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Features and Methods of Making Nanofibers by Electrospinning, Phase Separation and Self-assembly

Mohammadreza kheyrandish[1,2], Fahime Bafande[1,2], Mehdi Sheikh Arabi[1,2]*

1. Medical Cellular and Molecular Research Center, Golestan University of Medical Sciences, Gorgan, Iran
2. Department of Medical Nanotechnology, Faculty of Advanced Medical Technologies, Golestan University of Medical Sciences, Gorgan, Iran

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Correspondence:

Mehdi Sheikh Arabi

Medical Cellular and Molecular Research Center, Golestan University of Medical Sciences, Gorgan, Iran

drsheikharabi.m@goums.ac.ir

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Abstract

One of the major challenges in the field of tissue engineering is the production of scaffolding in nano-scale. The study of structural-functional connections in pathological and normal tissues with biologically active alternatives or engineered materials has been developed. Extracellular Matrix (ECM) is a suitable environment consisting of gelatin, elastin and collagen types I, II and III, etc., which are provided to cells for wound healing, embryonic development, cell growth and organogenesis, and. They also play a role in transmitting structural integrity and overall strength to tissues. In tissues, ECM manufacturers are structurally 50 to 500 nm in diameter; nanotechnology must be used to create scaffolds or ECM analogues. Recent advances in nanotechnology have led to the development of ECM-engineered analogues in various ways. To date, three self-assembly, phase separation and electrospinning techniques have been developed to activate nanofiber scaffolds. With these advances and the construction of a "biomimetic" environment, engineered tissue or scaffolding is now possible for a variety of tissues. This study will discuss the three existing methods for creating Tissue engineering scaffolds that are able to mimic new tissue, as well as the discovery of materials for use in scaffolding.

Keywords Nanofibers [MeSH], Tissue Scaffolds [MeSH], Nanocomposites [MeSH]

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Highlights

- Phase separation, self-assembly and electrospinning are common and easy methods for producing nanofibers.
- The imitation of extracellular matrix architecture is one of the challenges of cell culture.

Introduction

This review discusses the design of scaffolds having nanoscale features for collagen, fibrin, fibrinogen, chitosan, elastin, gelatin and cellulose are biopolymers nanofibers (are now emerging as one class of important nanomaterials) represent a practical approach to control cellular migration and orientation in cell culture (1,2). The advantage of natural polymers is that they are very similar to macromolecules in the body (3). The imitation of extracellular matrix architecture is one of the challenges of cell culture (4). In this regard, scientists are using the principle of nanotechnology to design and build nano scaffolds that are capable of replacing ECMs as well as to repair damaged tissues (5). One of the disadvantages of natural polymers, such as hyaluronic acid, is that they are mechanically weak and require processing to separate these polymers (6).

The study of cancer metastasis is limited due to weakness in tumor molecular progression. The types of biological molecules, such as growth hormones that are made up of scaffold have a positive effect on cell growth, proliferation, and function (7). Fine fibers made by electrostatic force have been discussed, investigated, and patented since the late 18th century (Table1). Nowadays, various types of natural and synthetic polymers are available in 3D fiber scaffolding (8) (Table 2). Three-dimensional (3D) culture platforms are able to mimic indoor environments, which are more physiologically important than conventional two-dimensional (2D) cultures (9). To connect cell-cell and cell-ECM, porous 3D structures are designed similar to natural ECM; the use of 3D culture medium has the ability to evaluate the impact of materials and environmental conditions that can be changed, which is an advantage of using 3D culture over the animal model (10). And the 3D cell culture model creates a bridge between the 2D cell culture and the animal model (11). Because of the advantage of nanofiber biopolymers for 3D cell culture template synthesis and It is not able to produce continuous fibers that can withstand the applied stresses, we introduce three main methods to produce nanofibers: electrospinning, self-assembly and phase separation (12).

Table 1. History of Electrospun development of nanofibers.

