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Three-Dimensional Nanofiber Scaffolds for Regenerative Medicine

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1. Introduction

Tissue engineering is an interdisciplinary technology of the basic concept of bioscience and biotechnology. It is aiming to make tissues that can replace or regenerate diseased tissues and organs after understanding of correlation between the structure and function of normal biological tissues. The regenerated tissues made in such way are improving, reviving, restoring, or substituting the functions of human body as well as maintaining the functions after transplanted into human body.[1]

The three elements of tissue engineering for regenerating the biological tissues are cell, growth factor, and scaffold. When the number of cells has been reduced due to some troubles of cell proliferation in the damaged tissue, cell can regenerate the damaged area by insertion of external cells, and the growth factor controls the growth and differentiation of cells. Scaffold is used to assist the growth and proliferation of cells. Scaffold helps cells growing and functioning in normal condition. If scaffold does not play its role properly, the replace or regenerate diseased tissues and organs must not success. Recent researches, therefore, have been focused on the development of scaffold that is influencing the proliferation and differentiation of cells.

Scaffold must have, particularly, the similar form of extracellular matrix that supports cells, which should have the following characteristics.[1,2] (1) Scaffold must connect tissue and blood vessel with each other by the appropriate size pores. (2) Scaffold must be able to adjust the biodegradability and bioabsorbability. (3) Scaffold must have the chemical surface where cells can achieve adherence, differentiation, and proliferation. (4) Scaffold must not induce other reverse functions or side effects. (5) Scaffold must be formed in various shapes and sizes, and it must be easy for the penetration or manipulation of various materials inside the scaffold.

Nanofiber is getting noticed the most intensively among the scaffolds with above characteristics (**Fig.1**).[3] Nanofiber is expected to overcome the limitation of conventional materials. So, it will be adopted in the new field with lots of advantages of high surface area per unit volume, high porosity, numerous fibers in the unit area, micro space created between fibers, and its flexibility. Nanofiber can be produced through phase separation, self-assembly method, electrospinning method, etc. Nanofiber can be used in various biomedical application such as high-functional filter and wound healing material, reinforced fiber of composite biomaterials, and scaffold for tissue engineering. Among them, the nanofiber as a scaffold for

tissue engineering can provide similar environment as collagen of the extracellular matrix (ECM). It is advantageous for the cell adhesion when cultivating the cells.
This chapter focuses on three-dimensional nanofiber scaffolds, and highlights their potential applications for regenerative medicine. The first section reviews the fabrication of nanofiber scaffolds that provide an optimal microenvironment for cell proliferation, migration, differentiation, and guidance for the reconstruction or replacement of damaged or diseased tissues and organs. The following section focuses on natural and synthetic biodegradable biomaterials that have been applied as nanofiber scaffolds. The last section focuses on the preclinical applications of nanofiber scaffolds in regenerative medicine.

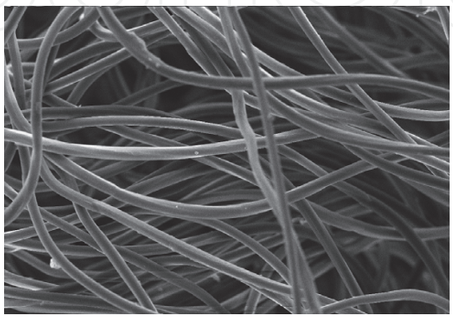

Fig. 1. Scanning electron micrograph (SEM) image of nanofibers.

2. Nanofiber manufacturing technology

Nanofiber manufacturing technology can be largely divided into 3 technologies of phase separation, self-assembly method, and electrospinning method. The nanofiber manufactured by phase separation and self-assembly method shows the limitation as a scaffold for the applications for tissue engineering. On the other hand, the nanofiber manufactured by electrospinning method shows various characteristics, which are suitable for the tissue engineering. This section introduces the nanofiber productions by phase separation, self-assembly method, and electrospinning method.

