its promise and makes the technique widely acceptable as a new medical therapy in the 21st century.

## Tissue regeneration

Tissue engineering can be classified into two main areas: tissue regeneration and organ substitution (bioartificial organs)[1] (Table 1). Once a tissue can be reconstructed on a large scale *in vitro*, it can then be supplied directly to patients requiring new tissue. This is an ideal therapeutic concept but, at present, only dermis, epidermis and articular cartilage have been successfully reconstructed *in vitro*. Recently, bioreactor systems have been developed in combination with three-dimensional (3D) cell scaffolds for *in vitro* tissue construction[2]. Tissue construction is greatly influenced by the microgravity in the cell culture system[3]. When seeded into a tube-shaped biodegradable scaffold and cultured *in vitro* under a pulsatile stress, smooth muscle cells (SMCs) give rise to a blood vessel, with the cells and their extracellular matrix (ECM) being normally arranged in the direction of pulsatile stress[4]. These findings clearly suggest that once an appropriate 'field' is given to stimulate cell arrangement, the cells spontaneously create the corresponding tissue while preparing their surrounding environment via ECM production. However, there are still practical limitations to *in vitro* tissue engineering because present technology only enables the proliferation of one cell type, and only the formation of simple avascular structures is possible.

Tissue regeneration *in vivo* utilizes the natural healing process of the body, which subsequently achieves natural regeneration of tissues and organs. If *in vivo* tissue regeneration works well, it could provide a more realistic and clinically acceptable approach than *in vitro* tissue regeneration. *In vivo* regeneration of tissues is currently being researched based on a combination of various technologies and methodologies[1].

In addition to the controlled release of growth factors and cell scaffolding, which is discussed later in this review, a third important technology in organ and tissue regeneration *in vivo* concerns a 'physical barrier'. When a tissue defect occurs, the surrounding area is gradually filled with fibrous tissue and subsequent repair of the defect by natural tissue cannot occur. However, a biomedical membrane inserted around the tissue defect can physically prevent undesirable tissue ingrowth, therefore permitting tissue regeneration. This technique has been demonstrated for guided tissue regeneration (GTR) of the peripheral nerve[5], and periodontium and alveolar bone[6].

Finally, *in vivo* tissue regeneration that utilizes angiogenetic growth factors and their

1359-6446/01/$ – see front matter  PII: S1359-6446(01)01753-6 

**Table 1. Recent technology and methodology for tissue engineering**

| | Purpose | Necessary technology and methodology |
|---|---|---|
| Tissue regeneration | *In vitro* production of tissue constructs | Cell scaffolding, bioreactor, microgravity |
| | *In vivo* assistance and promotion of natural healing process | Cell scaffolding, controlled release<br>Physical barrier |
| | Ischemia therapy | Angiogenesis |
| Organ substitution (Bioartifical organs) | Immunoisolation | Biological barrier |
| | Nutrition and oxygen supply for transplanted cells | Angiogenesis |
| | Temporary assistance for organ function | Extracorporeal system |

genes can be used in therapies for ischemic diseases such as myocardial infarction and arteriosclerosis obliterans (ASO)[7]; growth factors are discussed later in this review.

## Organ substitution

The second method of tissue engineering is the substitution of organ functions by using allo- or xenogeneic cells, leading to the development of 'bioartificial organs'. One of the key technologies for *in vivo* or *in vitro* organ substitution is immunoisolation, which provides protection of the transplanted cells from host attack of the humoral and cellular immune systems and also maintains the function of the transplanted cells. Permeation of active complement components into the transplant can be selectively blocked by incorporating polystyrene sulfonic acid into the immunoisolation hydrogel membrane, and a barrier material able to actively exclude complement has been reported[8]. One promising approach to this issue is to induce pre-vascularization at the site of cell transplantation. To date, the main target of *in vitro* organ substitution has been the liver, and the combination of various liver cells with immunoisolation membranes has been attempted. The bioartificial liver is clinically used as a temporary assist device until the functions of the natural liver recover[9]. Pancreatic islet and adrenal chromaffin cells are clinically implanted for cell therapy of diabetes and Parkinson's disease[8,10,11]. The next step in successful cell therapy is the supply of sufficient nutrients and oxygen to the transplanted cells in the body.

## Key factors involved in tissue engineering

### *Identification of a suitable cell type*

The main components required for tissue engineering are cells, scaffolds and growth factors. One major problem is the identification of a cell type that is both clinically viable and suitable for tissue engineering[12]. Until recently, it was believed that there are no 'key' cells (such as precursor or stem cells) involved in tissue regeneration in adult tissue other than skin, intestinal mucosa and blood cells. However, key cells have now been discovered in various tissues. For example, in addition to hematopoietic stem cells, mesenchymal stem cells (MSCs) are present in the adult bone marrow. It has been revealed that MSCs have an inherent potential to differentiate into osteogeneic, chondrogeneic, adipogeneic and myocardiac cell lineages. Isolated human MSCs are now commercially available[13] and there is a potential to industrialize this MSC system for medical therapy.