Year	Progress of Electrospinning technology
1902	Electrospinning as a solution
1980	Electrospinning as a melt
1999	Preparation of Electrospining Nanocomposites
2000	Electrospining Nanofibers for Tissue Engineering
2003	Electrospun Nanofibers on an axis
2005	Electrospinning Nanofibers as a emulsion
2012	Electrospun Nanoyarn
2014	Preparation of 3D porous electrospun compounded with freeze-drying technology

Table 2. Polymers in electrospun process

Polymer		Solvent	Applications	References
Polysaccharides and derivatives	Cellulose	N-methyl morpholine oxide or NMMO	Textile, as food additives, paper, plastic	(13)
	Cellulose acetate	Acetone	Textile, as food additives, paper, plastic	(14,15)
	Ethyl cellulose	Tetrahydro Furan (THF)/ Dimethyl Acetamide (DMA)	As a carrier for loading of functional material	(16)
	Propionyl cellulose	Acetone	As a carrier for delivery	(17)
	Methyl cellulose	Ethanol/water	As a carrier for delivery	(18)
	Hydroxypropyl cellulose	Anhydrous ethanol	As a carrier for delivery	(19)
	Hydroxypropyl methyl cellulose	Ethanol/water	As a carrier for delivery	(20)
	Carboxymethylcellulose	Methanol/water	As a carrier for delivery	(21,22)
	chitin	1,1,1,3,3,3-hexafluoro-2-propanol or HFIP	Tissue engineering and wound healing	(23)
	Practical grade chitin	1,1,1,3,3,3-hexafluoro-2-propanol or HFIP	Tissue engineering and wound healing	(24)
	Chitin/PGA	1,1,1,3,3,3-hexafluoro-2-propanol or HFIP	Tissue engineering and wound healing	(25)
	Chitin/silk fibroin	1,1,1,3,3,3-hexafluoro-2-propanol or HFIP	Textile and clothing	(26)
	Chitosan	Trifluoroacetic Acid (TFA) Ethanol/water	Wound healing	(27)
	Chitosan/polyvinyl alcohol (PVA)	Aqueous acetic acid	Wound healing	(28)
	Hexanoyl chitosan	Chloroform	Wound healing	(29)
	Dextran	Water and DMSO	Adhesion of some functional material	(30)
Protein	Collagen with gelatin/poly ethylene oxide	1,1,1,3,3,3-hexafluoro-2-propanol or HFIP	Tissue engineering and wound healing	(31)
	Silk/PEO	Formic acid	Fabric industry	(32)
	Casein/poly ethylene oxide	Tetrahydrofuran	Food supplement	(33)
	Zein/hyaluronic acid/PVA	Ethanol/water	Medical application	(34)
Nucleic acid	DNA	Ethanol or water	Gene Delivery	(35)
Synthetic and semisynthetic polymers	Polyurethanes	DMF	Protective clothing	(36)
	Polycarbonate	Dichloromethane	Sensor and filter	(37)
	Polyacrylonitrile	DMF	Carbon nanotubes	(38)
	Poly styrene	Tetrahydrofuran	catalyst	(39)
	PVP	Tetrahydrofuran	Antimicrobial agent	(40)
	Polylactic acid	Dichloromethane	delivery system	(41)
	Polyvinyl carbazole	Dichloromethane	Sensor, filter	(42)
	PLGA	Tetrahydrofuran	Scaffold for tissue engineering	(43)

Materials used in 3D culture

In 3D culture, there is a strong emphasis on the use of gelatin, elastin and collagen scaffolds. The structure of collagen provides a suitable biological space for the growth of cells, organs and fetal (44). The collagen in ECM can be separated from a native origin and does not excite the immune system (45). Cell-ECM binding, proliferation and differentiation in tissue engineering, is directly related to the structure of the fiber-like collagen, also the pattern of scaffold construction is the body's natural tissue ECM (46). Gelatin is a natural polymer that is almost identical in composition and biological properties to collagen. Gelatin can be used alone or in combination with a degradable polymer to produce nanofibers for tissue scaffolding, wound healing, and other medical applications (47). Gelatin's behavior is similar to that of other proteins in that it supports cellular supplement, migration and proliferation of cell (48). The structure of gelatin dissolves in water, but if we want to produce ultra-fine fibers for electrospinning, we cannot use a mixture of water and gelatin (49). Because the structure of gelatin should not be dissolved in water at 37°C or higher or congealing at low temperatures in the gel, its structure should be stable (50). Unfortunately, the combination of water with gelatin cannot be used in electrospinning. Moreover, Gelatin is a kind of colloidal solution that is not suitable for tissue scaffolding without cross-linking (51) (Figure 1). There are two types of protein in the human body that have amazing biological properties, like elastin that known as most linear elastic biosolid (52).

