

Chapter 1

Biological Events Occuring on the Biosis– Abiosis Interface: Cellular Responses Induced by Implantable Electrospun Nanofibrous Scaffolds

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Abstract Electrospun nanofibers have tremendous potential as novel scaffolds for tissue engineering of various soft and hard tissues because of their high surface area, surface functional groups, interconnected pores, and nano-scaled size. In this chapter, we reviewed the types of the nanofibrous scaffolds that have been used as implantable biomedical devices and used electrospun nanofibrous guided tissue regeneration membrane as an example to illustrate how the physiochemical properties of nanofibrous scaffolds influenced the biological events occurring on the scaffolds-host interface. It could be concluded that physical and chemical stimuli caused by nanofibrous scaffold would support in vivo-like three-dimensional cell adhesion and activate cell-signaling pathways. In terms of physical stimulus, the process of mechanotransduction may play an important role in influencing cellular behaviors. As a result, biological events such as cell-interface recognition, cell proliferation, and cell differentiation are altered. Nevertheless, the cellular and molecular mechanisms by which cells sense and respond to nanofibrous scaffolds remain poorly understood. More evidences are needed to reveal the underlying mechanisms whereby environmental cues alternated the cellular responses to the

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physiochemical properties of nanofibrous scaffolds. These future studies may help to design may help to design new generations of implantable biomedical devices that possess controllable cellular responses.

Keywords Biosis–abiosis interface • Electrospun nanofibrous scaffolds • Mechanotransduction • Mesenchymal stem cells

1.1 Electrospun Nanofibrous Scaffolds as Implantable Biomedical Devices

The electrospinning technique is being used to fabricate fibrous scaffolds for tissue engineering, with the aim of restoring and maintaining the biological function lost in host tissues [1]. The electrospun fibrous matrices can provide an ultrahigh surface for cell attachment with high porosity [2] for the exchange of nutrients, ions, and regulatory molecules between cells. The electrospun fibrous matrix holds great promise for tissue regeneration based on its morphology, which favors to support and guide cell growth [3].

Formhals first introduced electrospinning or e-spinning in 1938 [4]. Recently, numerous research groups have explored its use to generate fibrous scaffolds for tissue regeneration. A typical electrospinning apparatus includes a polymer solution/melt in a syringe, charged through a high voltage supply, and a grounded plate positioned at a predetermined distance from the tip of the needle. The potential difference overcomes the surface tension of the fluid droplet at the tip of the metal needle, which in turns results in the formation of the so-called Taylor cone. The fluid jet experience whipping instabilities and tends to dry and form fibers with an average diameter ranging from several microns to tens of nanometers. Processing parameters including voltage, distance from tip to collector, collector type (rotating or static), solution properties (e.g., concentration, viscosity and conductivity) and flow rate have significant influences on fiber formation and morphology. The solution must be sufficiently concentrated so that the polymer chains are continuous and entangled and of suitable viscosity to maintain a droplet and be pumped through the syringe. The resultant materials comprise biocompatible and degradable natural or synthetic polymers or blends and normally resemble the arrangement of the native extracellular matrix (ECM). The fibers can be collected at a random orientation when using a static collector or with high degree of alignment by using a rotating mandrel.

Maintenance of wound stability is a key factor for a successful outcome in regenerative periodontal surgery. Essentially, the three-dimensional (3D) structure shown by these e-spun membranes, with a high surface area of improved hydrophilicity and wettability, endow the structure with mechanical support and cell regulation functions that guide new bone formation into the defect. Li et al. have cultured different cells such as fibroblasts, cartilage cells, and mesenchymal stem cells on poly (lactic-co-glycolic acid) (PLGA) and poly-(caprolactone) (PCL) nanofibrous scaffolds and demonstrated the ability of the nanofiber structure to support cell attachment and proliferation.