If it is clinically possible to use a patient's own differentiated MSCs, immunological rejection is no longer an issue. Recently, various stem cells (e.g. neural stem cells) have been isolated from the fetus and adult body and both exhibit potential flexibility in cell differentiation[14]. Although stem cells are a promising cell source, they are not used clinically without their own problems in their application to tissue engineering. These problems include cell isolation, control of their differentiation and efficiency. The most probable cell source for tissue engineering is embryonic stem (ES) cells, which are more primodial than MSCs (Ref. 15). ES cells theoretically possess the potential to differentiate into every cell type present in the body. However, in relation to their application to tissue engineering, as with other stem cells, many problems still remain. Bone marrow transplantation of hematopoietic stem cells isolated from umbilical cord blood, and articular cartilage repair by autologous chondrocytes, are being performed clinically[16,17]. However, although the use of autologous cells from individual patients is ideal, such cell harvest is clinically difficult.

Undoubtedly, the two requirements for tissue regeneration and organ substitution are an increase in cell proliferation and the maintenance of their biological functions. Research into the identification of cell growth and

differentiation factors, and either the production of these factors on a large scale or improvement of the chemical components of cell culture media and substrates is still necessary.

### *Scaffold development*

The ECM that surrounds cells in the body not only physically supports cells but also regulates their proliferation, differentiation and morphogenesis[18]. Such a scaffold, therefore, needs to be developed for *in vitro* tissue reconstruction as well as for cell-mediated tissue regeneration *in vivo*. It is almost impossible for natural regeneration and repair of tissue defects to take place if the cells are not supplied with an ECM substitute. It is possible that placing a scaffold of artificial ECM at the defect in advance could facilitate a series of cell activities that results in tissue regeneration. This has been demonstrated with a type-I collagen sponge, which was shown to be an effective scaffold and promoted the regeneration of various tissues[1]. It is probable that once cells infiltrate a scaffold of a desired environment, they themselves can produce the intact ECM that accelerates their proliferation and differentiation resulting in natural tissue regeneration. For example, a collagen scaffold containing a basement membrane preparation plus gelatin microspheres containing basic fibroblast growth factor (bFGF) successfully induced *de novo* adipogenesis in fat-free subcutaneous tissue of mouse, in marked contrast to a scaffold containing basement membrane or bFGF-containing gelatin microspheres alone (Fig. 1). The combination of bFGF-containing microspheres and basement membrane was required to enable the mouse subcutis to form adipose tissue[19].

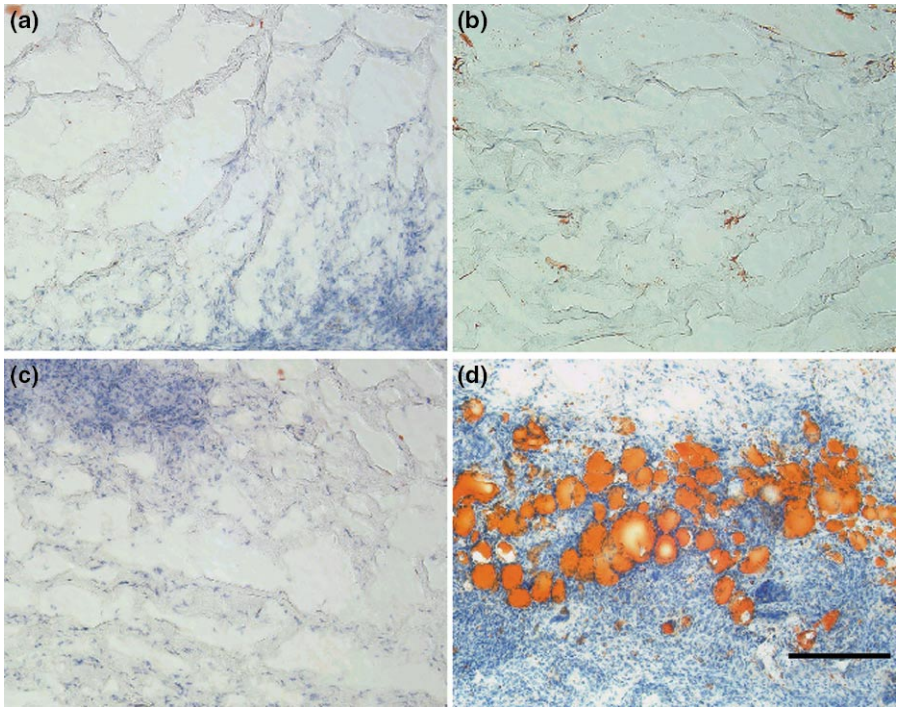


**Figure 1.** Lipid staining (Sudan III) of the mouse subcutis six weeks after implantation of **(a)** a type-I collagen sponge alone, **(b)** the sponge containing gelatin microspheres incorporating 0.1 μg of bFGF, **(c)** the sponge containing basement membrane and **(d)** the sponge containing basement membrane plus gelatin microspheres incorporating 0.1 μg of bFGF. The basement membrane was prepared by surfactant treatment of syngeneic mouse muscle. Scale bar, 200 μm.