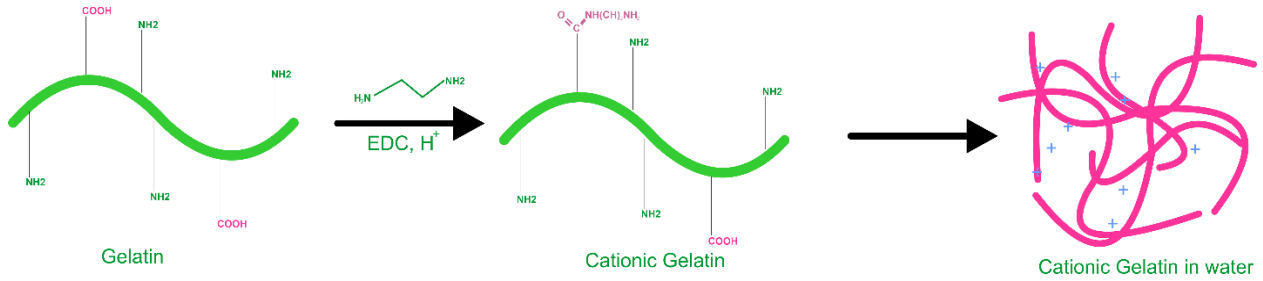


Figure 1. Dissolution of cationic gelatin in water and formation of cross-links between gelatin particles

Electrospinning

Nano-scale nanofibers are produced by electrospinning, in a high-voltage electric field a solution is thrown from the tip of a spinneret to the plate. The advantages of this method are low cost, simplicity and fastness, which are used for the production of nanoscale and micro-scale also, the products that produced by this method have high surface-to-volume ratio and porosity, that is required for three-dimensional culture (53). Electrospinning is widely used for the production of polymeric scaffold for 3D culture for tissue engineering because of its structure similar to ECM (54). The use of this technique gives us the opportunity to determine the thickness of the nanofibers, the porosity and the composition of the nanofibers. In this method, in a large area, the diameter of the nanofibers can be reduced from micrometers to nanometers (55). The high surface area and high porosity in this method allow optimal cellular interaction and therefore allow potential scaffolding. The use of an electric field to design a polymer solution or melt from a hole to a collector is the basis of electrospinning using electric potential in a polymer solution, nanofibers with a diameter of 50 to 1000 nanometers can be produced (56). Due to the surface tension and the electric potential for loading in the polymer solution the solution is kept at the tip of a tube with a capillary structure, and the repellent force absorbs the solution by the plate (57). The term Taylor cone means that the increase in electrical potential causes the surface of the soluble hemisphere at the end of the capillary to lengthen, resulting in such a conical shape. Its further

increase overcomes the electric potential on the surface tensile forces, which causes the formation of fiber coming out of the Taylor cone (58). The fiber that comes out of the tip of the Taylor cone becomes unstable and gradually becomes thinner in the air, which is mostly due to stretching and solvent evaporation. These nanofibers are formed randomly and can eventually be assembled on a fixed collector (59) (Figure 2). Parameters such as the distance between the tip and the collector, which is made of metal, determine the solvent evaporation size of the nanofibers and the precipitation on the collector, and on the other hand, the collector movement pattern during the precipitation determines the final shape (60). Electrospinning systems are used to produce nanofibers with several different layers of different polymer systems. Another way to produce nanofibers with several different compositions is to have several holes where different solutions come out at the same time (61).

