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Review

Potential of Drug Delivery Technology in Tissue Regeneration Therapy

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Abstract: As the third therapy following reconstructive surgery and organ transplantation, the therapy of regenerative medicine has been currently expected. The objective of regenerative medical therapy is to induce regeneration and repairing of defective and injured tissues based on the natural-healing potential of patients themselves. For successful tissue regeneration, it is undoubtedly indispensable to create a local environment that enables cells to efficiently proliferate and differentiate, resulting in the natural induction of tissue regeneration. Tissue engineering is a biomedical technology or methodology to build up this regeneration environment. For example, the technology of drug delivery system (DDS) enhances the biological functions of growth factors and the related genes for promoted tissue regeneration. This paper overviews the recent status of tissue regeneration based on the technology of growth factor release to emphasize significance of DDS technology in regenerative medical therapy.

Keywords: Tissue engineering, Drug delivery system (DDS), Growth factor, Plasmid DNA, Release technology, Cell genetic engineering, Tissue regeneration.

Need for tissue engineering in regenerative medical therapy

As surgical therapies currently available, there have been reconstruction surgery and organ transplantation. Although there is no doubt that these therapies have saved and improved the countless lives of patients, they have clinical limitations. For the former therapy, biomedical devices cannot completely substitute the biological functions even of a single tissue or organ and consequently cannot prevent progressive deterioration of injured tissue and organ, either. One of the biggest issues for organ transplantation is the shortage of donor tissues or organs. Additionally, the permanent medication of immunosuppressive agents often causes side-effects, while virus infection is not completely ruled out. In this circumstance, a new therapeutic trial, in which disease healing can be achieved based on the natural-healing potential of patients themselves, has been explored. To realize this therapy of regenerative medicine, it is necessary to provide cells a local environment suitable to their proliferation and differentiation for the natural induction of tissue regeneration. It is tissue engineering that is one of the biomedical engineering forms to build up the environment for regeneration induction. If it is possible to induce regeneration of defective or lost tissues as well as substitute the biological functions of damaged organ by making use of the tissue engineering concept, a new strategy of disease therapy will be achieved on the basis of the cell-mediated natural healing potential. For surgical tissue engineering, biomaterials with or without cells and / or drugs combination are surgically applied to a body tissue defect to induce tissue regeneration for disease therapy. On the other hand, drugs are physically applied to the fibrotic tissue of chronic diseases for digestion, leading to disease therapy based on the regeneration induction potential of the surrounding tissue, which is defined as tissue engineering of internal medicine.

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Fundamental technology and methodology of tissue engineering

Considering the components consisting body tissue, there are three key factors; such as cells, the natural scaffold for cell proliferation and differentiation, and biosignaling molecules (growth factors and genes). There are four important technologies or methodologies for tissue engineering. The first technology is to prepare an artificial scaffold of cells proliferation and differentiation for tissue regeneration. It is well known that the extracellular matrix (ECM) is not only a physical support of cells but also provides a natural environment for cell proliferation and differentiation or morphogenesis which contributes to tissue regeneration and organogenesis[1]. It is unlikely that a large-size tissue defect will be naturally regenerated and repaired only by supplying cells to the defective site. For example, one promising way is to artificially build an environment for cells suitable to

induce the tissue regeneration at the defect by in advance providing a scaffold of artificial ECM which initially assists cell attachment and the subsequent proliferation and differentiation. It is highly expected that cells residing around the scaffold infiltrate the scaffold and proliferate and differentiate therein if the artificial ECM is biologically compatible.

