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Chapter 4

Electrospun Zein-based Nanofibrous Membranes for Biomedical Applications

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Abstract

The electro spunzein-baednanofibrous membranes have been shown great potential in tissue engineering, wound dressings and drug delivery. This article reviews the recent progress of nanofiber matrices electrospun from zein and natural polymers, zein and synthetic polymers, zein and crosslinking agents,zein-based conjugates as well as their biomedical applications.

Keywords: Zein, Electrospunnanofibers, Natural polymers, Synthetic polymers, Crosslinking agents, Zein-based conjugates, Tissue engineering, Wound dressing, Drug delivery.

1. Introduction

Recent advances in electrospinning techniques have enabled the production of continuous fibers with dimensions on the scale of nanometers. Electrospun polymeric membranes have been shown great potential in biomedical applications [1-4]. Such materials have high porosity with excellent pore-interconnectivity. A wide range of natural and synthetic polymers can be electrospun into nanofiber matrices with structural integrity and specific fiber arrangements[5-8]. Up to now, various natural polymers have been used widely for this purpose because of their biological origin, nontoxicity, hydrophilicity, biocompatibility, biodegradabilityand low cost. Nanofiber matrices have been widely used as tissue engineering scaffolds, wound dressing and drug delivery systems [9-24].

Zein is a class of alcohol-soluble prolamine storage proteins in corn and one of the main

plant proteins [25]. Based on solubility and sequence homology, zein can be separated into four classes: α-zein, β-zein, γ-zein and δ-zein[26]. Among these, α-zein comprises 70–85% of the total fraction of zein mass, and γ-zein accounts for 10–20% as the second most abundant fraction [27]. It can form tough, glossy coating with antibacterial activity andtherefore is widely utilized in food and pharmaceuticalindustry [28, 29]. However, single-component zein solution is often insufficient for good electrospinnability or could not result in good fiber properties.

This review will discuss the recent progress of nanofiber matrices electrospun from zein and natural polymers, zein and synthetic polymers, zein and crosslinking agents,zein-based conjugatesas well as their biomedical applications.

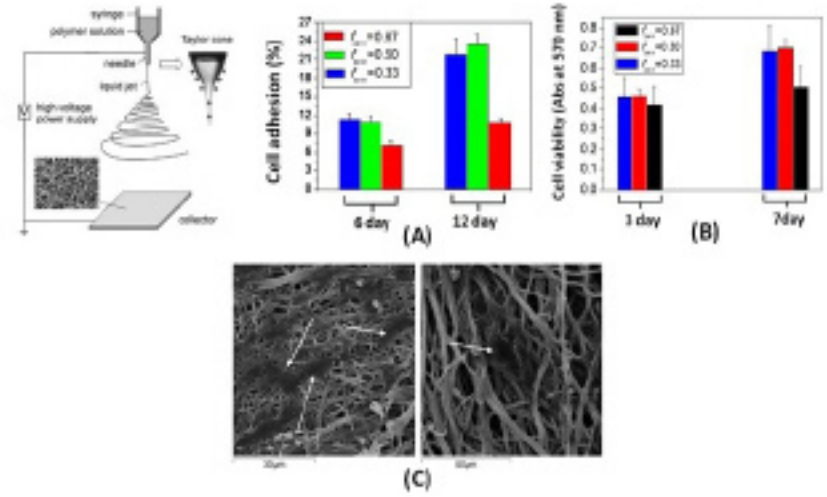

Figure 1: A laboratory setup for an electrospinning experiment, the cell adhesion (A) and cell proliferation (B) on the electrospunnanofibersfrom zein/ collagen blends with different weight fractions (fzein) of zein as well as typical SEM images (C) for the cell interactions with the nanofibers (fzein =0.50) (Reproduced from [30]).

In order to develop biocompatible nanofibrous membranes for wound healing, the electrospinning of zein and collagen, one of the main proteins, in aqueous acetic acid solution was investigated in our study [30]. **Figure 1** showed a laboratory setup for an electrospinning experiment, the cell adhesion and cell proliferation on the electrospunnanofibersfrom zein/ collagen blends with different weight fractions of zein. It was found that the combination of zein could improve the electrospinnability of collagen. For the resultant electrospun membrane, its fiber diameter, surface wettability, mechanical and in-vitro degradable properties as well as cell adhesive ability could be modulated by the change of zein/ collagen blending ratio. Moreover, berberine drug could be incorporated in situ into the electrospunnanofibrous membrane for its controlled release and antibacterial activity. The addition of berberine showed little effects on the fiber morphology and cell viability. In addition, the wound healing performance of the as-obtained nanofibrous membranes was examined in vivo by using female Sprague-Dawley rats and histological observation and was confirmed to be very effective [30].