Scaffolds that remain for longer time periods can, however, physically hinder tissue regeneration and the degradability of the scaffold *in vivo* needs to be controlled[20]. The pore size of spongy scaffold also has a great effect on cell infiltration and subsequent tissue regeneration[21]. Further research on the scaffold design will, therefore, be required because the chemical nature and structure of non-woven scaffolds affect tissue engineering both *in vitro* and *in vivo*. Hydrogels containing a peptide sequence have been designed to promote adhesion of specific cells[22].

### *Growth factors*

Successful tissue regeneration cannot always be achieved by the combination of cells and their scaffold alone. In such cases, a suitable growth factor to promote tissue regeneration is required. However, a successful method of supplying a growth factor that has, for instance, poor stability *in vivo,* remains to be found. One possible strategy is the controlled release of a growth factor at the site of regeneration over an extended time period. Drug delivery systems (DDS) enable growth factors and genes to exert their biological functions effectively *in vivo*[1]. Some researchers have found that the controlled release of growth factors and their plasmid DNA induces effective angiogenesis and bone regeneration.

## Future direction for tissue engineering

Although various cell types can be used, their application to *in vivo* tissue and organ regeneration based on recent technologies and methodologies is not always successful.

Every tissue and organ has its own individual metabolic rate. The heart, kidney, brain and lung, in addition to other organs in the body, perform extremely complex functions. These organs therefore consume three-quarters of the total body energy, although their total mass is only ~7.7% of total body weight. Conversely, the mass percentage of the muscle, skin and bone is ~92.3% of total body weight but their energy consumption is only about one-quarter of total body energy. For example, because the energy consumption rate of the skin and cartilage is low, only a small supply of oxygen is required for their survival and functional maintenance. Few blood vessels are observed in such simple tissues, which are composed of one or two cell types. As a result, *in vivo* regeneration works successfully for both tissues. The histological characteristics of tissues could, therefore, cause regeneration when artificially induced.

## Pre-vascularization

Regeneration of *in vivo* tissues or organs with high energy-demands requires the construction of a vascular network throughout the regenerated site to enable sufficient nutrition and oxygen supply. A recent trial involved the pre-vascularization of a site of cell transplantion that was induced by the controlled release of bFGF within a gelatin hydrogel[21]. Pancreatic islets and hepatocytes were then transplanted into the prevascularized site but could not survive for long time periods or maintain their biological functions[23]. However, this trial aimed to regenerate 2D or pseudo-3D tissue structures. A new methodology that arranges functional cells around sterically networked blood vessels is required to create a 3D tissue structure.

### *Development of multi-cell-type regeneration*

The liver, kidney and lung all comprise tubular structures in addition to a 3D vascular network. In these tubular structures, cell polarity is maintained and the various cell types that comprise the structure remain in communication. However, further technological and methodological progress will be needed because there is no way to create such a tissue structure using a combination of the technologies currently available. Cunha and colleagues reported that organ culture of epithelial and mesenchymal cells isolated at an embryonic stage gave rise to a prostate tissue[24]. Although embryonic cells are generally more potent than adult cells, there is further information to be gained about the morphogenesis of cells from detailed investigation of embryonic events.

### *'Internal medicine' tissue engineering*

Approaches to regenerative medicine based on conventional ideas and strategy is termed 'surgical' tissue engineering. The tissue regeneration ability of primates is not always inferior to that of lower-level animals. When injured, tissue is gradually repaired by the excessive formation of fibrous tissues (scar formation), which eventually suppresses natural tissue regeneration. This histological event is often observed in fibrotic diseases. If such fibrosis could be suppressed, or if the hypertropically formed tissue could be excluded, it is envisioned that natural tissue could regenerate at the treated site. This strategy is defined as 'internal medicine' tissue engineering; this would not only contribute to a complete cure for fibrotic diseases but would also permit natural tissue regeneration thereafter. It has been reported that injection of hepatocyte growth factor represses the fibrotic change in murine lung fibrosis, which is induced by bleomycin[25]. Therefore, drugs composed of proteins or genes are required for internal medicine tissue engineering and there is no doubt that DDS technologies will have a major role in this. In addition to drug release, DDSs can also prolong the lifetime of a drug and target drugs to a specific site. For example, DDS technologies could assist internal medicine tissue regeneration by targeting a drug to the necessary site and prolonging its lifetime *in vivo*.