When the tissue around the defect does not have any inherent potential to regenerate, the tissue regeneration cannot be always expected only by supplying the scaffold. The scaffold should be used in combination with cells or/and bio-signaling molecules (growth factors and genes) which has the potential to accelerate tissue regeneration. Although there are cases, where growth factor is required to promote tissue regeneration, the direct injection of growth factor in solution into the site to be regenerated is generally not effective. This is because the growth factor is rapidly diffused from the injected site and is enzymatically digested or deactivated. To enable the growth factor to efficiently exert its biological function, a technology or methodology is required. This is the drug delivery system (DDS), the second key technology of tissue engineering (Fig. 1). Among the DDS technologies, the controlled release of growth factor at the site of action over an extended period is achieved by incorporating the factor into an appropriate carrier which is very important for tissue engineering. It is also highly possible that the growth factor is protected against its proteolysis, as far as it is, at least, incorporated in the release carrier, for prolonged retention of the activity *in vivo*. The release carrier should be degraded in the body since it is not needed after the growth factor release is completed. Other than the controlled release of drug, the objectives of DDS include the prolongation of drug half-life, the improvement of drug absorption, and drug targeting. For example, it is a promising approach to promote tissue regeneration by targeting a growth factor with a prolonged half-life to the tissue site to be regenerated.

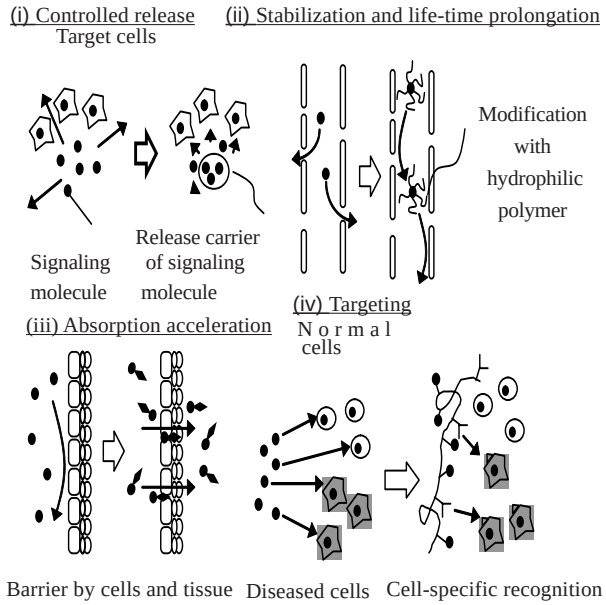


Fig. 1 DDS technology and methodology with biomaterials applicable for tissue engineering.

It is no doubt that cells with high proliferation and differentiation potentials, so-called precursor and stem cells, are important to induce tissue regeneration. However, one of the major problems is the shortage of cells clinically available. Therefore, it is necessary to increase the number of stem cells with a high quality up to a level clinically applicable. For this purpose, cell isolation and *in vitro* cell culture methods are required. The cell scaffold mentioned above can be utilized as the substrate for cell culture. The third technology is for the isolation and proliferation of cells. The fourth is for a physical barrier to protect the cells transplanted and the area to be regenerated from immunological attack and fibroblast infiltration, respectively. When a body defect is generated, the defect space is generally occupied rapidly with the fibrous tissue produced by fibroblasts which are ubiquitously present in the body and can rapidly proliferate. This is one of the typical wound healing processes observed in the biological system. However, once this ingrowth of fibrous tissue into the defective area to be regenerated takes place, the regeneration and repairing of a target tissue at the space cannot be expected any more. To prevent the tissue ingrowth, a barrier membrane is highly required to secure a space for tissue regeneration. The immunoisolation membrane used to protect the cells transplanted from the biological attacks of humoral and cellular components of the body is one such example. Thus, it is tissue engineering that by making use of cell scaffold, barrier, and DDS technologies, biomedical technology or methodology to create an environment for the proliferation and differentiation of cells to induce tissue regeneration.

