CHAPTER 2

Tissue Engineering of Internal Medicine for Regeneration Therapy of Chronic Fibrotic Diseases

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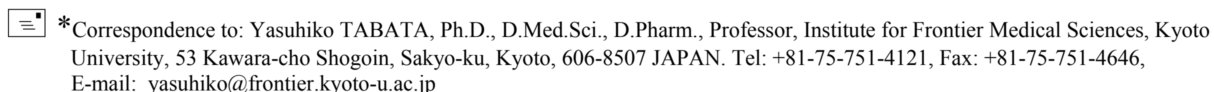
Summary

The objective of tissue engineering is to induce tissue regeneration at damaged tissues or organs for medical therapy by making use of the self-healing potential of the living body. This tissue regeneration can be achieved with cells and the local environment which promotes their natural process of proliferation and differentiation. To build up the local environment of cell-induced tissue regeneration, it is important to develop drug delivery system (DDS) which allows bio-signal molecules (growth factors and genes) to deliver to the target cells for a certain period of time at a desired concentration. DDS technologies enhance and prolong the in vivo biological functions of bio-signal molecules for tissue regeneration. When applied to the site to be regenerated in surgically (surgical tissue engineering), DDS technology has achieved the regeneration of various tissues. The current chapter introduces a new concept of DDS-based tissue engineering for therapy of chronic fibrotic diseases. For this physical tissue engineering, fibrotic tissue is loosened or digested by the physical drug therapy to convert into an in vivo environment which can be naturally repaired by the regeneration potential of the surrounding healthy tissue. The DDS of bio-signal molecules facilitates the regeneration and repairing processes of disease fibrosis.

KEYWORDS: Chronic fibrosis, Physical tissue engineering, Drug delivery system, Controlled release, Bio-signal molecules.



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INTRODUCTION

The basic idea of tissue engineering is to induce tissue regeneration at defective tissues or organs with cells and their environment prepared by biomaterials. To successfully achieve cell-induced tissue regeneration, it is necessary to create a local environment that enables cells to enhance the natural proliferation and differentiation (1). It has been recognized that in the living body, an environment is formed by a well-organized combination of bio-signal molecules, extracellular matrix (ECM) molecules, mechanical stress, and cell-cell interactions (2). Tissue engineering is one of the biomedical forms to build up the environment to induce cell-based tissue regeneration by an appropriate combination of the biological cues. Many tissue engineering approaches have been investigated to induce regeneration of various tissues and organs (3-9). Cell scaffolds and drug delivery systems (DDS) of bio-signal molecules have been explored with biomaterials, and surgically applied to a defective or lost tissue for the regeneration on the basis of cell-mediated natural healing potential. This idea of regeneration therapy can be applied to treat chronic fibrotic diseases. If the fibrotic tissue is loosened or digested by the physical drug therapy, it is possible that the natural healing of fibrosis is induced by the regeneration potential of the surrounding tissue. This is defined as the physical tissue engineering of internal medicine which is different from the surgical tissue engineering. To enhance the therapeutic efficacy of drug-based therapy in chronic fibrotic diseases, it is important to develop DDS technology and methodology that allows a drug to specifically be delivered to target cells in an appropriate time and concentration sequences. If regenerative medical therapy based on tissue engineering is realized in surgical or the physical fashion, it does not only provide us with new therapeutic methods, but it also increases the therapeutic choices for clinicians and patients.

This chapter describes physical tissue engineering to carry out regenerative therapy for the treatment of chronic fibrotic diseases on the basis of the natural healing potential of the living body. We, briefly, explain the molecular mechanisms on the tissue fibrosis and introduce some experimental data to emphasize the importance of DDS technologies in the new therapeutic trial.

CHRONIC FIBROTIC DISEASES

Recently, chronic diseases, such as heart disease, cancer, and diabetes, are among the most prevalent, costly, and preventable of all the health problems and the leading causes of death and disability. Prolonged course of illness and disability from these chronic diseases results in

extended pain and suffering and decreases the quality of life of patients. Tissue fibrosis is a characteristic of most types of chronic diseases in different organs, such as liver, kidney, lung, pancreas, and heart (Table 1), which is the natural result of wound-healing responses of organs to a repeated injury in conjunction with the accumulation of ECM proteins. The accumulation of ECM proteins distorts organ microarchitecture by forming a fibrous scar, leading to organ failure. The accumulation of ECM proteins results mainly from both their increased synthesis and decreased degradation.

Table 1. Chronic fibrotic diseases in different organs.