1.1.1 Categories of Electrospun Nanofibrous Scaffolds: Classified by Chemical Components

1.1.1.1 Single Component Nanofibers

The choice of material for tissue engineering applications depends upon the type of scaffold required. The correct material to fulfill the requirement of specific mechanical properties and degradation times required for the particular application [5]. Polymer are the main source materials of electrospun nanofibers. Certain of the synthetic and natural polymers have been introduced widely to electrospinning technique for regenerative medicine. Aliphatic polyesters, such as PCL, poly (lactide) (PLA), and their copolymers and blends, are some of the many biodegradable synthetic polymers that have been electrospun. By adjusting electrospinning parameters such as voltage, distance between the electrodes, and flow rate of the solution during electrospinning, and polymer solution properties, such as viscosity and conductivity, most of the biocompatible polymers can be electrospun (e.g. Poly (L-lactic acid) (PLLA), PLGA).

Compared with synthetic polymers, natural biopolymers have good biocompatibility and provide many of the instructive cues required by the cells for attachment and proliferation; however, they tend to display poor processability, which needs to be modified for better electrospinnability. For instance, Zhang et al. have tried to improve the electrospun processability of gelatin by modifying the solubility of gelatin at elevated temperature and the degree of cross-linking of the resultant gelatin fibers [6].

Besides polymer nanofibers, ceramic nanofibers were also fabricated using electrospinning. For example, Zhang et al. developed woven-bone-like β -TCP fibers by sol–gel electrospinning [7]. Optimization studies were conducted in terms of sol–gel synthesis and the electrospinning process, to fabricate electrospun nanofibrous scaffolds with pure β -TCP fibers that mimic the mineralized collagen fibrils in woven bone in size [7].

1.1.1.2 Composite Nanofibers

Organic/Organic Composite Nanofibers

The combination of synthetic and natural polymers is considered advantageous not only for tuning the solubility of natural polymers but also for easy surface modification. Many synthetic (e.g. aliphatic polyester) and natural polymers (e.g. proteins and polysaccharides) have been reported to possess the tissue regenerative potential [8]. However, the innate concerns associated with synthetic polymers are their poor cell affinity [9], while biopolymers are rarely considered as scaffold materials for tissue engineering applications without any special treatment (e.g. cross-linking, or hydrophobic modification) [10, 11]. Mixing synthetic and natural polymers is a

feasible approach to theoretically circumvent the shortcomings of the individual materials and produce new biomaterials with good performances for tissue engineering applications. Jiang et al. firstly reported the preparation of co-electrospun composite membranes composed of PLGA and dextran [12]. After that, different synthetic/natural polymer pairs were coelectrospun, including Poly(L-lactic acid) (PLLA)/gelatin (GE), polycaprolactone (PCL)/GE and cellulose acetate/polyurethane, exhibiting desired cell behaviors and degradation properties [13–15].

Organic/Inorganic Composite Nanofibers

Recent efforts have focused on the development of composite nanofiber scaffolds which can better mimic the composition and mechanical properties of natural bone. Incorporating inorganic phase material (e.g. hydroxyapatite (HA) or β -tricalcium phosphate (β -TCP)), which is one of the compositions of natural bone or bone precursors, into an organic phase material (e.g. biodegradable polymeric nanofibers) is generally used to enhance the mechanical property and osteoconductivity of nanofiber scaffolds in recent years. Sui et al. developed a kind of electrospun membranes composed of HA and PLLA and reported their applications for periodontal tissue regeneration and guided bone regeneration [16, 17]. More recently, Mei et al. developed a novel electrospinning nanofibrous membrane which contained ceramic nano-HA, carbon nanotubes (CNTs), and PLLA matrix [18]. Zhang et al. have fabricated gelatin/ β -TCP composite nanofibers using electrospinning technique, which could regulate Ca ions release by altering the content of β -TCP nanoparticles in gelatin matrix [19]. These composite membranes exhibited excellent biocompatibility, biodegradability, and mechanical properties.