Tissue regeneration based on DDS technologies of biosignaling molecules

Tissue engineering for clinical regenerative medicine can be classified into two categories in terms of the site where tissue regeneration or organ substitution is performed: *in vitro* and *in vivo* tissue engineering. I*n vitro* tissue engineering involves tissue reconstruction and organ substitution which has been known as bioartificial hybrid organ. If a tissue can be reconstructed *in vitro* in factories or laboratories on a large scale, we can supply the tissue constructed to patients when it is needed. If possible, this will be available for commercialization. However, it is quite difficult to completely reconstruct the *in vivo* event using the present knowledge of biology and medicine or cell culture technologies. It is difficult to complete *in vitro* tissue engineering at present, as far as it is impossible to artificially arrange a biological environment for cell-based tissue reconstruction. Another application of *in vitro* tissue engineering is the substitution of organ functions by use of allo- or xenogeneic cells. Such engineered organs are called bioartificial hybrid organ and many

Table 1. Regeneration induction of body tissues and organs based on the controlled release of bioactive growth factors from biodegradable hydrogels.

Materials	Growth factor	Animal	Effect	Objective	Reference
Acidic gelatin	bFGF	Mouse, Rat, and Dog	Angiogenesis	Transplantation of Langerhans islands for diabetes therapy	15, 26)
(pI 5.0)		Rat	〃	Transplantation of hepatocytes for therapy of enzyme deficiency disease	27)
		Rat	〃	Transplantation of renal epithelial cells	17)
		Rat and dog	〃	Transplantation of cardiomyocytes	16)
		Rat and guinea pig	〃	Promoted repairing of skin dermal layer	28)
		Rat and Pig	〃	Treatment of cardiac infarction	13, 29)
		Rabbit	〃	Treatment of lower limb ischemia	14)
		Rat, Dog, and Monkey	Osteogenesis and angiogenesis	Repairing of sternum and connective tissue	19, 30, 31)
		Rat, Rabbit, and Monkey	Osteogenesis	Repairing of skull and long bone	32,33)
		Mouse	Adipogenesis	Repairing of breast and soft tissue reconstruction	20)
		Mouse	Angiogenesis and activation of hair follicle tissue	Promotion of hair growth	34, 35)
		Dog	Periodontium repair	Repairing of periodontium	36)
		Dog	Peripheral nerve repair	Nerve repairing	37)
		Dog	Osteogenesis	Repairing of mandiblular bone	38)
	TGF- β 1	Rabbit and Monkey	〃	Repair of skull bone	39-42)
		Sheep	Chondrogenesis	Repairing of tracheal cartilages	43)
	HGF	Mouse	Angiogenesis and activation of hair follicle tissue	Promotion of hair growth	35)
		Rat and Pig	Angiogenesis and inhibition of apoptosis	Treatment of dilated cardiomyopathy	
	bFGF/TGF- β 1	Rabbit	Osteogenesis	Repairing of skull bone	
	CTGF	Rabbit	Chondrogenesis	Repairing of articular cartilage	44)
Basic gelatin (pI 9.0)	BMP-2	Rat, Dog, and Monkey	Osteogenesis	Repairing of skull and mandiblular bone	45)
		Dog	Chondrogenesis	Repairing of tracheal cartilages	46)
Collagen	TGF-β1	Rabbit	Osteogenesis	Repairing of skull bone	47)
		Rabbit	〃	Promotion of engraftment of soft tissue grafts	48)
		Mouse	Angiogenesis and hair elongation	Promotion of hair growth	35, 49)

bFGF: fibroblast growth factor, TGF-β1: transforming growth factor β1, HGF: hepatocyte growth factor, CTGF: connective tissue growth factor, VEGF: vascular endothelial growth factor, BMP-2: bone morphogenetic protein 2, pI: isoelectric point

researchers are interested in designing bioartificial liver and pancreas. Distinct from the *in vitro* tissue engineering, *in vivo* tissue engineering has an advantage for cell-induced tissue regeneration. It is likely that most biological components essential for tissue regeneration are naturally supplied by the host. Therefore, almost all the approaches of tissue engineering have been performed *in vivo* with or without biodegradable scaffolds. This *in vivo* approach is more realistic and clinically acceptable if it works well. There are several examples where *in vivo* tissue regeneration is achieved by use of cell scaffolds or the combination with cells[2].