Organ and tissue	Diseases
Heart	Dilated cardiomyopathy
Lung	Idiopathic pulmonary fibrosis
Liver	Liver cirrhosis
Kidney	Chronic nephritis
Pancreas	Chronic pancreatitis
Gastrointestinal tract	Crohn's disease
Skin	Keloid, Scleroderma

The molecular mechanisms of the accumulation of ECM proteins have been extensively investigated in different tissues and organs, including kidney (10, 11), liver (12-16), and lung (17-20). Briefly, fibroblasts are the key players for the generation, deposition, and remodeling of ECM proteins during the development, response to injury and tissue fibrosis. Fibroblasts undergo a change in phenotype from their normal relatively quiescent state in which they are involved in slow turnover of ECM proteins, to a proliferative and contractile phenotype of myofibroblasts. Myofibroblasts show some of the phenotypic characteristics of smooth muscle cells and have been shown to contract *in vitro*. Myofibroblasts are present during tissue repairing process or in the response to injury in various tissues, including the liver, kidney, and lung. During normal repair process, myofibroblastic cells are lost by apoptosis. In pathological fibrosis, transforming

growth factor-β (TG-β) stimulates fibroblast differentiation into a myofibroblast phenotype and suppresses myofibroblast apoptosis in the site of developing fibrosis (20-24). As a result, myofibroblasts persist in the tissue and are responsible for fibrosis via increased ECM synthesis and for the contraction of the tissue.

On the other hand, matrix degradation is generally maintained ultimately through the balance of matrix metalloproteinases (MMP) and tissue inhibitors of metalloproteinases (TIMP) (25, 26). In chronic fibrotic tissues, decreased activity of ECM-removing MMP is mainly due to overexpression of TIMP.

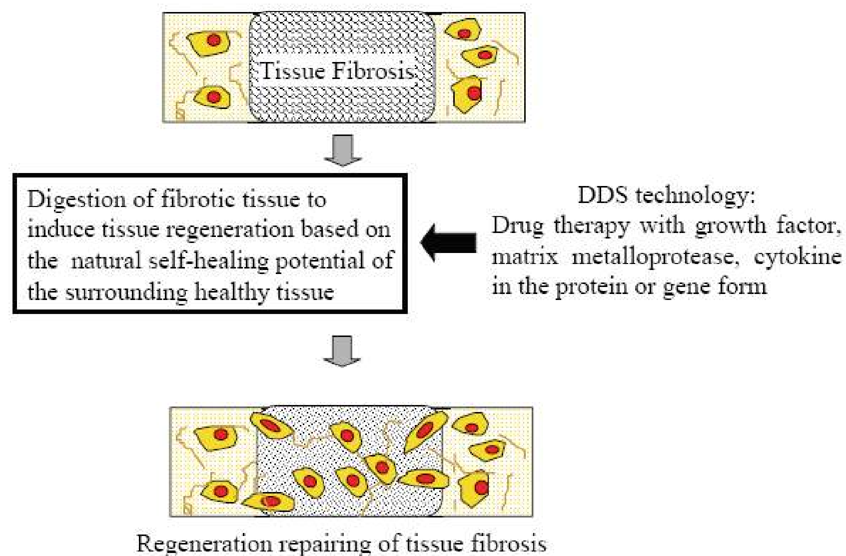

Fig. 1. The concept of physical tissue engineering of internal medicine.

FUNDAMENTAL STRATEGY FOR PHYSICAL TISSUE ENGINEERING OF INTERNAL MEDICINE

Physical tissue engineering of internal medicine is defined as the therapeutic approach to treat chronic fibrotic diseases based on the natural healing potential of healthy tissue around a fibrotic tissue, following loosening and digestion of the fibrotic tissue by drug treatment (Figure 1). In other words, the natural healing capability at a disease site is induced by removing the pathogenic cause to therapeutically cure the fibrosis and delay or suppress the deterioration of

chronic disease. In general, chronically injured tissue is gradually repaired by the excessive formation of fibrotic tissues (scar formation), which eventually suppresses the natural process of

Table 2. Anti-fibrotic therapeutic trials for pulmonary, liver and renal fibrosis