1.1.2 Categories of Electrospun Nanofibrous Scaffolds: Classified by Electrospinning Techniques

1.1.2.1 Coaxial Electrospinning

The formation of core/shell nanofibers by coaxial electrospinning was first reported by [20]. This technique proved to be very versatile not only for the encapsulation of biorelevant molecules and nanocomposites but also for modifying the surfaces of electrospun fibers. The effect of nanofiber surface coatings on the cell-proliferation behavior was studied by Zhang et al. studied the effect of nanofiber surface coatings on cell-proliferation behavior the coaxial electrospinning technique [21]. They produced collagen coated PCL nanofibers. Coatings of collagen on PCL were shown to favor proliferation of human dermal fibroblasts and also encouraged cell migration inside the scaffolds. Using a similar approach, biodegradable fibrous scaffolds composed of gelatin coated PCL were prepared by Zhao et al. by coaxial

electrospinning [22]. More recently, Sun et al. developed core–shell PAN–PMMA nanofibers by coaxial electrospinning [23].

1.1.2.2 Coaxial or Emulsion Electrospay

Bioactive factors-loaded microparticles can generally be achieved by two different electrospay approaches: coaxial or emulsion electrospay. Coaxial electrospay was first reported by Loscertales et al. [24]. Two immiscible solutions are coaxially and simultaneously electrospayed through two separate feeding channels into one nozzle. The eventual jet, by which the outer polymeric solution encapsulates the inner proteinaceous liquid, breaks into droplets to generate microparticles with core–shell structure. This technique is preferred for preparing protein-loaded microcapsules, because it totally eliminates the emulsion step that is basically unsuitable for sensitive biomacromolecules [25, 26]. Coaxial electrospay is envisioned a promising approach to prepare biomacromolecule-loaded microcapsules for controlled drug delivery applications.

Emulsion electrospay involves mixing of an aqueous solution containing proteins with immiscible polymeric solution by ultrasonication [27, 28]. Compared with other conventional manufacture methods, the second emulsion or high temperature is omitted in emulsion electrospay. It increases the drug-loading efficiency and is suited for encapsulation of thermosensitive bioactive compounds.

1.1.3 Biofunctionalization of Nanofibrous Scaffolds

Electrospun nanofibrous membranes are considered to have great potential in the field of tissue engineering because they can closely mimic the ECM architecture [29, 30]. However, for some polymer nanofibers with the relative hydrophobicity and surface inertia, such as poly (methyl methacrylate) (PMMA) and PLLA, bioactive treatment will usually be needed to improve their cellular affinity and facilitate osteogenesis.

1.1.3.1 Plasma Treatment

Low-temperature plasma treatment has been shown to be a convenient and effective way to modify surfaces to improve the hydrophilicity of biomaterials, thus increasing their biocompatibility and facilitate cell attachment [31, 32]. Wan et al. reported that ammonia plasma treatment significantly increased the hydrophilicity of PLA scaffolds and resulted in enhanced cell adhesion and proliferation of mouse 3T3 fibroblasts [33]. D'Sa et al. reported that improved hydrophilicity of PMMA surfaces by plasma treatment increased adsorption of proteins and promoted actin stress fiber formation [34]. Moreover, plasma technique was found to modify levels

of chemical groups, such as $-\text{COOH}$, $-\text{OH}$, or $-\text{NH}_2$ on scaffold surfaces, thereby influencing the cell–substrate interactions. For example, human umbilical vein endothelial cell (HUVEC) adhesion was improved by plasma treatment of PLA through the control of carbon and oxygen concentration [35], and human embryonic palatal mesenchyme (HEPM) cell proliferation was increased by plasma-treated poly(ether ether ketone) (PEEK) through assembling amino groups on the surface [36]. Amino-rich PLA surfaces created by plasma treatment were also reported to promote osteogenic differentiation of MC3T3-E1 cells [32]. More recently, Liu et al. investigated the effects of plasma treatment on the surface of PLLA nanofibrous membranes, and the subsequent dose-dependent cellular response and osteogenesis of MSCs were clarified preliminary [37]. These results support the feasibility of plasma technology to regulate the biological functions of biomaterials.