As described above, if the tissue to be repaired has a high activity toward regeneration, active and immature cells from the surrounding healthy tissue infiltrate the matrix of biodegradable scaffold implanted resulting in formation of a new tissue. However, additional means are required if the regeneration potential of tissue is very low, because of, for instance, low concentration of cells and biosignaling molecules like growth factors responsible for new tissue generation. The simplest method is to supply a growth factor to the site of regeneration for cell differentiation and proliferation in a controllable fashion. As described above, it is undoubtedly necessary for the induction of tissue regeneration with a growth factor of *in vivo* instability to make use of DDS technology, for example a controlled release system of the factor. Recent research on tissue regeneration through combination of growth factors with the DDS carriers has indicated that a carrier is absolutely necessary to allow growth factor to exert the biological activity for *in vivo* tissue regeneration. Although such significance of DDS in tissue regeneration is claimed, the controlled release of growth factor for tissue regeneration has not been studied extensively. In place of growth factor protein itself, recently the gene encoding the growth factor has been applied to promote tissue regeneration [3]. For tissue engineering with gene, there are two future directions of research and clinical therapy. The first is conventional gene therapy by using plasmid DNA and viruses. The plasmid DNA solution is directly injected into the body. The secretion of protein expressed by the plasmid DNA around the injected site is expected to be achieved in this protein-based disease therapy. However, to improve the efficacy of gene transfection and the consequent gene expression, DDS technologies for plasmid DNA are needed. The angiogenesis[4] and bone tissue regeneration[3] have been attempted by use of the corresponding growth factor genes. If the gene injected is transfected into cells existing in the site of regeneration, the cells will secrete growth factor for a certain time period, resulting in promoted tissue regeneration. Basically this approach is also one of protein therapies which can be achieved by gene-transfected cells. The second direction is to genetically activate cells for enhanced efficacy of cell therapy. Stem cells are sometimes not powerful for cell therapy. As one trial to activate the stem cells, it will be a promising way to genetically engineer cells for biological activation. A DDS technology or methodology assists to develop a system of non-viral gene transfection at the efficiency as high as that of viral system[5].

Successful tissue engineering by conttrolled release technology

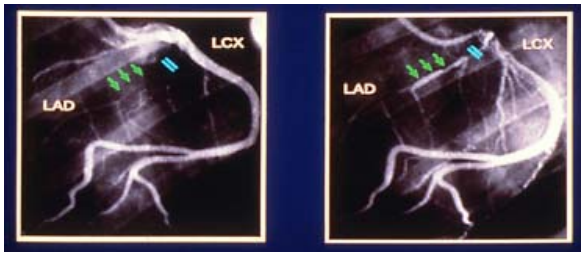
We have succeeded in inducing the regeneration of various tissues and organs by the controlled release of various growth factors with the biological activities remaining as shown in Table 1. This hydrogel system permits the controlled release of plasmid DNAs. The controlled release technology enabled a plasmid DNA and small interference RNA (siRNA) to enhance the level of gene expression and prolong the time period of gene expression[6-7]. In addition, the hydrogel system can release not only one type of growth factor, but also two or multi-types of growth factor at the same time or in a time-order fashion. Upon applying a hydrogel incorporating low doses of either bFGF or TGF-β1 to a bone defect of rabbit skulls, no bone was regenerated at the defect. However, a synergistic effect on bone regeneration and angiogenesis was observed by the simultaneous release of two factors[10].