2.1 Phase separation

Phase separation is the porous polymer membrane forming technique used for years. Phase separation can control the pore structure of nanofiber by using two or more materials of different physical characteristics, and porous fiber is obtained when using polymer and highly volatile solvent. So, pore size can be changed by control of the volatility of solvent. Also it is possible to manufacture the nanofiber of which hydrophilic property has been adjusted, as pore structure can be changed by the interaction of solvent and water molecules in the air. However, there happens rapid phase separation between solvent and solute, when using the volatile solvent, due to the radical solidification of polymer with the volatilization of solvent. So it is not easy to control the concentration of polymer solution. It also has a problem that mass production of nanofiber is difficult as it can be applied only for limited numbers of polymer.

2.2 Self-assembly method

Self-assembly means that each component forms orderly structure voluntarily by the noncovalent bond. The universal method to make nanofiber is synthesizing the Peptide

Amphiphile (PA). When attaching PAs consisted with dialkyl chain (tail part of hydrophobicity) to the N-α amino group in the end of peptide chain, the peptides become similar to the base sequence of collagen amino acid of human ECM. However, self-assembly method is limited only for several polymer arrays (two block copolymers, three block copolymers, peptides-amphiphilic three block copolymers, and dendrimers). Another problem is that its mass production is not easy because of complicated manufacturing process and low productivity.

2.3 Electrospinning method

Electrospinning had been proposed in 1930s together with electrospraying method. But it has not been commercialized since its development as its application was limited (**Fig.2**). Electrospinning method gets spotlighted again later in mid of 1990s when Reneker succeeded in the manufacturing of nanofiber with various polymers after simplifying the electrospinning device.[4] The porosity, thickness, and components of nanofiber can be adjusted with simple experimental equipment in the electrospinning method. So it has been able to produce continuous nanofiber not only with polymer but also with ceramics at low process cost. It can produce nonwoven fabric type nanofiber at the same time of spinning, so its spinning time is short and its construction is simpler than general spinning facilities. Also, nanofiber can be produced with various polymers, and the spinning is available with just a little amount of polymer. The biggest feature of electrospinning method is that its nanofiber has the similar structure of ECM in terms of morphology. ECM forming collagen is consisted with micro fibril of 50~100 nm, and the ECM-similar nanofiber can be produced by using the electrospinning method.

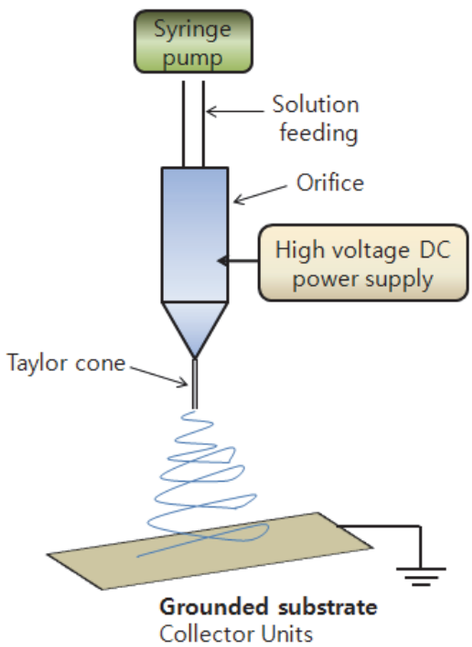

Fig. 2. Schematic image of the electrospinning method.

Reviewing the electrospinning principle, polymer fine bubbles are forming the taylor cone by the mutual repulsion of induced electric charges when applying high electric field to the polymer solution hanging on the end of a charged jet. Then, polymer solution is emitted when electric repulsion is higher than surface tension. Solvent is volatilized while emitted solution is in the air, then fibers of 50~100 nm diameter are laminated in 3-dimensional network structure to form the mesh structure. The nanofiber made in this way has high ratio of surface area compared to its volume, and it has high porosity to constitute favorable environment for cells to live in. As complex polymers can be used to produce nanofiber, it has been possible to manufacture the nanofiber that has overcome the limit of properties of existing polymers.

3. Nanofiber manufacturing materials

The scaffold is transplanted into the living body for treatment, so it is necessary to pay a lot of attention in selecting the materials. Both natural polymer and synthetic polymer have been used for treatment as the highly absorbing materials for over 30 years. It is important in recent tissue engineering to make scaffold that decomposes easily in the body without intermediate products or other side effects. Natural polymer is frail in its property but it is superior in the cell affinity and cell compatibility. Synthetic polymer can adjust the property and degradation period easily, but it is less biocompatible as there are no molecules to which cells can adhere. This section will describe the characteristics of each material applied for the production of nanofiber.