Drug	Activity	Representative paper		
		Kidney	Liver	Lung
Corticosteroids	Inhibition of neutrophil and lymphocyte migration into the lung (less therapeutic potential)			27
	Reduction of TGF-β signaling in hepatic stellate cells		28	
Azathioprine	Inihibition of adenine deaminase, which impairs the proliferation of cells, especially leukocytes and lymphocytes			29
Cyclophosphamide	Suppression of lymphocyte function by activation in the liver			29
Cyclosporin	Inihibition effect on T-cell			30
Interleukin (IL)-1 receptor antagonist	Blockade of fibroblasts activation	31		
Etanercept (TNF-α antagonist)	Inhibition of fibroblasts differentiation and collagen synthesis			32
IL-10	Up-regulation of MMP and down-regulation of α1(I) collagen and TIMP	33	34	35
Sirolimus, rapamycin	Inhibition of hepatic stellate cell proliferation	36	37	
SDZ RAD (rapamycin analogue)	Blockade of cytokine-dependent cell proliferation			38
α-tocopherol	Down-regulation of collagen type I, α-smooth muscle actin (SMA), and Proliferation cell nuclear antigen (PCNA) expression.		39	40
S-adenosylmethionine	Blockade of collagen type I production		41	
N-acetylcysteine	Blockade of smad signaling pathway		42	43
curcumin	Reduction in cell proliferation, induction of apoptosis and suppression of extracellular matrix gene expression	44	45	46
Fluvastatin	Reduction in fibroblasts proliferation and suppression of collagen type III production	47		
Losartan (angiotensin II type I receptor antagonist)	Inhibition of angiotensin-converting enzyme, fibroblasts proliferation, and apoptosis	48	49, 104	50
Captopril (angiotensin-converting enzyme inhibitor)	Inhibition of angiotensin-converting enzyme, fibroblasts proliferation, and apoptosis	51, 105	52	53
Bosentan (endothelin ET(A/B) receptor antagonist)	Inhibition of TGF-beta secretion and fibroblasts proliferation	54	55	56
Imatinib mesylate (tyrosine kinase inihibitor)	Inhibition of protein-tyrosine kinases	57	58	59
Interferon (IFN)-α	Inhibition of collagen production by fibroblasts	60	61	62
PEG-IFN-α2β+ribavirin	Inhibition of collagen production by fibroblasts		63	
IFN-γ	Inhibition of collagen production by fibroblasts	64	65	66
IFN-β1α	Inhibition of collagen production by fibroblasts		67	68
colchicine	Inhibition of pro-collagen secretion and conversion to collagen synthesis.	69	70	
Lovastatin, simvastatin	Inihibition of protein synthesis of pro-collagen	71	72	73, 74
CTGF small interfering RNA (siRNA)	Suppression of CTGF expression		75	
	Suppression of TGF-β expression			
Antisense oligonucleotide		76	77	
	Suppression of TGF-β activation			
TGF-β antibody		78		
Decorin		79, 80		
TGF-β receptor-IgG chimera		81		
	Suppression of TGF-β receptor activation			
TGF-β receptor antibody			82	
TGF-β receptor siRNA		83		
Bone morphogenetic protein (BMP)-7		18	84	
	Suppression of Smad signal transduction			
Smad 7 overexpression		85	86	87
Pirfenidone	Reduction in fibroblasts proliferation and suppression of collagen production	88	89	90
Hepatocyte growth factor (HGF)		91	92	93
Keratinocyte growth factor (KGF)				94
Anti-tissue inihibitor of metalloproteinase (TIMP)	Suppression of collagenase inactivation		95	
Matrix metalloproteinase (MMP)	Digestion of extracellular matrix	96	97	

tissue regeneration. If such a fibrosis can be suppressed or excluded by the drug treatment of internal medicine, it is physiologically expected that the fibrotic tissue is naturally repaired by the regeneration potential of the surrounding tissues.

Many pre-clinical and clinical researches have been conducted by using a variety of drugs with different biological functions (27-97) (Table 2). Among them, TGF-β is a potential target molecule to ameliorate tissue fibrosis, since TGF-β functions as one of the primary mediators to accelerate ECM accumulation (78, 98-105). TGF-β is a multifunctional cytokine acting in many physiologic and pathologic processes, regulates the proliferation and differentiation of cells in several tissues, and plays a central role in fibrogenesis (102). TGF-β increases the production and deposition of ECM proteins, reduces matrix degradation accompanied with a decreased protease production and increased inhibitors production, and stimulates the synthesis of ECM protein receptors (98). Therefore, it is possible that blocking the TGF-β action on ECM may suppress tissue fibrosis (Table 3).

Table 3. Suppression of TGF-β activity for anti-fibrotic therapy.

Suppression of TGF-β activity	Therapeutic method
Suppression of TGF-β expression	Angiotensin II type I receptor antagonist (AT1RA) Angiotensin converting enzyme inhibitors (ACE-I) Antisense oligonucleotide, Small interfering RNA
Suppression of TGF-β activation	TGF-β antibody Decorin TGF-β receptor-IgG Fc chimera
Suppression of TGF-β receptor activation	TGF-β receptor antibody Antagonist
Suppression of Smad signal transduction	Smad7 overexpression

It has been demonstrated that the biological inhibition of TGF-β protein by the use of angiotensin II type I receptor antagonists (AT1RA) (103, 104), angiotensin converting enzyme inhibitors (ACE-I) (105), neutralizing antibody (78), antisense oligonucleotide (76), decorin (79, 80), TGF-β receptor-IgG Fc chimera (81), and TGF-β receptor small interfering RNA (siRNA)

(83) suppressed the accumulation of ECM in the animal models of fibrosis. For example, Terui *et al*. demonstrated that losartan, an angiotensin II type 1 (AT1) receptor antagonist decreases the plasma TGF-β concentration and the fibrosis score in patients with early stages of hepatic fibrosis of chronic hepatitis C (103, 104). Sharma *et al*. reported that an angiotensin-converting enzyme inhibitor, captopril decreased TGF-β1 levels in diabetic nephropathy and captopril-induced reduction of serum levels of TGF-β1 correlates with long-term renoprotection in insulin-dependent diabetic patients (105). These results suggest that drugs commercially available, such as ACE-I and AT1RA, are promising for anti-fibrosis therapy on the basis of the concept on the physical tissue engineering of internal medicine.