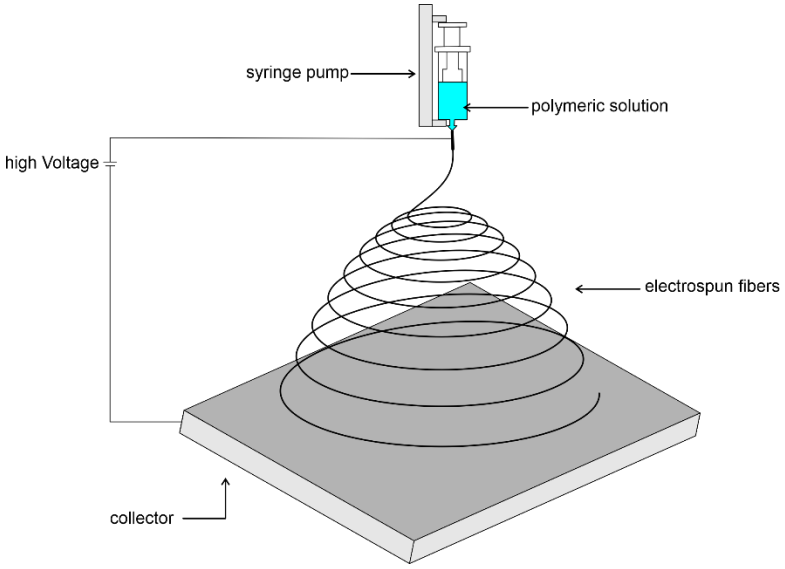


Figure 2. Schematic view of nanofiber preparation with electrospinning device

Self-assembly

Organizing in the self-assembly method is such that there are weak interactions such as electrostatic and hydrogen bonds that bind the atoms of the molecules together and create stable structures in the nanoscale (62). For the production and formation of nanostructures, the self-assembly method can be used for micelles, capsules, nanoparticles, etc. Expanding the application of nanoscale self-assembly to unconventional materials is a new way to multi-purpose systems customized for specific applications (63). However, our goal is to make scaffold to simulate the environment inside the body so that cells can grow on it. To improve the stability and adhesion of the structure in the connecting area, there are 3 glycine amino acids at the head of the pedal flexibility groups (64) (Figure 3).

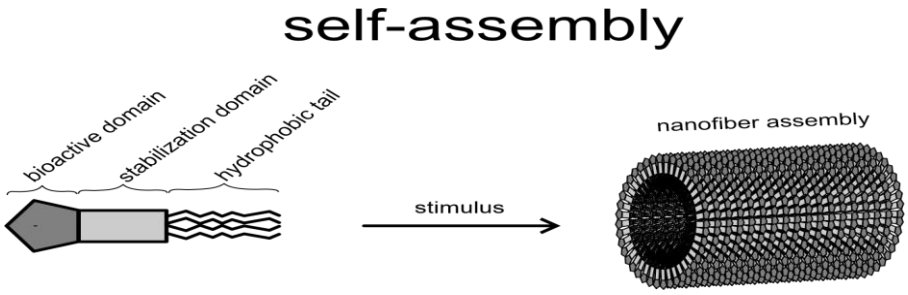
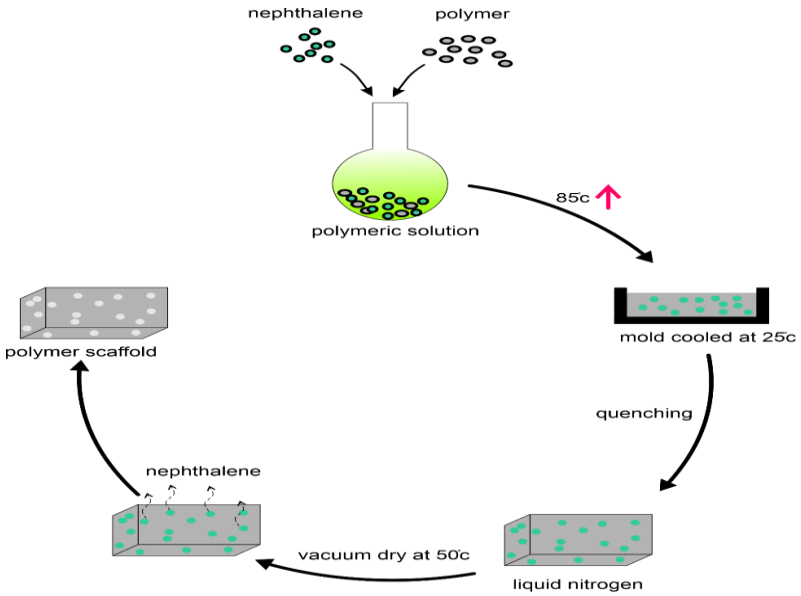