1.1.3.2 Biomineralization

In addition to encapsulation of inorganic materials to improve the properties of fibrous materials, depositing inorganic phase materials i.e. biomineralization on the surface of polymeric nanofibers to form uniform coatings is an alternative methods. Biomineralization on the nanofiber surface can not only enhance its mechanical properties, but also provide a favorable substrate for cell proliferation and osteogenic differentiation. Ramakrishna and co-workers mineralized electrospun PLGA/collagen fibrous scaffolds, and the presence of the functional groups of collagen significantly hastened n-HA deposition in comparison with pure PLGA fibrous scaffolds [38]. The use of simulated body fluid (SBF), a kind of solution with ionic concentration closely resembling human blood plasma to biomimetically coat the composite fibers with apatite layers should be a good choice.

In principle, the morphology and grain size of minerals deposited on the nanofibers can be tailored by controlling the composition of the mineralized solution, the surface charge of substrate and the surface chemical properties. Recently, Cai et al. reported biomineralization of electrospun poly(L-lactic acid)/gelatin composite fibrous scaffold by using a supersaturated simulated body fluid with continuous CO_2 bubbling [39]. They found that the mineralites could be formed heterogeneously in the $5 \times$ SBF with CO_2 bubbling.

1.1.3.3 Biomagnetism

Nature is a source of inspiration for scientists and engineers to design advanced functional materials. Very weak local magnetic fields exist in living organisms and various organs in humans. Earlier clinical research showed that the magnetic field might be beneficial for enhancing bone tissue regeneration though mechanisms that have not yet been clarified.

In recent years, interest in magnetic biomimetic scaffolds for tissue engineering has increased considerably. Magnetic nanoparticles are of great interest owing to

their potential biomedical applications [40, 41], such as cell expansion, cell sheets construction, magnetic cell seeding, as drug delivery vehicles and for hyperthermia treatment. For bone regenerative research, electrospinning technique has been used successfully to fabricate magnetic fibrous scaffolds, including $\text{Fe}_3\text{O}_4/\text{PVA}$ nanofibers [42], $\text{Fe}_3\text{O}_4/\text{CNF}$, and FePt/PCL nanofibers. More recently, Wei et al. fabricated magnetic biodegradable $\text{Fe}_3\text{O}_4/\text{CS}/\text{PVA}$ nanofibrous membranes, which promoted MG63 cells adhesion and proliferation on these membranes [43]. These results support the feasibility of incorporating magnetic nanoparticles into polymer nanofibers to regulate the biological functions of biomaterials.

1.2 Cellular Responses Influenced by Electrospun Nanofibrous Scaffolds

Electrospun nanofibrous scaffolds are able to recapitulate both the topographical features of the ECM, and biochemical cues via various modifications to the fiber material or surface. This type of artificial scaffold with enhanced biofunctionality would comprise a more biomimetic microenvironment for ex vivo stem cell culture. The cell/nanofibers interface exerts considerable influence on MSC functions and differentiation.

1.2.1 *Biological Events Occuring on the Biosis–Abiosis Interface: The Role of Chemical Cues*

The chemical matrix of nanofibers may create and maintain specialized functional properties in the local microenvironment for cell function. Hybrid scaffolds comprising synthetic and natural organic polymers take advantage of the physical properties of the synthetic components and the bioactivity of the natural constituents while minimizing the disadvantages of both, resulting in more favorable biocompatibility than those with a single component [16, 44]. The incorporated nanoparticles in nanofibers could provide multiple binding-ligands for amino and carboxyl groups of serum proteins to facilitate cell attachment [44] and bone matrix deposition [16, 43], and introduce magneto-electrical effect (Fe_3O_4 , $\text{r-Fe}_2\text{O}_3$, BeTiO_3 , ...) to benefit cell proliferation [43, 45]. The simultaneously incorporation of multi-walled carbon nanotubes (MWCNTs) and HA nanoparticles in PLLA nanofibers selectively increased adhesion of osteoblast cells and decreased the adhesion of osteoblast competitive cell lines, which was a valuable feature for GBR application [17]. The small trace amount of Mg [46, 47], Si [48–50], and Zn ions [51, 52] integrated in nanofibrous scaffolds have been proved to accelerate cell adhesion and proliferation by delivering the mitogenic stimuli and enhancing channel sensitivity. In addition, chemical signal molecules in the form of growth

and differentiation factors [53, 54] or plasmid DNA [55, 56] incorporated in the nanofibers in a spatially defined manner could achieve corresponding bioactivity in promoting specific differentiation to orchestrate the growth of new tissue.