PHYSICAL TISSUE ENGINEERING OF INTERNAL MEDICINE BY PROTEINS AND GENES

At present, there is no effective therapy for chronic fibrosis diseases, although some drugs that area commercially available exhibit the capability to prevent progression of tissue fibrosis as described above. However, recent progress in basic biology and medicine has demonstrated the molecular mechanisms of tissue regeneration in chronic fibrotic diseases. Therefore, molecular-based therapies using proteins and genes have attracted much attention as an alternative therapeutic approach with a high specificity for chronic fibrotic diseases.

As described above, TGF-β is one of the primary mediators to accelerate ECM accumulation and have been investigated as a molecular target to modulate its activity and signal transduction for suppressing the accumulation of ECM proteins and the progression of tissue fibrosis. For example, Border *et al*. reported that intravenous injection of antiserum against TGF-β suppressed the progression of renal fibrosis in an experimental glomerulonephritis model (78). *In vivo* transfection of antisense oligonucleotides for TGF-β receptor (82) and TGF-β (77) by non-viral and adenovirus vectors showed significant decrease in the expression level of TGF-β receptor and TGF-β, resulting in suppressing progression of liver fibrosis. As another molecular target, Yokoi *et al*. have demonstrated that blocking the expression of connective tissue growth factor (CTGF) by an antisense oligonucleotide for CTGF results in prevention of the accumulation of collagen type I and reduction of the tissue area of fibrosis in an experimental glomerulonephritis model (106).

On the other hand, MMP-1 digestion allows a fibrotic tissue to convert to a physiological state where the natural process of tissue regeneration can function to heal fibrosis. Iimuro *et al*. demonstrated that transfection of pro-MMP-1 gene using an adenovirus vector, histologically improved tissue fibrosis in the liver in a rat cirrhosis model (97). It is suggested that the possible healing mechanism is associated with the suppression of hepatic stellate cells and proliferation of hepatocytes. Indeed, several approaches to reduce collagenous ECM proteins in tissue fibrosis using proteins and genes could successfully prevent the progression of tissue fibrosis in different organs. However, it is highly expected to use a biological molecule that can achieve both the reduction of accumulated ECM proteins and the induction of tissue regeneration. Hepatocyte growth factor (HGF) has emerged as a potent, endogenous antifibrotic factor that shows an impressive efficacy in ameliorating tissue fibrosis in a wide variety of animal models by both the inhibition of TGF-β-mediated ECM accumulation and the stimulation of tissue regeneration (91).

HGF was first identified, purified, and cloned as a potent mitogen for fully differentiated hepatocytes about two decades ago. HGF has multiple biological activities for a wide variety of cells, including mitogenic, motogenic (enhancement of cell movement), morphogenic, and anti-apoptotic activities (107). Besides its well-described regenerative property, many researches have indicated that HGF is an endogenous, antifibrotic factor that is capable of improving fibrotic lesions and preserving organ functions in a wide variety of experimental animal models (91). Nakamura *et al*. have demonstrated that the supplementation of exogenous HGF could lead to a restoration of the balance between HGF and TGF-β in fibrotic tissues, thereby suppressing the fibrogenic actions of TGF-β (93). This HGF antagonistic activity against TGF-β inhibits myofibroblast activation and subsequent tissue fibrosis, leading to suppression of chronic fibrosis in a variety of organs, including liver, lung, kidney, and heart.

PHYSICAL TISSUE ENGINEERING OF INTERNAL MEDICINE BASED ON DRUG DELIVERY SYSTEM

In the previous section, we claimed that biologically active proteins and genes have a great potential to improve chronic fibrotic diseases in different organs. However, multiple injections of proteins in solution or genes in viral vectors with a high transfection activity should be required to achieve their high biological activities for chronic fibrosis treatment because proteins and

genes rapidly diffuse from the injection site and enzymatically digested or deactivated. In addition, there are several drawbacks to be resolved for viral vectors, such as the limited size of DNA molecules that can be inserted into viral vectors, high immunogenicity and toxicity, or the possible mutagenesis of transfected cells. To enable proteins and genes to exert the biological functions efficiently in clinical applications, it is highly expected to develop DDS technologies to enhance their *in vivo* therapeutic efficacy. There are four objectives of DDS, the controlled release of drugs, the stabilization of drugs, the acceleration of drug absorption and permeation, and the targeting of drugs to the site of action. Among them, so far only the release technology has been applied to growth factors and genes for the induction of tissue regeneration (109, 110). For example, controlled release of growth factor and gene at the site of action over an extended period of time is achieved by incorporating them into an appropriate carrier. It is also possible that growth factors and genes are protected against enzymatic digestion when incorporated in the release carrier, for a prolonged retention of activity *in vivo*. To achieve these requirements for the release carrier, we have explored a biodegradable hydrogel carrier of gelatin derivatives for controlled release of proteins and genes *in vivo* (109, 110).