bFGF was originally characterized *in vitro* as a growth factor for fibroblasts and capillary endothelial cells and *in vivo* as a potent mitogen and chemoattractant for a wide range of cells. In addition, bFGF is reported to have a variety of biological activities[11] and is found to be effective in enhancing wound healing through induction of angiogenesis as well as regeneration of various tissues, such as bone, cartilage, and nerve. Recently, bFGF of human type in a solution form has been on the Japanese market for remedy of decubitus, a chronic ulcer of the skin caused by prolonged pressure on it, from Kaken Pharmaceutical Co. Ltd., Tokyo (product name: Fibrast® spray). When a gelatin hydrogel incorporating bFGF was subcutaneously implanted into the mouse back, significant angiogenic effect was observed around the implanted site, in marked contrast to controls injected with bFGF solution or higher doses or the site implanted with bFGF-free, empty gelatin hydrogel[12].The controlled release of growth factors other than bFGF has been achieved with biodegradable hydrogels to realize the regeneration of various tissues (Table. 1).

There are two important objectives of angiogenesis in tissue engineering, the therapy of ischemic disease and in advance angiogenesis for cell transplantation. As the former example, when injected into the ischemic site of myocardial infarction[13] or leg ischemia[14], gelatin microspheres incorporating bFGF induced angiogenesis in a level that is therapeutically acceptable (Fig. 2). This angiogenic therapy for leg ischemia has been permitted by the ethics committee of university and a clinical study has been started in different hospitals. The bFGF-induced angiogenic therapy has shown the good results.

It is no doubt that sufficient supply of nutrients and oxygen to cells transplanted into the body is indispensable for cell survival and the maintenance of biological functions. For successful cell transplantation, it is practically promising to induce in advance

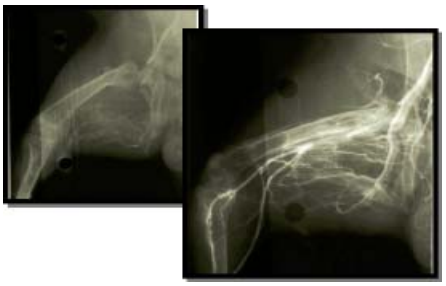
Regeneration of coronary artery



bFGF solution

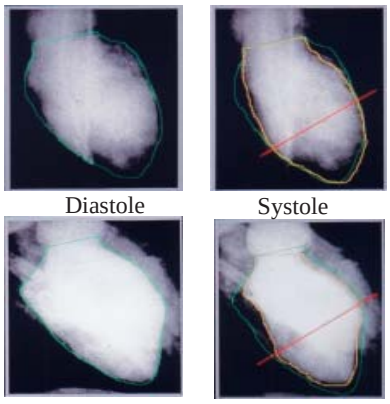
Gelatin microspheres incorporating bFGF

Regeneration of blood vessels in ischemic leg (angiogenesis)

