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Nanofibres for blood vessel tissue engineering: A review

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ABSTRACT

Currently, there are three techniques available for the synthesis of nanofibres: electrospinning, self-assembly, and phase separation. Of these, electrospinning is the most widely studied technique and also seems to exhibit the most promising results for tissue engineering applications. Nanofibres synthesized by self-assembly and phase separation have had relatively limited studies that explored their application as scaffolds for tissue engineering. Electrospinning is an enabling technology that can architecturally and biochemically fabricate engineered cellular scaffolds that mimic the native extracellular matrix (ECM). This is especially important as it forms one of the essential paradigms in the area of tissue engineering. While biomimesis of the physical dimensions of native ECM's major constituents (example collagen) is no longer a fabrication- related challenge in tissue engineering research, conveying bioactivity to nanofibrous structures will determine the efficiency of utilizing nanofibres for regenerating biologically functional tissues. This article gives a brief overview on the current development and application status of employing nanofibres for constructing biomimetic and bioactive tissue scaffolds. This review details the use of nanofibres to produce scaffolds that would promote vascular tissue growth through surface modification by both chemical and physical methods. It also entails the scaffold cell interaction and cell harvesting and seeding techniques.

KEYWORDS

Electrospinning;
Phase separation;
Self-assembly;
Nanofibre;
Extracellular matrix (ECM);
Scaffold.

INTRODUCTION

Blood vessels have different sizes, mechanical and biochemical properties, cellular content, and ultra-structural organization depending on their location and specific function. It is necessary to control the fabrication of vascular grafts for obtaining desirable characteristics of blood vessel substitutes. Vascular grafts are needed in many types of surgery, especially to maintain blood flow to certain areas. In some surgeries like coronary bypass, an autologous saphenous vein or an internal mammary artery is harvested to replace the occluded blood vessel. Harvesting these may harm the patient, so it is better to develop an artificial blood vessel as a replacement. In development of an artificial blood vessel, the newly created vessel must be able to mimic the

capabilities of a real blood vessel. It should have a similar extracellular matrix (ECM) to allow cellular proliferation and vessel regeneration, mimic the mechanical properties of a blood vessel containing collagen and elastin, contain an inner layer that prevents occlusions from occurring, and to degrade for the development of the new blood vessel[1-5].

Blood vessel scaffolds can be created using electrospinning technique. Scaffold designing using electrospinning allows the use of creating similar dimensions of the ECM along with fibre orientation. Nanofibrous scaffold could therefore provide environmental or physical cues to the cells and promote cell growth and function well towards the synthesis of genuine extracellular matrices over time.

NATURAL VASCULAR STRUCTURE

Currently there is research on tissue engineered small-diameter vascular grafts which focuses more on the development of native blood vessel-like tubes (or conduits)[6,7]. The development of biomimetic vascular grafts relies on the understanding of the anatomical structure and the biological function of blood vessels[8]. Normal blood vessels, except capillaries, have tri- lamellar structures, with each layer having specific functional properties. The intima (tunica intima) contains the endothelium, which is a single layer of ECs functioning to prevent spontaneous blood coagulation[9,10].

As an interface between dynamic blood flow and static blood vessel wall, ECs are directly exposed to flow and the associated shear stress and blood pressure, which make ECs elongate in response to flow and orient their major axis with the direction of flow[11]. ECs attach to a sub endothelial layer which is a connective tissue bed, called the basement membrane (BM). This is adjacent to the internal elastic lamina which is a band of elastic fibres, found most prominently in larger arteries. The media layer (tunica media) is composed of smooth muscle cells (SMCs) and variable amount of connective tissues such as collagen, elastin, and proteoglycans. Specially, SMCs and collagen fibres have a marked circumferential orientation to withstand the higher pressures in the blood circulation, as well as their abilities to contract or relax in response to external stimulus. The adventitia layer (tunica adventitia) is composed primarily of fibroblasts and loose connective tissue fibres. In arteries with diameter greater than 1 mm, the innermost layer of the wall (intima) is nourished from blood flow in the lumen while the outer layers (the adventitia and part of the media) are supplied from small blood vessels called vasa vasorum[9,10].

NANOFIBRE MANUFACTURING TECHNOLOGY

Nanofibre manufacturing technology can be divided into 3 technologies namely phase separation, self-assembly method, and electrospinning method. The nanofibre 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 nanofibre manufactured by electrospinning method shows various characteristics, which are suitable for the tissue engineering[12,13]. This section introduces the nanofibre productions by phase separation, self-assembly method, and electrospinning method.

Phase separation

Phase separation is the porous polymer membrane forming technique that has been used for years. Phase separation can control the pore structure of nanofibre by using two or more materials of different physical characteristics, and the porous fibre is obtained when using polymer and highly volatile solvent. Therefore the pore size can be changed by controlling volatility of the solvent. Also it is possible to manufacture the nanofibre 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 nanofibre is difficult as it can be applied only for limited numbers of polymer [14,15].