Gelatin has been extensively used for industrial, pharmaceutical, and medical applications. The biosafety has been proved through its long clinical usage as surgical biomaterials and drug ingredients. Another unique advantage is the electrical nature of gelatin which can be readily changed by the processing method of collagen in the preparation (111). For example, an alkaline processing allows collagen to structurally denature and hydrolyze the side chain of glutamine and asparagine residue. This results in generation of "acidic" gelatin with an isoelectric point (IEP) of 5.0. On the other hand, the acidic processing of collagen produces "basic" gelatin with an IEP of 9.0. We have prepared hydrogels by crosslinking gelatin for controlled release of growth factors. Growth factors with IEPs higher than 7.0, such as basic fibroblast growth factor (bFGF) (112) and transforming growth factor beta1 (TGF-β1) (113), are immobilized into the biodegradable hydrogels of "acidic" gelatin mainly through the electrostatic interaction force between the growth factor and gelatin molecules. In this release system, the growth factor immobilized is not released from the gelatin hydrogel unless the hydrogel carrier is degraded to generate water-soluble gelatin fragments. Therefore, the time profile of growth factor release could be controlled only by changing that of hydrogel degradation which can be modified by the extent of hydrogel crosslinking (112). The key point is to chemically modify the property of

gelatin to permit the physicochemical interaction with the growth factor to be released. Chemical derivatization allows gelatin to change the electric and hydrophobic natures and consequently interact with different factors (92, 109, 110, 112-126). Hydrogels prepared from gelatin derivatives can be applied for controlled release of various bioactive substances including growth factors and genes, such as plasmid DNA, decoy DNA, and siRNA (83, 96, 127-139).

Based on controlled release technology for various bioactive substances, we have investigated the feasibility of the hydrogel system for anti-fibrotic proteins and genes in enhancing their biological activities *in vivo*. For example, controlled release of HGF protein from gelatin hydrogels prevented the progression of fibrosis and induced significant regeneration and repair in animal models of liver cirrhosis (92) and dilated cardiomyopathy (114). Oe *et al*. have reported therapeutic efficacy of HGF release in the liver cirrhosis (92). Biodegradable gelatin microspheres incorporating HGF were intraperitoneally injected into a rat model of liver cirrhosis which has been prepared by the intraperitoneal injection of thioacetamide every other day for 10 weeks. Histological observation of the rat liver revealed that the injection of gelatin microspheres incorporating HGF effectively allowed a histological decrease in the area of liver fibrosis and to induce liver regeneration (Figure 2).

Sakaguchi *et al*. have investigated the feasibility of gelatin hydrogels incorporating HGF in preventing the progression of heart failure in stroke-prone spontaneously hypertensive rats (114). When applied on the left ventricular free wall of stroke-prone spontaneously hypertensive rats of 25 weeks old, a gelatin sheet incorporating HGF improves cardiac function, reversed left ventricular remodeling, and markedly improved survival in spontaneously hypertensive rats. These beneficial effects are associated with angiogenesis and reduced fibrosis in the left ventricular myocardium (Figure 3).