3.1 Natural biomaterials

As natural polymer has similar structure to the macromolecules in the body, it is used a lot as the biomaterial. For the production of nanofiber, chitosan, alginate, and elastin as well as collagen are used representatively (**Fig.3**).

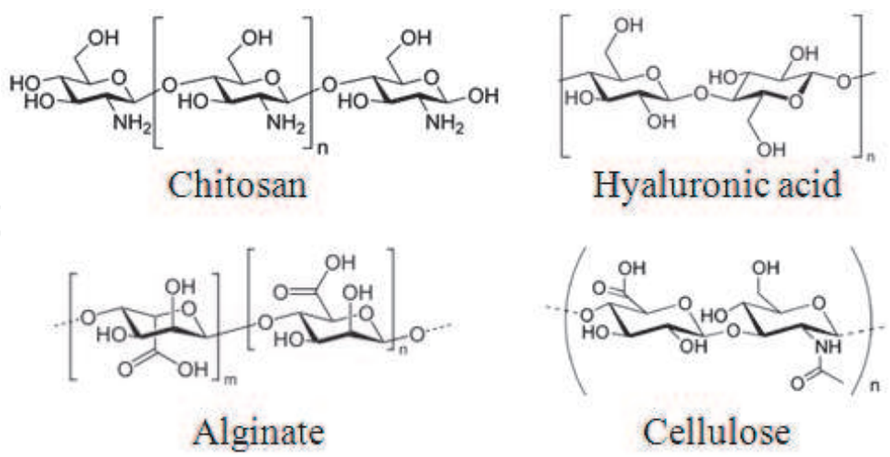

Fig. 3. Structures of natural biomaterials.

Collagen

Collagen occupies 20~30% of total protein in vertebrate animals, which is a main component of ECM. At least no less than 22 kinds of collagens are existing in the human body to keep up the tissues or organs and to maintain the figure of human body. Collagen has higher cell

affinity and less immune reaction, moreover, as it contains lots of chemical inducers which are related to the adhesion, proliferation, and differentiation of cells. Collagen is also reported to have effectiveness in improving the physiology such as cell proliferation and organ formation, promotion of wound healing, and so on. It is disclosed that collagen nanofiber is inducing the biological tissue switching effect of nanocomposites in the connective tissues, accordingly, its applications are getting wider.

Chitosan

Chitosan, a naturally abundant antibacterial polymer, is found in the microbe cell walls and in the exoskeleton of crustaceans. Chitosan is manufactured by removing acetyl out of chitin, a kind of polysaccharide. The chitosan based nanofiber production is under research as it has less stimulation, excellent biocompatibility and biodegradability, and blood coagulating function. Also the chitosan nanofiber is being researched as antibacterial wound dressings in the tissue engineering.

Alginate

Alginate is one of natural polymers, which is safe without toxicity. Alginate is produced in fiber form and processed in the forms of woven fabric, nonwoven fabric, and composite material for the treatment of wound area. Calcium ion is exchanged with sodium ion in the body fluids, when alginate nanofiber contacts with wound exudation, so it is used widely for the absorbent wound dressing material. As alginate is an electrolyte of very high conductivity and high viscosity, however, it is not easy for electrospinning. So, it must be electrospinning processed after mixing with water-soluble substances.

Elastin

Elastin is a major component of elastic tissues such as blood vessel, lungs, ligament, and skin, which plays an important role to maintain the elasticity of tissues. Elastin is being proposed now as elastin nanofiber in 3-dimensional structure to produce excellent bioabsorbability, although it had not been easy to form and process elastin due to its stiff cross-linked structure.

3.2 Synthetic biomaterials

Synthetic polymer is being developed for over 30 years in various forms of surgical suture or screw form, mesh structure, etc. Synthetic polymer is manufactured according to the unique characteristics of each material, and it is being produced to minimize the immune reactions in the tissue engineering (**Fig.4**). As the biodegradation period of nanofiber made of synthetic polymer can be adjusted, its commercialization is being progressed partially as a tissue engineering scaffold (**Table 1**).