Surface modification, including chemically grafted surface-functional-groups and microcontact printed peptides or proteins, could initiate specialized cell-nanofibers interactions. The enrichment of specific $-OH$, $-NH_3$ or $-COOH$ chemical groups on nanofibers may lead to improved hydrophilicity and reversible albumin adsorption, facilitating focal adhesion assembly and matrix deposition [57–59]. Attachment of adhesion-promoting peptides, such as RGD, 52 GRGDS, and GEFYFDLRLKGDK could increase the selective interactions between nanofibers and cells in terms of adhesion, spreading, and proliferation [60, 61]. The coated structural ECM proteins of collagen, fibronectin and laminin may present cells with a myriad of recognition sites for binding integrins, heparin sulfate proteoglycans, growth factors and cytokines, these biologically active nanofibers can better support cell attachment and growth [24, 25]. Interaction of these modified cell-nanofibers interaction could in turn exert a considerable influence on the osteogenic differentiation of mesenchymal stem cells (MSCs).

1.2.2 Biological Events Occuring on the Biosis–Abiosis Interface: The Role of Topographical Features

Topography cues of electrospun nanofibers have been demonstrated to provide actual osteogenic niches in various aspects. Their fibrous structure could mimic the structure of ECM-derived scaffolds [50]. And, their dimension seems to simulate the structure of woven bone, which is the initial bone phenotype formed in the healing procedure after a fracture [49]. The apparent porosity of nanofibers was considered to favor efficient mass transportation of nutrients, oxygen, and waste products [48]. Recent studies have indicated a powerful role of the nanotopographic cues from nanofibers in regulating the osteogenic behavior of stem cells [51, 52].

1.2.2.1 Temporal Changes in the Osteogenic Behaviors on Diversely Arranged Nanofibrous Scaffolds

Phenotype observation showed that the cell shape, nuclear morphology and focal adhesion were modulated by nanofiber orientation; all these three aspects are considered to be closely correlated with the differentiation state of stem cells [62, 63]. The relatively isotropic random nanofibers may favor the growth of human bone mesenchymal stem cells with a highly branched morphology with spherical nuclei and large focal adhesion, while the aligned nanofibers result in a polarized morphology with elongated oval nuclei and small focal adhesion. Such a highly branched cell shape is thought to have an “osteocyte-like” morphology to

push hBMSCs toward an osteogenic lineage [62]. The small and immature focal adhesion (FA) of MSCs on aligned nanofibers was considered to represent a migratory cell status, while the large and super-mature FA of MSCs on random nanofibers indicated the cell status of sensing the mechanical properties to act on cell lineage [54, 64]. The more osteogenic-specific fate of MSCs on random nanofibers than those on aligned ones was corroborated by many studies. Hu et al. reported that MSCs cultured on random PLLA nanofibers exhibit an enhanced osteogenic differentiation phenotype involving higher bone sialoprotein (BSP) and osteocalcin expression and increased alkaline phosphatase (ALP) activity [53]. Yin et al. reported that randomly oriented nanofibers induce higher ALP activities and more calcium deposition, which is related to integrin- and myosin-mediated mechanotransduction [56]. Wang showed that ALP activity and the production of collagen type I and osteocalcin all increased in MG63 cells cultured on random PLLA nanofibers [55]. These observations demonstrated that the nanotopographic features of electrospun nanofibers might provide essential niches to guide MSCs osteogenic behavior.