## Conclusions

There is no doubt that tissue engineering has an enormous potential to make significant contributions to society over the next decade. However, tissue engineering is still in infancy and it will take time and further progression in technology and methodology to come to full fruition in the 21st century. The scientific, clinical and social benefits of regenerative medicine have yet to be realized.

## References


1 Tabata, Y. (2000) The importance of drug delivery systems in tissue engineering. *Pharm. Sci. Technol. Today* 3, 80–89

2 Freed, L.E. and Vunjak-Novakovic, G. (2000) Tissue engineering bioreactors. In *Principle of Tissue Engineering* (Langer, R. and Vacanti, J.P., eds), pp. 143–156, Elsevier

3 Unsworth, B.R. and Lelkes, P.I. (1998) Growing tissues in microgravity. *Nat. Med.* 4, 901–908

4 Niklason, L.E. *et al.* (1999) Functional arteries grown *in vitro*. *Science* 284, 489–493

5 Valentini, R.F. *et al.* (1987) Collagen- and laminin-containing gel impede peripheral nerve regeneration through semipermeable nerve guide. *Neurology* 98, 350–357

6 Ishikawa, I. and Arakawa, S. (1998) Awareness of periodontal disease – the role of industry. *Int. Dental J.* 48, 261–267

7 Isner, J.M. and Asahara, T. (1999) Angiogenesis and vasculogenesis as therapeutic strategies for postnatal neovascularization. *J. Clin. Invest.* 103, 1231–1236

8 Iwata, H. *et al.* (1999) Control of complement activities for immunoisolation. *Ann. New York Acad. Sci.* 875, 7–23

9 Watanabe, F.D. *et al.* (1997) Clinical experience with a bioartificial liver in the treatment of severe liver failure; a phase I clinical trial. *Ann. Surg.* 225, 484–494

**10** Date, I. and Ohmoto, T. (1999) Neural transplantation for Parkinson's disease. *Cell Mol. Neurobiol.* 19, 67–78

**11** Soon-Shiong, P. (1999) Treatment of type I diabetes using encapsulated islets. *Adv. Drug Deliv. Rev.* 35, 259–270

**12** Health, C.A. (2000) Cells for tissue engineering. *Trends Biotechnol.* 18, 17–19

**13** Pittenger, M.F. *et al.* (1999) Multi-lineage potential of adult human mesenchymal stem cells. *Science* 284, 143–147

**14** Svendsen, C.N. *et al.* (1999) New prospects for human stem-cell therapy in the nervous system. *Trends Neurosci.* 22, 357–364

**15** Fuchs, E. and Segre, J.A. (2000) Stem cells: a new lease on life. *Cell* 100, 143–155

**16** Kurzberg, J. *et al.* (1996) Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients. *New Engl. J. Med.* 335, 157–166

**17** Brittberg, M. *et al.* (1994) Treatment of deep cartilage defects in knee with autologous chondrocyte transplantation. *New Engl. J. Med.* 331, 889–895

**18** Ross, J.M. (1998) Cell–extracellular matrix interactions. In *Frontier in Tissue Engineering* (Patrick, C.M. *et al.*, eds), pp. 15–27, Elsevier

**19** Tabata, Y. *et al.* (2000) *De novo* formation of adipose tissue by controlled release of basic fibroblast growth factor. *Tissue Eng.* 6, 279–289

**20** Hong, L. *et al.* (2000) Bone regeneration at rabbit skull defects treated with transforming growth factor-β1 incorporated into hydrogels with different levels of biodegradabilities. *J. Neurosurg.* 92, 315–325

**21** Yamamoto, M. *et al.* (2000) Promotion of fibrovascular tissue ingrowth into porous sponges by basic fibroblast growth factor. *J. Mater. Sci. Mater. Med.* 11, 213–218

**22** Hubbell, J.A. (1999) Bioactive biomaterials. *Curr. Opin. Biotechnol.* 10, 123–129

**23** Ogawa, K. *et al.* The efficiency of prevascularization by bFGF for hepatocyte transplantation using polymer scaffolds in rat. *Tissue Eng.* (in press)

**24** Cunha, G.R. *et al.* (1992) Developmental response of adult mammary epithelial cells to various fetal and neonatal mesenchymes. *Epithelial Cell Biol.* 1, 105–118

**25** Yaekashima, M. *et al.* (1997) Simultaneous or delayed administration of hepatocyte growth factor equally represses the fibrotic change in murine lung injury induced by bleomycin. A morphological study. *Am. J. Respir. Crit. Care Med.* 155, 625–639