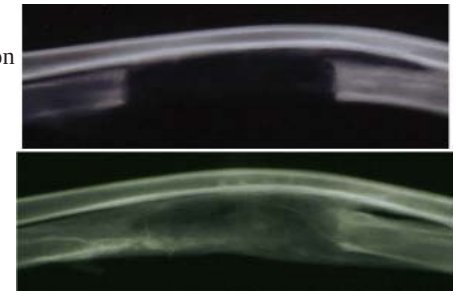


Gelatin microspheres in corporating bFGF

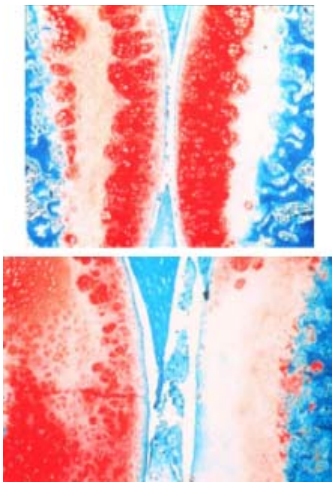
Recovery of heart muscle motion



Regeneration of long bone



Regeneration of articular cartilage to treat osteoarthritis



Gelatin hydrogel microspheres incorporating CTGF

Promotion of hair shaft elongation

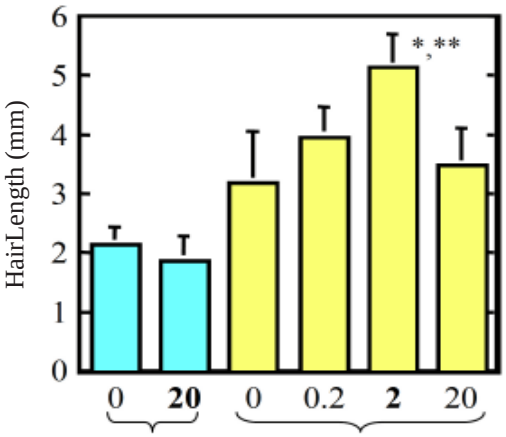


Fig. 2 Controlled release technology of growth factors to realize the regeneration induction of various tissues.

angiogenesis throughout the site where cells are transplanted, by using the release system of bFGF. This technology of in advance angiogenesis efficiently improved the biological functions of pancreatic islets[15], cardiomyocytes[16], and kidney cells transplanted[17] as well as enhanced the engrafting rate of bio-artificial dermis-epidermis skin-tissue construct. We have succeeded in improving the cardiac functions of ischemic rat hearts by combination of cardiomyoblasts implantation with in advance angiogenesis induced by gelatin microspheres incorporating bFGF. These findings clearly indicate that the prior induction of angiogenesis at the transplanted site was effective in successfully engrafting cells transplanted and tissue construct grafted. The release system enabled bFGF, TGF-β1, and BMP-2 to enhance their activity of bone renegeration as well as bone regeneration

induced by mesenchymal stem cells of bone marrow[18]. For the grafting surgery of heart, the bilateral sternum artery is normally used because of the high potency. However, in spite of successful graft surgery, the sternum repairing is often delayed, much worse the infection at the resection area of sternum often takes place, while wound healing of the surrounding soft tissue is also poor due to surgical elimination of their nutrient artery. As one trial to tackle the issue, we have applied the bFGF release system to this surgical therapy because bFGF has an inherent potential to induce bone regeneration as well as angiogenesis. A hydrogel sheet incorporating bFGF was applied to the soft tissue around the sternum of diabetic rats of which sternum was cut and the bilateral arteries were ligated. As expected, bone regeneration at the cut line of sternum was achieved together with enhanced angiogenesis and the recovery of blood flow at the surrounding soft tissue[19]. This bFGF-induced simultaneous regeneration of bone and the surrounding blood vessels was also observed in a clinical study. De novo adipogenesis was succeeded by the preadipocytes isolated from human fat tissues, gelatin microspheres incorporating bFGF, and a collagen sponge of cell scaffold [20]. Appropriate combination of all the three materials are needed to induce this adipogenesis.

We have found that a plasmid DNA could be released from a biodegradable hydrogel of cationized gelatin derivative to enhance the level of gene expression as well as prolong the time period expressed [6-7]. When intramuscularly injected into the ischemic leg of rats, the cationized gelatin microspheres incorporating a plasmid DNA of FGF-4 induced angiogenesis to a significantly higher extent than the plasmid DNA solution even at the dose 100 or 1000 times less than that of solution type[21]. The microspheres incorporating plasmid DNA was effective in genetically activating cells and consequently enhancing the efficacy of cell therapy. Cationized microspheres incorporating the plasmid DNA of adrenomedulin were prepared to allow them to internalize into endothelial progenitor cells. Intracellular controlled release of plasmid DNA enhanced the efficiency of gene transfection to the level higher than that of adenovirus transfection. The cells genetically engineered also cells functioned well to achieve higher therapeutic efficacy[5].