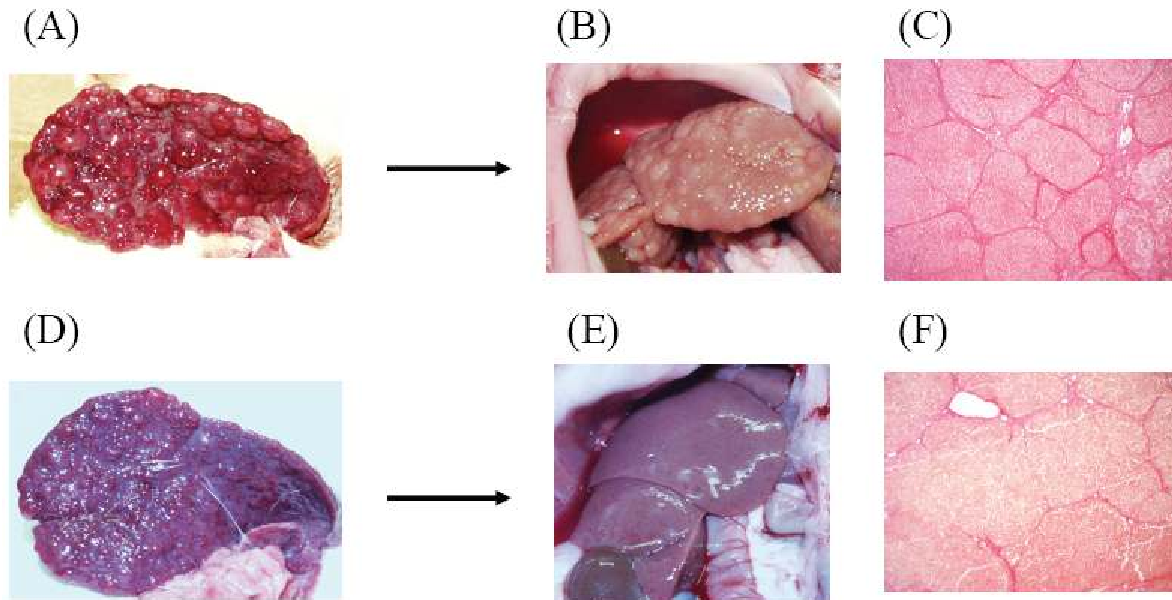

Fig. 2. Physical tissue engineering of internal medicine for liver cirrhosis by controlled release of HGF. Gelatin microspheres incorporating HGF (0.4 mg) and 0.4 mg of free HGF were intraperitoneally injected into the liver cirrhosis model rats after 13 weeks of thioacetoamide injection. Gelatin microspheres incorporating HGF (A, B, C) and free HGF (D, E, F). Macroscopic views of the liver (A, D) before and (B, E) 2 weeks after treatments. Histological sections of the liver were stained with Sircol collagen dye.

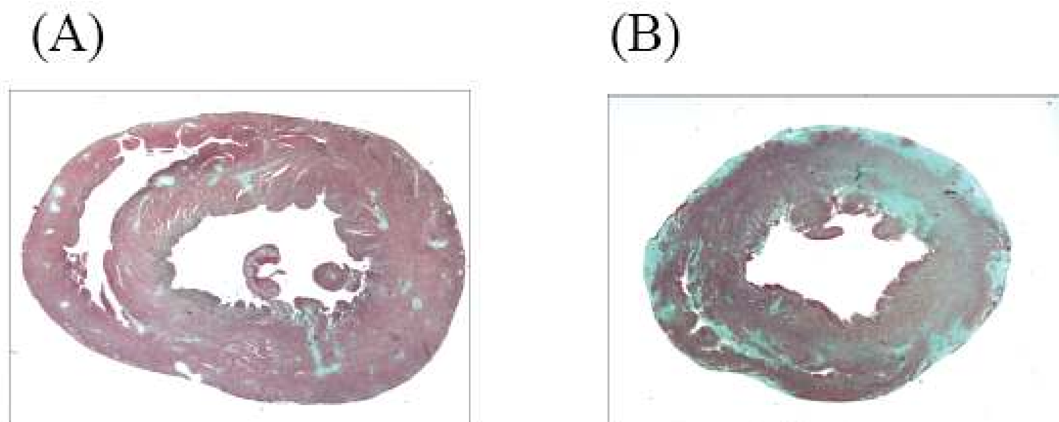

Fig. 3. Myocardial sections of rats receiving injection of gelatin hydrogel sheets incorporating HGF or free HGF into cardiac muscle at four weeks after surgery were stained with Masson's trichrome. (A) Gelatin hydrogel sheets incorporating HGF (50 μg/site) and (B) free HGF.

Renal interstitial fibrosis is the common pathway of chronic renal disease, while it causes end-stage renal failure. TGF-β is well recognized to be one of the primary mediators to induce

the ECM accumulation in the fibrotic area. We have demonstrated the enhanced anti-fibrotic activity of a plasmid DNA of TGF-β type II receptor siRNA expression vector for a mouse model of renal fibrosis by complexation with a cationized gelatin (83). The injection of plasmid DNA-cationized gelatin complex significantly decreased the level of TGF-β receptor and *α*-smooth muscle actin over-expression, the collagen content of fibrotic kidney, and the fibrotic area of renal cortex, in remarked contrast to free plasmid DNA injection (Figure 4). In addition, Miyazaki *et al*. have demonstrated the enhanced anti-fibrotic activity of a siRNA of heat shock protein 47 (HSP47) by the controlled release from cationized gelatin microspheres for a mouse model of peritoneal fibrosis (127). The injection of cationized gelatin microspheres incorporating HSP47 siRNA significantly decreased the collagen content of fibrotic peritoneal membrane in a mouse model, in contrast to non-silencing siRNA injection.