Materials	Product names	Degradation period
Polycaprolactone (PCL)	MONOCRYL	> 20 months
Poly L-lactic acid (PLLA)	BioScrew, PL-FIX	20~60 months
Polydioxanone (PDO)	PDS BIOSYN	6 months
Polyglycolic acid (PGA)	DEXON	1~4 months
Poly (lactic-co-glycolic acid) (PLGA)	VICRYL (90% Glycolide 10% Lactide)	2 months

Table 1. Degradable periods of synthetic polymer.[5]

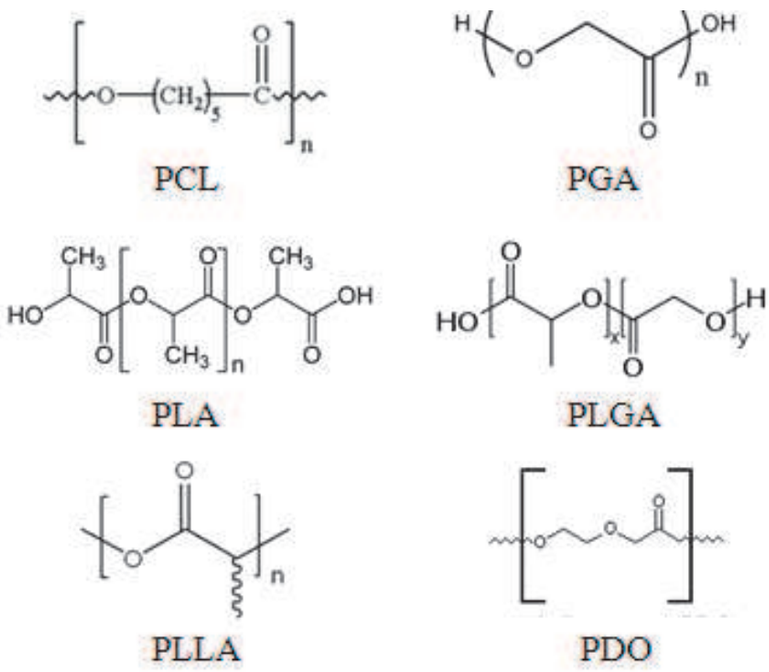

Fig. 4. Structures of synthetic biomaterials.

Polycaprolactone (PCL)

PCL is a polyester polymer of high elasticity without toxicity, which is superior in biocompatibility but its degradation period is rather slow for 1~2 years. It is aliphatic polyester with repeating 5 nonpolar methylene groups and 1 polar ester group, which is hydrophobic as of numerous carbons in its structure. It has disadvantages that initial protein absorption capacity is low, as the surface of PCL nanofiber is hydrophobic, the cell adhesion is slow, and the cell differentiation and tissue regeneration is slow. But, it is used as a good absorbent material for either soft or hard tissues as its properties can be adjusted.

Polyglycolic acid (PGA)

PGA is biodegradable aliphatic polyester, which had been developed for surgical suture in 1970s. Its biological absorptiveness can be predicted, and it takes 2~4 weeks for degradation as it is hydrophilic in the body. Its strength is reduced by 60%, for the first 2 weeks, also the pH and crystallinity are lowered down due to the generation of hydrolysis. The PGA nanofiber has high strength and elasticity initially, which is biodegraded rapidly by the diffusion of water and hydrolysis at the body temperature. However, it may cause undesirable reactions in the tissue as the pH of topical area is increasing rapidly while

degradation speed is getting faster. To solve such a problem, researches are progressing for better biocompatibility by improving cell's adhesion capability. The PGA nanofiber is pretreated with acid in order to hydrolyze the ester bond and to expose the carboxylic acid and alcohol base.

Polylactic acid (PLA)

PLA is a synthetic polymer with steric hindrance, more hydrophobic than PGA, as it contains methyl base. It is highly soluble in the organic solvents and slow in hydrolysis, and its degradation period is as long as 30~50 weeks. There are L-type and D-type stereoisomers in PLA. PLLA (Poly L-lactic acid), which is an L-type stereoisomer, has a merit of excellent mechanical property in the polymerized form of lactic acid that is synthesized in the living body. PLLA nanofiber has problems as it is hydrophobic, fragile, and slow in degradation speed. But it has been proved, as a scaffold imitating collagen, to be highly capable in the cell adhesion and differentiation.