The high hydrophobicity of zein is unfavorable for cell adhesion and thus greatly limits its use. The electrospunzein/gelatin nanofibrous scaffolds in order to take full advantages of the two natural materials were fabricatedin our study[31]. Zein and gelatin in four groups of

different mass ratios (100:00, 100:20, 100:34, 100:50) were given.The scaffolds were smooth and homogeneous, as shown in scanning electron micrographs [31]. The diameter of hybrid fibers was increased from 69 nm to 950 nm, with the proportion of gelatin increase. The cell affinity of zein/gelatin nanofibers was evaluated by using human periodontal ligament stem cells. The hydrophilicity and cytocompatibility of zeinnanofibers were improved by blended gelatin. The electrospunzein / gelatin nanofibers had sufficient mechanical properties, satisfied cytocompatibility, and could be utilized as biological scaffolds in the field of tissue regeneration. In bone tissue engineering, it was important for biomaterials to promote the osteogenic differentiation of stem cells to achieve tissue regeneration. Therefore, it was critical to develop biomaterials with excellent cytocompatibility and osteoinductive ability. In our study, we found a zein/gelatin electrospinning scaffold with good biocompatibility [31], but low osteoinductive ability for human periodontal ligament stem cells (hPDLSCs). Therefore, we fabricated novel zein/gelatin/nanohydroxyapatitefibrous membranes to overcome the drawbacks of the zein/gelatin scaffold [32]. Moreover, the inclusion of nanohydroxyapatitefacilitated the attachment, proliferation, and osteogenic differentiation of hPDLSCs. The zein/gelatin/nanohydroxyapatitefibrous membranes showed good biocompatibility and osteoinductive activity for hPDLSCs in vitro and in vivo.

To overcome the poor electrospinnability of aqueous zein solution, the electrospunzein / hordein/ cellulose nanowhiskersnanofabrics possessed significantly improved mechanical properties and had potential to be used as tissue engineering scaffold materials or natural delivery systems for biomedical applications [33].A simple and cost-effective way to prepare water-stable zein-based nanofibers for potential drug delivery was presented by Luet al.[34]. Zein was co-electrospun with hydrophobic ethyl cellulose. Indomethacin, as a model drug, was incorporated in situ into the composite nanofibers. The electrospun blends composed of zein/silk fibroin with unique mechanical properties and excellent biocompatibility were produced by Wang et al. [35] to fabricate nanostructured bicomponent scaffolds. The as-spun blend fibrous membranes with a combination of protein nanofibers showed the structural stability in aqueous media.The electrospunzein / proanthocyanidinsnanofibers showed a good controlled release toward proanthocyanidins [36]. Novel antimicrobial ultrathin structures of zein/chitosan blends obtainedby electrospinning were produced by Torres-Giner et al. [37]. The formulation, morphology and biocide properties of electrospunzein-based ultrathin fiber structures were described. Zein-based nanofibers containing cyclodextrins were produced byKayaci and Uyar [38] via electrospinning. The incorporation of cyclodextrinsin zein improved the electrospinnability and bead-free nanofibers were obtained at lower zein concentrations.

3. Electrospunnanofibers of zein and Synthetic Polymersfor Biomedical Applications

For soft tissue engineering, zein has inferior mechanical properties and lacks aqueous stability. The electrospun scaffolds from zein blended with poly(glycerol sebacate) were fabri-

cated [39, 40].The addition of poly(glycerol sebacate) to zein resulted in a seven-fold increase in ultimate tensile strength and a four-fold increase in failure strain, whereas the Young's Modulus did not change significantly (**Figure 2**). It was found by Vogt et al. [40] that the relative humidity influenced the electrospinnability of the blends. Degradation tests in phosphate buffered saline revealed the morphological instability of zein containing fiber mats in contact with aqueous media [40].