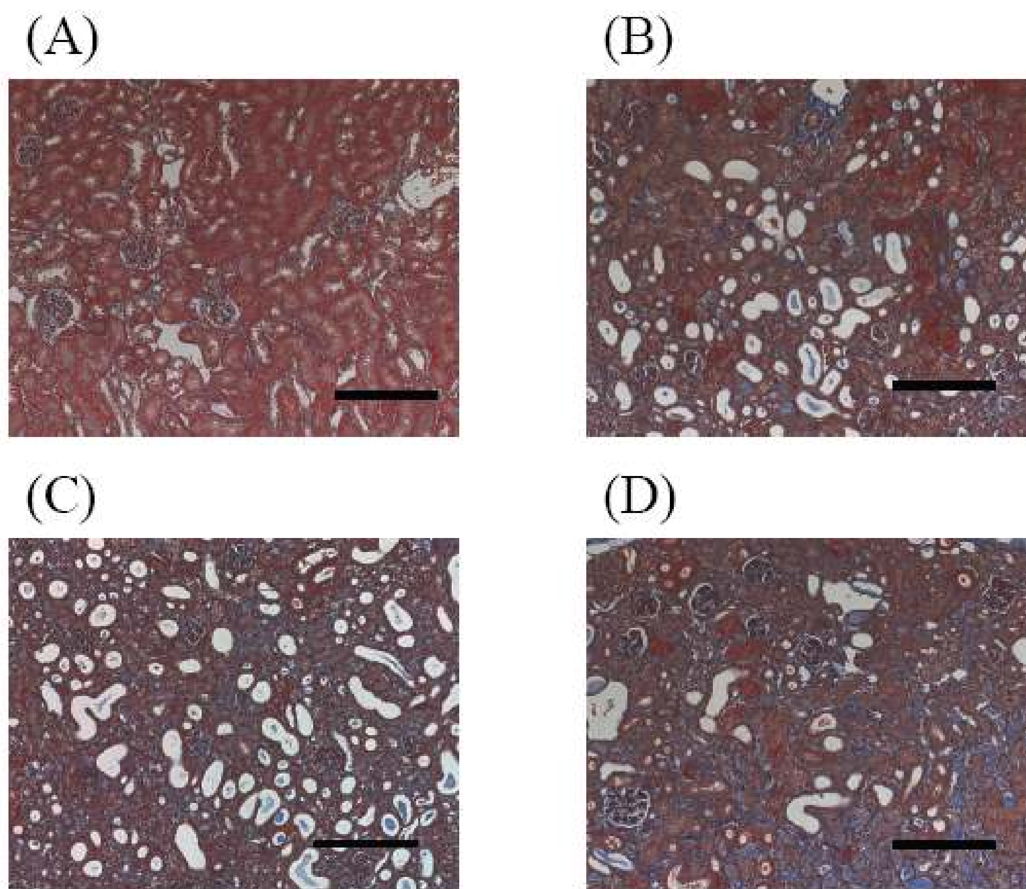

Fig. 4. Histological sections of renal cortex of unilateral ureteral obstruction (UUO) model mice after TGF-β receptor typeII shRNA expression plasmid (pSUPER-TGF-βRII) application. The section was stained with Masson's trichrome seven days after injection of (A) pSUPER-TGF-βRII complexed with cationized gelatin, (B) free pSUPER-TGF-βRII, (C) empty pSUPER, (D) saline. Bar length is 200 μm.

On the other hand, MMP-1 digestion could convert a fibrotic tissue to a physiological state where the natural process of tissue regeneration can initiate to function for fibrosis healing. As described in the previous section, Iimuro *et al*. demonstrated that transfection of pro-MMP-1 gene with an adenovirus vector, histologically improved tissue fibrosis in the liver in a rat cirrhosis model. However, as far as viral vectors are used for gene expression, the therapeutic trial cannot be clinically applied. This is because it is practically impossible to use viral vectors in the clinic. Therefore, a new method of non-viral gene transfection should be developed. As one trial, controlled release technology of plasmid DNA has been explored. Cationized gelatin microspheres incorporating a MMP-1 plasmid DNA were injected into the subcapsular space of mouse kidney in advance, and then the mice received streptozotocin (STZ) to induce the onset of diabetic renal disease. It was reported that advanced lesion of STZ-induced diabetic kidney mimics some findings of early-stage clinical diabetic nephropathy. Figure 5 shows the histological renal sections of mice pre-injected with microspheres incorporating MMP-1 plasmid DNA 28 days after STZ injection. Renal fibrosis was histologically suppressed by the application of cationized gelatin microspheres incorporating MMP-1 plasmid DNA, compared with that of free MMP-1 plasmid DNA. The injection of plasmid DNA-free cationized gelatin microspheres was not effective and the tissue appearance was similar to that of the saline-injected control group (96).