Self-assembly method

Self-assembly means that each component forms an orderly structure voluntarily by the noncovalent bond. The universal method to make nanofibre 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[16,17].

Electrospinning

Electrospinning uses an electric field to draw polymer solution or melt from an orifice to a collector. High voltages are used to generate sufficient surface charge to overcome the surface tension of the solution and a jet that erupts from the tip of the spinneret. The jet is only stable near the tip of the spinneret, after which the jet undergoes bending instability. As the charged jet accelerates toward regions of lower potential, the entanglements of the polymer chain will prevent the jet from breaking up while the solvent evaporates resulting in fibre formation. A grounded plate is usually used to collect the fibres. The diameters of the electrospun fibres are at least one order of magnitude smaller than those made by conventional extrusion techniques[18,19].

A non-woven sheet with a flat profile is usually produced. The ejection rate of the polymer solution through the orifice must be controlled at a low value to produce desired nanoscaled fibre diameter. A 3-D non-woven fibrous mesh can be obtained if the electrospinning time is long enough. The thickness of the nanofibre increases with the electrospinning time at a typical speed of 20 μm/h. The use of more than one orifice simultaneously can increase the thickness of the non-woven sheet, in which the electrospinning speed will be proportional to the number of the orifices. This method may produce 3-D nanofibrous scaffold in a short time in the future[18-20].

NANOFIBRE SCAFFOLDS

The scaffold should mimic the structure and biological function of native extracellular matrix (ECM) as much as possible, both in terms of chemical compositions and physical structures[21]. ECM is mainly composed of three major classes of biomolecules, thus structural proteins (collagen and elastin), specialized proteins (fibronectin and laminin), and proteoglycans composed of a protein core and glycosaminoglycans (GAGs)[22]. ECM also consists of various protein fibrils interwoven within a hydrated network of GAG chains. The ECM provides a physical support for cells and it also provides a substrate with specific ligands for cell adhesion and migration. The ECM also regulates cellular proliferation and functions by storing and presenting various growth factors. An ECM- mimic tissue engineered scaffold is expected to play a similar role to promote tissue regeneration in vitro as the native ECM does in vivo[23,24]. Polymer nanofibre scaffold is promising biomaterials for native ECM. Electrospun nanofibre scaffolds can mimic the nanoscaled dimension of the natural ECM and mesoscopic scale of ECM's spatial organization through fibre orientation and spatial placement. Nanofibre scaffolds can emulate chemical compositions of ECM by including biomolecules into fibres[25].

Biodegradable polymers like PLGA or PCL and water soluble polymers like poly (ethylene oxide) (PEO) and poly (vinyl alcohol) (PVA) can be easily electrospun into nanofibres using organic or water solvent. DNA has also been electrospun into nanofibres. Electrospinning of the natural occurred biomaterials is much more challenging compared with the synthetic polymers due to the difficulties in looking for appropriate solvent. In addition to the ECM-like architecture, polymer nanofibres have other desired features for tissue engineered scaffolds such as biocompatibility, high porosity for tissue ingrowth, high surface area-to-volume ratio, adjustable mechanical and biodegradable properties, capability of surface modification, and flexibility of loading drugs or genes[26-32].

The role of architecture in tissue engineering

The matrix contributes to how a cell transduces input from the external physical environment into biochemical signals that dictate cell response. Research has indicted that cells grown in 3-D culture systems reveals altered morphologies and gene expression compared to traditional 2-D platforms[33,34]. A cell's physical environment may be used to ultimately control cell

behaviour and fate. Thus, the structure-function relationships that govern normal physiology are equally instrumental during the repair process. The tissue engineered replacements should emulate the natural order of the body. In the architectural schemes in vivo, two elements found in high frequency are fibrils and tubules. Most body tissues are hierarchal fibrillar or tubular arrangements. It is the variation in size, organization and composition of these simple building blocks that dictates the wide range of observed mechanical and biophysical properties. Replicating these diverse structures from the macroscopic to the nanoscale level is a significant scientific undertaking. Advancements in micro and nanofabrication have paved way for constructing biologic analogs beginning at the molecular level[35,36]. As geometric features become smaller, changes in cell morphology and fate can be observed. Micrometer based lengths induce more 2-D (planar) geometries, while cells are more spatially interactive on 3-D nanoscaled meshes[35].

Effect of nanofibre orientation on scaffold

Conventional electrospinning produces randomly oriented nanofibres. Nanofibrous scaffold can be developed using a rotating collector disc for collection of aligned electrospun nanofibres. Aligned nanofibres have been explored to fabricate tubular scaffolds that could be used for engineering blood vessels[13]. The aligned nano-sized fibres have been found to mimic the dimensions of natural ECM, provide mechanical properties comparable to human coronary artery, and form a well-defined architecture for smooth muscle cell adhesion and proliferation.