To explore the biological mechanisms underlying the osteogenic behavior of MSCs in response to nanofibrous scaffolds, full-scale, high-throughput and high-efficient global microarray analyses were carried out [37]. The temporal gene expression profiles demonstrated that the dynamic cellular behaviors of MSCs on nanofibers occur in a time-dependent pattern. At day 4, genes representing in cell adhesion molecules, extracellular matrix receptors, and integrin-mediated signaling pathways were up-regulated. At day 7, expression of genes associated with cytoskeletal organization and mechanical stimulation was observed to notably increased. At day 14, osteogenic pathways, including TGF- β /BMP, MAPK, and Wnt, were up-regulated. At day 21, genes associated with skeleton development, ossification and mineralization were up-regulated. Taken together, a lower extent but similar rhythm of dynamic cellular behavior was induced on random nanofibers when compared with the osteogenic supplement condition. Furthermore, this temporal dynamic rhythm suggested that mechanotransduction might be the underlying mechanism of nanofibrous topography driven osteogenic differentiation of MSCs.

1.2.2.2 Mechanisms of Electrospun Nanofibrous Scaffolds-Induced Cellular Responses

The Nanometer Effects of Nanofibrous Scaffolds on Cellular Responses

Nanofibers have distinct advantages over conventional scaffolds as its topographic structure mimics the *in vivo* extracellular milieu. For example, the fiber diameters of electrospun nanofibers are in the range about 100 nm. The mineralized type I collagen fibrils, constituting ~90 % of the bone structure, are nano-sized (50–500 nm in diameter) [65], and may thus be well mimicked by the synthetic nanofibers. In previous studies, it was demonstrated that the nanometer effects of

nanofibrous scaffolds could induce up-regulated focal adhesion kinase signaling and increased cellular elastic modulus for osteoblastic cells and enhanced fate direction into osteogenesis for hBMSCs.

The Role of Focal Adhesion Formation and Cellular Cytoskeleton Arrangement

Knowledge of cells-ECM interactions might help to understand the various cellular responses to the diversely arranged electrospinning nanofibrous scaffolds (aligned or randomly distributed). It is well known that cells anchored to the extracellular matrix through focal adhesions, which allow the cells to “communicate” with the ECM [66]. Thus, the properties of the ECM, including its mechanical character, are transmitted via focal adhesions to the cytoskeletal network of a cell [67]. In general, the cytoskeleton is composed of three distinct components: actin microfilaments, microtubules, and intermediate filaments [68]. The organization of the cell cytoskeleton actively participate in the ability of cells to sense and convert mechanical cues into biological responses. It cells display highly elongated cell morphologies when growing on aligned nanofibers and spread cell morphologies when growing on randomly distributed nanofibers [37, 65]. These phenomena might be associated with the spatial distribution of the focal adhesion formation of the attached cells which arises from the “communication” between cells and diversely arranged nanofiber scaffolds. Cells on fiber networks, developed longer and more concentrated focal adhesion clusters compared with cells on flat control substrates [69]. In addition, the highly elongated cell morphology also means the greater cytoskeletal tension, and relevant signaling such as ROCK may be up-regulated in cells on aligned nanofibers [65].

The Role of Mechanotransduction

Mechanotransduction describes the molecular mechanisms by which cells respond to changes in their physical environment. Cells can sense mechanical stimulation and changes in their physical environment through force-induced conformational changes at the molecular level; however, of the molecular mechanisms are still incompletely understood. Kris et al. [68] summarized that the underlying mechanisms as: extracellular forces might stimulate stretch sensitive ion channels and force-driven activation of transcription factors might stimulate the downstream cellular pathways. For instance, opening of these ion channels could result in changes in intracellular ion concentrations, which would have different downstream effects including activation of signaling pathways that leading to changes in gene transcription [70]. Moreover, transcription factors, such as nuclear factor NF- κ B, translocate from the cytoplasm to the nucleus on mechanical stimulation, and protein cascades such as the mitogenactivated protein kinase (MAPK) cascade could be activated following molecular events.

1.3 Future Prospective

In this review, we discussed the biochemical and biophysical cues given by the nanofibrous scaffolds that could influence cellular behaviors on the biosis–abiosis interface. These cues include the chemical composition of the nanofibers, the surface biofunctionalization of the nanofiber scaffolds and the arrangement of the nanofibers in three-dimension. Future studies are needed to fully understand the molecular and biophysical basis of this direct form of nuclear mechanotransduction and to understand how these processes are integrated with chemical diffusion-based signaling mechanisms [68].

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