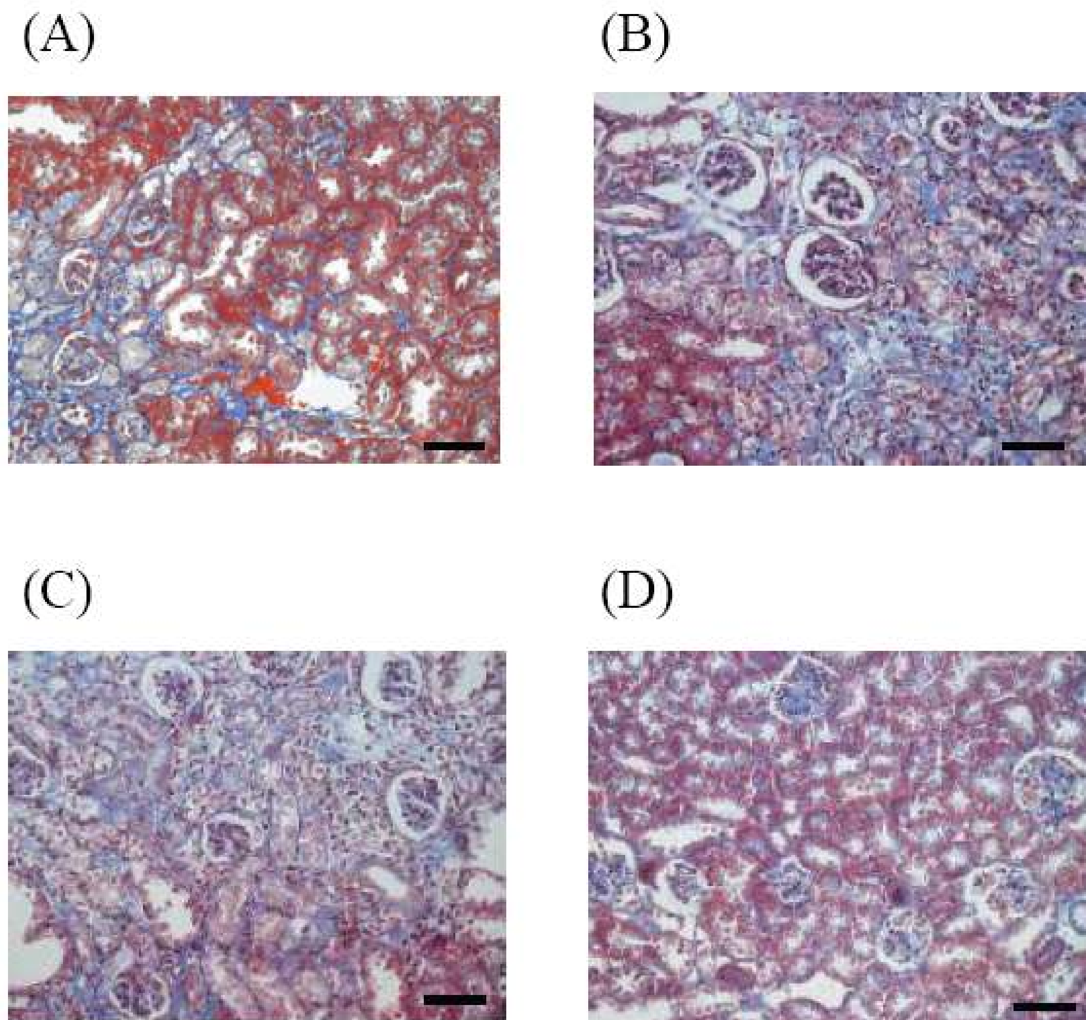

Fig. 5. Renal histological sections of mice receiving injection of gelatin microspheres incorporating MMP-1 plasmid DNA or other agents into the renal subcapsule 28 days after STZ injection were stained with Masson's trichrome. (A) saline, (B) cationized gelatin microspheres, (C) free MMP-1 plasmid DNA (50 μg/site), and (D) cationized gelatin microspheres incorporating MMP-1 plasmid DNA (50 μg/site) □□Each bar corresponds to 50 μm.

CONCLUSIONS

Tissue engineering technology can not only be used surgically to induce tissue regeneration in a body defect, but it can also be applied to therapeutically treat chronic fibrosis diseases of pulmonary fibrosis, liver fibrosis, dilated cardiomyopathy, and chronic nephritis in a physical manner by making use of anti-fibrotic drugs, such as proteins and genetic substances. The fibrotic tissue is loosened or digested by making use of the drugs combined with DDS technologies and convert to a physiological state susceptible to natural regeneration based on the

self-healing potential of the body. This is the basic concept of the physical tissue engineering of internal medicine. This therapeutic trial is an application of regenerative medical therapy to the filed of internal medicine. This is a just-started trial, but the *in vivo* therapeutic possibility has been experimentally confirmed by different animal models of diseases. If it can be applied clinically, it will be possible to treat the fibrotic diseases for which there has been so far no efficient therapy. To further develop this anti-fibrotic therapy, the research advance of biology and medicine on the molecular mechanisms of fibrosis is highly expected to identify and enable the use of new drug targets with anti-fibrotic nature. Active combination of drugs with DDS technologies needs specific cell and tissue targeting of anti-fibrosis and newly developed method of regeneration therapy based on the natural self-healing potential of patients themselves. DDS-based suppression of tissue fibrotic progression will significantly improve the quality of life of patients. It is no doubt that increasing the therapeutic choice for clinicians brings about large medical benefits to patients.

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