In electrospinning, a charged solution is drawn from the tip and the residual random fibres collect on a grounded plate. A spinning disc technique commonly employed to create aligned electrospun fibres. Fibres aggregate on the disc edge. Corresponding random and aligned fibres produced from shown setup[37-39]. Aligned fibres not only give structural integrity but also maintain vasoactivity as they provide necessary mechanical strength needed to sustain high pressure of the human circulatory system[39].

SURFACE MODIFICATIONS OF POLYMER NANOFIBRES

The interactions between cells and their environments are meditated by the bio- recognition processes, thus the specific binding of the receptors on cell surfaces with their corresponding ligands. In native tissues in vivo, cell attachment on ECM is mediated by the binding between integrins (receptors on cell surfaces) and ECM adhesion proteins such as collagen, fibronectin, vitronectin, and laminin. On biomaterial surfaces in vitro, the same mechanisms also apply. When foreign materials come into contact with body fluids or cell culture mediums, the initial response is protein adsorptions on material's surfaces. Thus materials interact with cells through the adsorbed protein layer. The composition and structure of this protein layer play critical roles in determining subsequent cell behaviours. A successful tissue engineering scaffold should have cell compatible surface to allow cell attachment and proliferation. The use of both synthetic and natural polymers in tissue engineering scaffolds has advantages and disadvantages[40,41].

Synthetic polymers often do not possess surface properties needed for tissue engineering applications. Most mechanically strong and chemically stable synthetic polymers often have inert surfaces both chemically and biologically. On the other hand, natural polymers having active surfaces usually do not possess excellent mechanical properties which are critical for their successful applications as tissue engineering scaffolds. Surface modifications of synthetic polymer nanofibres are done to improve surface properties of the nanofibres. Functionalization of polymer nanofibres is typically carried out either through direct incorporating bio molecules in to the spinning solution during electrospinning process or through immobilizing biomolecules onto the surface of nanofibres during the post processing step[40,41].

Biodegradable polymer nanofibres have to be protected from rapid degradation and destruction during surface modification process. Strong reaction conditions such as plasma, ultraviolet (UV), γ radiation, high temperature, and acidic or basic environments may destroy degradable nanofibres to some extent. Because of the high surface area-to-volume ratio, biodegradable polymer nanofibres may degrade much faster than bulk materials due to the larger contact area between nanofibres and the environment. Some surface modification methods, such as physical coating and "layer by layer" electrostatic interaction, can immobilize

biomolecules onto nanofibre surfaces under very moderate conditions[40,41].

Physical methods

The easiest way to modify polymer surfaces is coating biomolecules onto polymer surfaces or blending biomolecules into bulk polymers. The biggest limitation of this technique is the instability of the polymer surface compositions which is caused by the losing of biomolecules from polymers. The losing of bio molecules should not be too slow to be considered, or not too fast to affect applications of surface modified polymers. For example, collagen, fibronectin and laminin have been coated onto the electrospun silk fibroin (SF) nanofibre surfaces to promote cell adhesion[42]. Human keratinocytes and fibroblasts showed spreading morphology and good attachment on the modified SF nanofibres. Similar results were also reported on collagen-coated poly (ε-caprolactone) (PCL) nanofibres, which showed improved smooth muscle cell growth and attachment. Blending is another physical method of modifying nanofibre surfaces. Contrarily, blending is carried out during the electrospinning process, while coating is carried out in the postprocessing of electrospun nanofibres[43-47].

Chemical methods

ECM protein like collagen and gelatin can be covalently grafted onto nanofibre surface to develop a biocompatible tissue engineering scaffolds. For initiation of graft copolymerization, radicals or groups which can produce radicals like peroxide groups must be introduced onto polymer surfaces first. For most of chemically inert polymers, this can be achieved via irradiation (γ-ray, electron beams, UV), plasma treatment, and Ozone or hydrogen peroxide orxidation or Ce^{4+} oxidation. Then the polymer to be surface modified is usually immersed in a monomer solution, so that the radicals produced on the polymer surface can immediately initiate the copolymerization of the monomer[45,48,49].

CELLS-NANOFIBRE SCAFFOLDS INTERACTIONS

Any implant should be studied in vitro first before implantation in vivo for the interactions of cells and biomaterials in terms of cell adhesion, proliferation, phenotype maintenance, and functional development. There is a significant effect of nanoscale-textured surface roughness on cell response in terms of cell adhesion and proliferation. It is known that cells attach and organize very well around fibres with diameters smaller than cell size.