Poly (lactic-co-glycolic acid) (PLGA)

PLGA, which is a copolymer of polyglycolide and polylactide, is a synthetic polymer approved by FDA. It is widely used as the biomaterial for porous scaffold, drug delivery system, etc. in tissue engineering. PLGA is harmless to human body as it is changed to lactic acid and glycolic acid by hydrolysis in the living body and changed to carbon dioxide and water when discharging out of the body. As it is highly biocompatible, biodegradable, and processable, it is used widely in tissue engineering and drug delivery system. PLGA is a synthetic polymer with various degradation periods when the amount of monomers is adjusted. The PLGA nanofiber is being applied the most widely as a scaffold imitating biological tissues or drug delivery system, and biosensors. Especially, PLGA is spreading its applications after controlling its properties by adjusting the amount of PLA and PGA.

Polydioxanone (PDO)

PDO is biodegradable polyester of 55% crystallinity, which has been developed originally as a biodegradable surgical suture. Its degradation speed in the body is 6 months, intermediate period between PGA and PLA. PDO is superior in flexibility as it contains ester oxygen in the chains of monomer. PDO nanofiber is suitable for the biomaterial as it carries the property between minimum elasticity coefficient of collagen and maximum elasticity coefficient of elastin.

4. Nanofiber applications for tissue engineering

For the applications of nanofiber scaffold to the human body, it must provide processability and appropriate conditions for adhesion, proliferation, and differentiation of cells in the tissue as well as the properties of conventional scaffolds. As the nanofiber satisfying such requisites is widely applied to the artificial tissues such as skin, blood vessel, bone, etc., wound dressings, and drug delivery system, this section is going to describe the applications of nanofiber.

4.1 Artificial skin

Skin is the biggest tissue that is covering the surface of human body. It prevents loss of moisture, adjusts the body temperature, blocks bacterial invasion, and protects human body from radiant rays and ultraviolet rays. Skin grafting technology has been developed to

regenerate the skin tissue when it had been damaged by a burn, external wound, carcinoma resection, skin disease, etc. It is proved that nanofiber is greatly effective as a tegaderm in the adhesion and proliferation of human skin cells, and it has been reported that nanofiber can be used as an artificial skin.

4.2 Artificial blood vessel

Scaffold of excellent biocompatibility must be used in the vascular grafting, and it must satisfy several specific requirements such as mechanical elasticity and durability that can put up with repeated inflations and compressions. The nanofiber produced by electrospinning can imitate similarly the component, structure, and mechanical characteristics of blood vessel. It is reported that the adhesion, proliferation, and differentiation of cells have been improved when cultivating the nonstriated muscle cells with the scaffold made of copolymer P(LLA-CL) of PLLA and PCL. Its result has shown that P(LLA-CL) nanofiber is potential to be an ideal artificial blood vessel.

4.3 Artificial bone

In order to regenerate the bone in tissue engineering, the human regeneration mechanism promoting the bone formation and restoration should be increased or the tissue similar to the bone in living body must be developed. For the past several decades, autograft or allograft has been used for the damaged bones by disease or traumatic injury. However, there was limitation in such bone transplantations. So bioactive materials such as hydroxyapatite or tricalcium phosphate have been used for its substitution, or glass and ceramic have been used in dentistry or in orthopedics as alternative bones. Bioactivity, tissue integration, and mechanical strength are requested for such materials. Various nanofiber scaffolds have been developed recently as bone substitutions to fulfill such requirements. Ramakrishna et al. has made the scaffold by electrospinning of collagen, which has similar structure of extracellular matrix, and hydroxyapatite at the mixing ratio of 1:1. It is proved, when osteoblast was cultured on the nanofiber that had been electrospinning processed with hydroxyapatite, that it has been mineralized as of high differentiation of cells and high concentration of calcium and phosphorus.