Tissue engineering of internal medicine based on DDS technology

Presently, there is no effective therapy for chronic fibrosis diseases, such as lung fibrosis, cirrhosis, dilated cardiomyopathy, and chronic nephritis. For these diseases, the injured site of tissue and organ is normally occupied with fibrous tissue of excessive collagen fibers and fibroblasts. It is highly possible that this tissue ingrowth occupation causes impairment of natural healing process at the disease site. Therefore, if the fibrosis can be digested by any method to loosen or disappear, it is highly expected that the disease site is repaired based on the natural regeneration potential of the surrounding healthy tissue. It has been demonstrated that the injection of virus encoding a matrix metaloprotease (MMP) enzyme suppresses the tissue fibrosis to promote healing[22]. The finding strongly suggests that when collagen in the fibrous tissue is enzymatically digested, fibrosis is naturally improved or repaired due to the body potential to induce tissue regeneration which is naturally equipped in the surrounding healthy tissue. It is a new direction of tissue engineering that is called this regeneration therapy for chronic fibrosis diseases based on the natural potential of regeneration induction. This is defined as tissue engineering of internal medicine (Fig. 3), because disease therapy induced by tissue regeneration potential is achieved by the drug treatment of internal medicine. We have demonstrated that the controlled release of a MMP-1 plasmid DNA at the medulla of chronic renal sclerosis induced the histological regeneration of kidney structure, in contrast to the plasmid DNA solution[23]. When gelatin microspheres incorporating hepatocyte growth factor (HGF) was intraperitoneally injected into rats with liver cirrhosis, the liver fibrosis was histologically cured[24]. However, the injection of HGF solution was not effective at all and the tissue appearance was similar to that of un-treated controlled group.

Necessity of tissue engineering technology in future regeneration therapy

Without using precursor and stem cells with high proliferation and differentiation potentials, presently, it has been possible to induce tissue regeneration only by using the controlled release system of biological active growth factors. Depending on the type of target tissue or organ and the site, it is necessary to make use of cells, their scaffold, growth factor, and the barrier membrane or their appropriate combinations. For the therapeutic approach of tissue engineering with growth factor, it is no doubt that the DDS technology or methodology is useful and will be indispensable in future. From the viewpoint of disease therapy based on the natural healing potential of patients themselves, two approaches of tissue engineering in the surgical and internal medicine will be extensively carried out in future.

If a key growth factor is supplied to the target site at a right time over an appropriate period of time and at a right concentration, we believe that the living body system will naturally direct toward the process of tissue regeneration. Once the right direction is given, it is highly possible that the intact biological system of the body starts to physiologically function, resulting in natural achievement of tissue regeneration. There is no doubt that whenever growth factors and genes are used *in vivo*, their combination with DDS technology is essential. However, the present technology of controlled release doe s not always regulate accurately the amount and time period of growth factor release. It is practically impossible, however, to artificially control the process of cell differentiation only by the release technology of growth factors currently available, since the differentiation process is regulated

by the complicated network of growth factor in the restricted time, site, or concentration manner.

Regenerative medical therapy, which is a new therapeutic trial based on the promoted potential to induce tissue regeneration with cells and tissue engineering, is the third therapy following reconstructive surgery and organ transplantation. To achieve the therapy of regenerative medicine by use of tissue engineering technology and methodology, substantial collaborative research between material, pharmaceutical, biological, and clinical scientists is needed. Even though superior stem cells with high potential of proliferation and differentiation can be obtained to use by development of basic biology and medicine of cells, it is impossible to directly apply the cells and the related scientific results to medical therapies for patients (regeneration therapy) unless an environment suitable for cell proliferation and differentiation is created and efficiently combined with the cells to use. However, one of the large problems is the absolute shortage of biomaterial researchers of tissue engineering, such as scaffolding and DDS especially release technology, aiming at tissue regeneration and the biological substitution of organ functions. Such researchers must posses knowledge in medical, dental, biological, and pharmacological fields, in addition to material sciences. It is indispensable to educate the researchers of interdisciplinary field who have engineering background and can also understand basic biology and medicine or clinical medicine necessary for research and development of tissue engineering. One of the representative interdisciplinary research fields is DDS. The DDS technology is also applicable to create the non-viral vectors to prepare genetically-engineered cells for regenerative medicine. Research and development of non-viral vectors with a high efficiency of gene transfection for stem cells are highly required. Tissue engineering technology is not only used surgically to the tissue defect for regeneration induction therapu, but also applied to newly develop a therapeutic method for chronic fibrosis diseases by making use of methodology of internal medicine.