Figure 3. An example of nanofiber formation through self-assembly

Phase separation

The phase separation method is that the polymer solvent, which is homogeneous, is converted to polymer-rich and poor phases of polymer, and this method is accomplish by exposed to an insoluble solution or by decrease temperature of the solution under the solubility curve (65). In this method, increasing the temperature separates the phase. The polymer solution that is submerged under the freezing point is used for produce a spongy-like structure by regulation of the thermal and kinetic parameters, porous structures such as the porous nanofluid matrix are easily obtained through this technique (66). Using the phase separation method, floor scaffolding is manufactured in five stages; in the first step, the solubility of the polymer is examined, then the separation of the phase, after that the solvent is extracted by water, in the next stage, finally freeze-dry in vacuum conditions (67) (Figure 4). The gelation stage is a key step in controlling the nanofiber matrix as well as the degree of porosity. High temperature is required to produce a plate-like structure, while low temperature is required to create a network nanofiber structure (68). The limitation of the plate-like structure has been solved by increasing the cooling rate of the generators that produce uniform nanofibers. Various factors affect the properties of nanofibers, such as the concentration of the polymer (69). To reduce the porosity of the material and increase some properties such as mechanical properties, the polymer concentration must be increased. Other parameters such as thermal activities, solvent type and polymer type affect the appearance of nanofibers scaffolding (70). In the process of phase separation, a porous structure is formed within a strong, durable collagen-like fibrous network. To increase the porosity, macropores are combined by adding salt, sugar or paraffin as a porogen (Each particle volume, with a specific size and shape, is used in molded structures for tissue engineering to create pores) to the polymer solution during phase separation (71). To improve and increase cell implantation, dispersion and transfer of materials such as molecular signals, food and waste, and organizing cellular connections such as cell to cell and cell to extracellular matrix, this structure is built in the nanofibril phase separation matrix. At the cell membrane surface, there are adhesive proteins such as laminin and fibronectin that interact with nanofibers that are made similar to ECM (72). Phase separation is used to create a three-dimensional culture structure with interconnected pores. These nanofibers are characterized by a higher surface-to-volume ratio than traditional scaffolding, which improves connection, migration, proliferation, and cell differentiation (73).

Figure 4. Schematic diagram of formation of polymer scaffold using naphthalene



Results and discussion

The use of biopolymer nanoparticles, which have the physical, chemical and mechanical properties of natural ECM tissues in the body tissues, as well as with high porosity and surface to volume ratio, the tendency to use these biopolymers is increasing (74). Even as an electrospun nanofibrous scaffold this proves that homogenized nanofibers show a useful approach to controlling cell orientation and migration. Contact guidance theory for nanofibers used shows that a cell is most likely to migrate in a direction where the cell tends to have chemical or physical structural properties (75). The reason for the lack of two-dimensional cell culture compared to the three-dimensional that shown in the studies is that the physical and chemical properties of the environment, cellular behavior, and gene expression are significantly affected. The use of 3D culture patterns and its development is increasing (76). The challenge of using three-dimensional culture is lack of connection between the cell and scaffold and prolonging the construction time of the 3D culture (77) (Table 3).

Table 3. Advantages and disadvantages of three techniques preparation nanofiber scaffolds.

Technique	Advantages	Disadvantages
Phase separation	Control the diameter of the structures and also their formation	A limited number of short-lived polymers can be produced
Self-assembly		This method is expensive. Also, the fibers produced can be fragmented and absorbed
Electrospinning	This method has advanced mechanical properties, as well as inexpensive and nanofibers produced with very small diameters in microns	Scaffolding production is limited and cannot be produced in high scale

Conclusion

One of the most important nanoscale biopolymer particle design techniques for cell culture is their construction. Electrospun collagens promote the growth, migration and penetration of cells into the scaffold. Biodegradable nanofibers, which have a controlled molecular surface and structure, can be electrospun to create a three-dimensional culture medium with the special arrangement of fibers and the integrity of the structure. To control and guide cell growth, these nanofibers can provide mechanical simulation of signals, and can also use appropriate and flexible nanofibers to regulate cellular behavior and some functional biopolymers. Electrospinning is also used to design nanofibers with collagen structures for scaffolding, such as natural ECM for tissue engineering. The imitation of nanoscale natural tissue architecture has increased through the development of nanofibers. The porous structure and surface-to-volume ratio of nanofibers help adhesion, migration, proliferation, and cell proliferation. If the structure of the scaffold has a high porosity, the exchange of nutrients also the excretion of cells is better between the structure of the scaffold and the surrounding fluid. Therefore, today, research into the construction of identifying and using nanofibers can be used as 3D culture scaffolding.

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Conflict of interest

There are no conflicts of interests to declare.

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