4.4 Artificial cartilage

Peculiarly, there is no blood vessel, nerves and lymphoid tissue in the articular cartilage. There is no inflammation reaction when damaged, therefore, and it is difficult to supplement the cells to recover the damage. So, it is highly limited to recover or regenerate the articular cartilage when it has been damaged. Nanofiber is applied to make hyaline articular cartilage tissue, therefore, by cultivating articular cartilage cells or adipose-derived stem cells in the appropriate 3-dimensional scaffold. Nanofiber scaffold promotes the generation of ECM by the transplanted cells. The nanofiber is also able to adjust the chondrogenesis of human cartilage cells and that nanofiber can be applied widely in the chondral resurfacing by adjusting the shape and size of nanofiber.

4.5 Wound dressing

Dermis is exposed as epithelial tissue is peeled off in the affected part of skin tissue by wound such as external injury, burns, diabetes, and clogging of venous blood flow. Wound dressing is the first-aid kit to stop bleeding, which promotes the injury protection as blood

or serum out of the wound is penetrating the wound dressings. If the wound dressing is constituted with nanofiber of micro diameter, the body fluids such as blood cannot penetrate the nanofiber, and so blood flow stays in the wound and coagulated. As there is no blood coagulation in the nanofiber, therefore, it is easy to remove the nanofiber. Also it is easier to exchange the oxygen and evaporate the water-vapor between the wound surface and air when porosity is higher. As nanofiber is a mat of very tiny diameter and pores with high specific surface area, its moisturization and breathability is good enough, it protects wound from germs and prevents body fluids from penetrating, and it is easy to remove. So its application for various wound dressings is also being reviewed actively.

4.6 Drug delivery system

Drug delivery system is being developed to reduce the number of medications, to enhance the drug effectiveness and stability by adjusting the initial emissions through continuous emission of constant amount of drug to the medicating area. Numbers of researches are reported recently for using nanofiber produced with biodegradable polymers in the drug delivery systems. The drug delivery system made of nanofiber carries out the drug delivery function with fewer side effects by installing physical barriers using its wide surface area. Also the fibrosis of biocompatible polymers is easy and it does not need to raise the temperature when electrospinning. It is advantageous as drug is not decomposed in the nanofiber.

5. Conclusion and prospect

Nanofiber made of biomaterials in various methods is getting noticed for its very wide application potential as a scaffold imitating the ECM. Nanofiber shows significant effect in the adhesion, proliferation, and differentiation of cells as of its wide surface area and high porosity. Moreover, nanofiber is researched for the scaffold for the musculoskeletal tissues such as bone, spine, ligament, and skeletal muscle and for the tissues of skin, nerves, and blood vessel. It is also expanding its applications up to adjusting the delivery of drug, protein, and DNA. It is expected obviously that nanofiber will be used as an important scaffold in tissue engineering based on these researches. However, researches must be accompanied simultaneously for productivity and safety of nanofiber and securing application safety. It is also for easy biological application of nanofiber and for assessment of long-term regeneration capability in long-term view. Nevertheless, interdisciplinary researches of the experts in the fields of nanotechnology, biomaterials, medicines, and clinics are currently progressing so as to apply nanofiber clinically in the near future.

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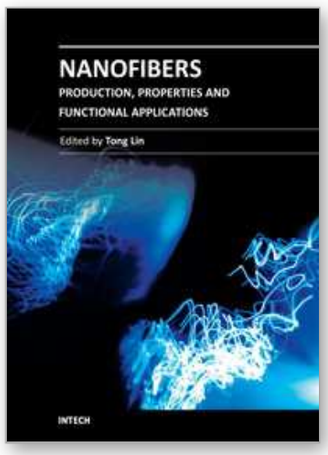

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As an important one-dimensional nanomaterial, nanofibers have extremely high specific surface area because of their small diameters, and nanofiber membranes are highly porous with excellent pore interconnectivity. These unique characteristics plus the functionalities from the materials themselves impart nanofibers with a number of novel properties for advanced applications. This book is a compilation of contributions made by experts who specialize in nanofibers. It provides an up-to-date coverage of in nanofiber preparation, properties and functional applications. I am deeply appreciative of all the authors and have no doubt that their contribution will be a useful resource for anyone associated with the discipline of nanofibers.

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