A gross change in ECM affects cell behaviours. A little is known about how cell behaviours will be affected by fine changes at the nanometre scale in the synthetic ECM. Research has shown that cells attach and organize well around fibres with diameters smaller than size of cells. Nanoscaled surface topography has been found to promote osteoblast adhesions. Nanoscaled surface roughness with dimensions ranging from 20 to 50 nm can be produced by chemical etching on Silicone wafer enhanced neural cell adhesion and hydroxylase activity. Recent studies reported that osteoblast adhesion, proliferation, alkaline phosphatase activity, and ECM secretion on carbon nanofibres increased with deceasing fibre diameters in the range of 60-200 nm, while the adhesion of other cells like chondrocytes, fibroblasts, and smooth muscle cells is not affected. The nanoscaled surface is said to affect the conformation of the adsorbed adhesion proteins like vitronectin to affect the cell behaviours[50-53].

CELL HARVESTING AND SEEDING METHODS

Cell seeding is essential for tissue engineered vascular grafts. There are a number of cell harvesting and seeding techniques. The traditional approach of placing cells on a scaffold for TEVG creation is static cell seeding, in which the patient's cells are pipetted directly onto a graft before being given several hours to attach. There are a number of recognized shortcomings of the static seeding method, including lower efficiency and inter-operator variability. A number of alternatives have been proposed, including dynamic, magnetic, vacuum, electrostatic, and centrifugal seeding. The leading option at this point seems to be vacuum seeding in a specially designed chamber, which is both more standardized and more effective in that it allows for rapid, operator-independent, and self-contained cell seeding. Cell seeding by passive, dynamic and hybrid techniques can

achieve a seeding density of about 1×106 cells/cm^2. These involve implementing static forces or gravity in the case of the passive seeding techniques. Passive seeding techniques have several limitations like unpredictable seeding, longer duration, and poor seeding efficiency. These limitations can be eliminated by the use of biological glues such as fibronectin, fibrin, collage, lamini, and plasma. Dynamic seeding utilizing rotational systems, vacuum systems, and magnetic field based systems, electrostatic systems, photopolymerized hydrogel systems and hybrid systems thereby demonstrating improved seeding efficiencies. Quantitative measures of seeding efficiency with hemocytometer, quantitative histology, scanning electron microscopy, picogreen (DNA) detection assay and metabolic activity measurements have been implemented to evaluate cell viability[54,55].

Appropriate cell source selection

The cell source is the least controlled factor, but the most important for the quality of the living part of the replacement. The quality of cells varies from patient to patient depending on the individual tissue characteristics and co-morbidities. The choice of the right cell source is of major importance for the success of cardiovascular tissue engineering. Besides cell growth and expansion capacity, an important issue is the possibility to develop a cell phenotype that matches the native counterpart. The safest approach is using cells originating from the tissue to be replaced[56,57].

In the case of heart valve tissue engineering, the usage of valvular interstitial cells obtained by biopsy has been shown feasible. Several alternative human cell sources have been investigated for use in cardiovascular tissue engineering. In cardiovascular replacements a development of the extracellular matrix is crucial. The choice of cells which are responsible for production of extracellular matrix is also an important factor. Two cell types are routinely used for the fabrication of cardiovascular tissues. These are cells with the capacity to synthesize extracellular matrix elements, commonly myofibroblast/fibroblast-like cells, and endothelial cells forming a monolayer endothelium with antithrombogenic characteristics. The seeding procedure onto three-dimensional scaffolds is mostly performed sequentially. The first step is seeding of the myofibroblast/fibroblast-like cells, followed by the endothelial cells. Other most promising cell sources investigated in the previous years are vascular-derived cells, bone marrow-derived cells, blood-derived cells and umbilical cord-derived cells, particularly for paediatric applications[56,57].

CONCLUSION

Mimicking the architecture of ECM is one of the major challenges of tissue engineering. Amongst all the approaches used to prepare ECM synthetically, the approach using nanofibres has shown the most promising results. Nanofibres can be formed using either one of the three prevailing techniques: electrospinning, self-assembly, or phase separation. Electrospinning is the most widely studied technique and has also shown the most promising results. The availability of a large range of natural and synthetic biomaterials has fuelled the area of nanofibre synthesis, especially using the electrospinning technique.

Nanofibres, irrespective of their method of synthesis, have provided for scaffolds with high surface area and enhanced porosity. These properties have been demonstrated to have a significant effect on cell adhesion, proliferation, and differentiation. Hence nanofibrous matrices are currently being explored as scaffolds for musculoskeletal tissue engineering (including bone, cartilage, ligament, and skeletal muscle), skin tissue engineering, neural tissue engineering, vascular tissue engineering, and controlled delivery of drugs, proteins, and DNA. The results of all these studies clearly indicate that nanofibre-based scaffolds show excellent potential to be developed for a variety of tissue engineering applications.

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