As tissue engineering is still in its infancy, it will take a long time to become well established although a part of the research projects has already come close to the stage of clinical applications. Increasing significance of drug delivery in future will further help progress of tissue engineering. We will be happy if this short review stimulates readers' interest in the idea and research field of tissue engineering to assist understanding of the importance of release technology in tissue engineering.

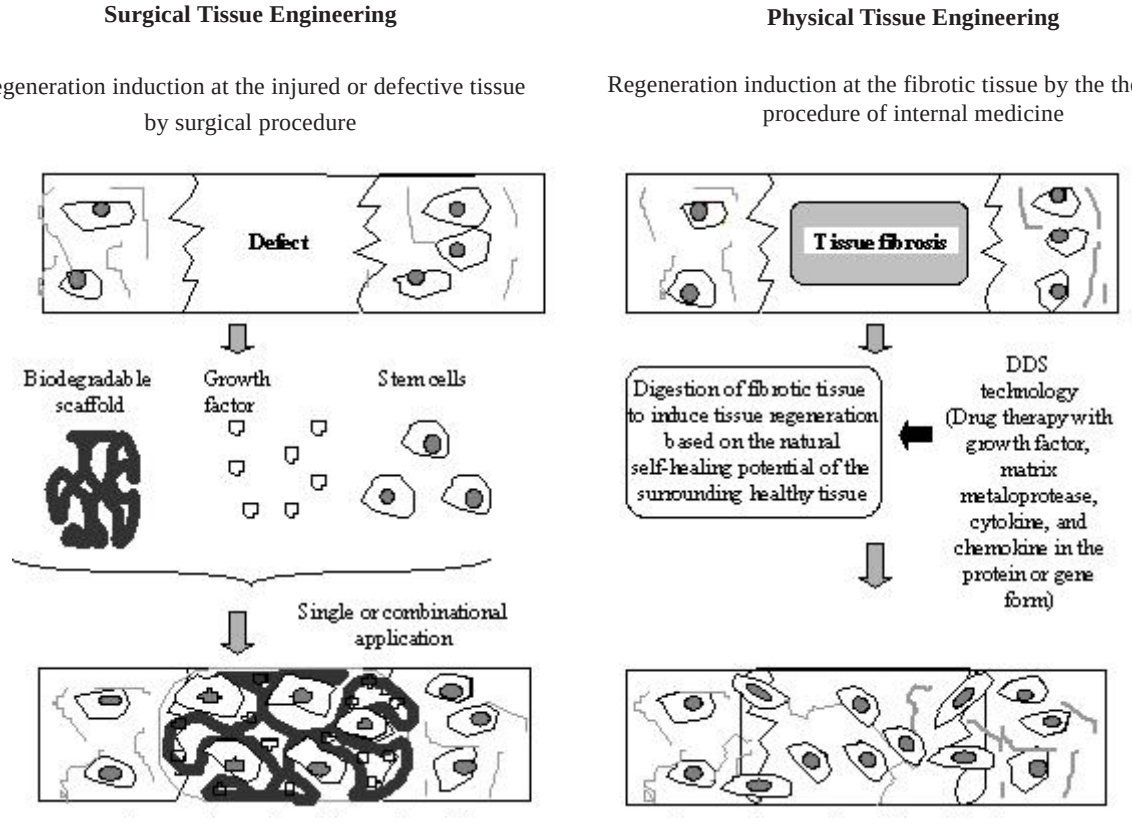


Fig. 3 The Concept of surgical tissue engineering and physical tissue engineering of internal medicine.

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