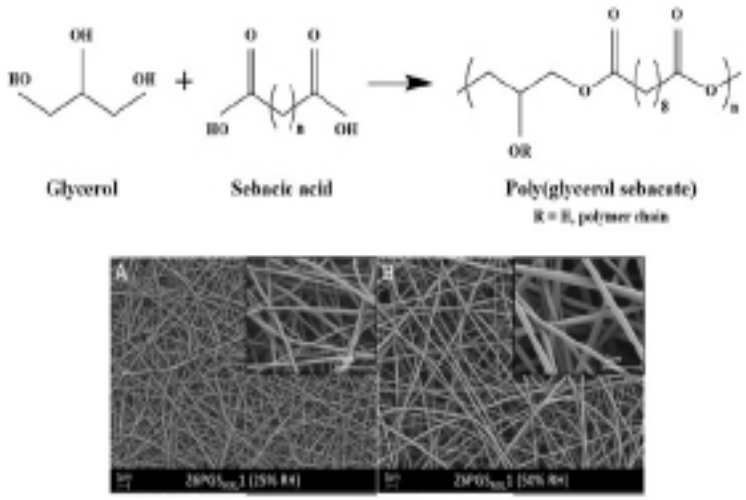

Figure 2: Poly(glycerol sebacate) obtained by polycondensation of glycerol and sebacic acid and SEM micrographs of electrospunzein / poly(glycerol sebacate) nanofibers (A,B) at 25% and 50% relative humidity(Reproduced from [40]).

The electrospun nanofibers of zein/ branched polyethyleneimine (PEI) and zein/PEI/ ZnOwere synthesized by Mascia et al. [41]. The PEI / ZnOsuspensions in ethanol were mixed in an ethanol/watersolution of zein and used for electrospinning of different types ofzein-modified fibers [41].Zinc acetyl acetonatedihydrate (ZnAcAc·2H2O) was chosen as the precursor for the related sol−gel reactions, leading to the nucleation and growth of wurtzite crystals within the PEI matrix. Differential scanning calorimetry analysis showed that PEI acted as a plasticizer for zein. The presence of the ZnO nanoparticles conferred antibacterial properties to the zein/PEI electrospun mats [41].Osteochondral tissue was hard to regenerate after injuries or degenerative diseases. Traditional treatments still had disadvantages, such as donor tissue availability, donor site morbidity, implant loss, and limited durability of prosthetics.Thus, recent studies focused on tissue engineering strategies to regenerate osteochondral defects with different scaffold designs. The electrospunzein/ polyhedral oligomericsilsesquioxanes (POSS) nanofiber layer were designed by Tamburaci et al. [42] to mimic a bone−cartilage tissue interface. POSS nanocages did notalter the zeinfiber morphology. This could be attributed to thehomogeneous distribution of POSS nanocages in the zeinmatrix [42].

For biomedical applications, zein and synthetic polymershad also shown potential in the electrospinning process. A series of zein /poly(3-hydroxybutyrate-co-4-hydroxybutyrate) blend fiber scaffolds were prepared by Cai et al. [43]. Theseelectrospunfibers showed a circular and uniform morphology with random distribution. The blend fiber scaffolds possessed well interconnected porous fibrous network structurewith high porosity and large aspect surface

areas. Novel electrospunzein and polyethylene glycol scaffolds with fibers oriented randomly and evenly in three dimensions including in the thickness direction were developed by Cai et al. [44] based on the principle of electrostatic repulsion. The unique structure was different from most electrospun scaffolds with fibers oriented mainly in one direction. Babitha and Korrapati [45] reported that the electrospunzein/polydopamine polymeric scaffold impregnated with TiO2 nanoparticles for skin tissue engineering. Karthikeyan et al. [46] reported that the aceclofenac/pantoprazole loaded zein/eudragitnanofibers were developed using a single nozzle electrospinning process. The morphological analysis revealed the uniform and smooth surface of the drug loaded nanofibers. The zein /poly(ε-caprolactone) (PCL) core/shell nanofiber membranes nanofibers were developed by He et al. [47] via coaxial electrospinning for guided tissue regeneration. Moreover, Jing et al. [48] reported that the electrospunzein/ PCL scaffolds exhibited remarkable improved mechanical strength in terms of Young's modulus and yield stress compared with PCL scaffolds.

4. Electrospunnanofibers of Zein and Crosslinking Agents for Biomedical Applications

For tissue engineering applications, Xu et al. [49] reported that the three-dimensional ultrafine fibrous zein scaffolds were crosslinked with non-toxic oxidized sucrose and citric acid. As seen in **Figure 3**, SEM and confocal laser scanning microscope (CLSM) images of electrospunzeinscaffolds with the crosslinkings of 5 wt% oxidized sucrose and 10 wt% citric acidwere demonstrated. The protein sorption and mechanical properties of citric acid and oxidized sucrosecrosslinkedzein scaffolds were not significantly different. The oxidized sucrosecrosslinkedzein scaffolds were found to show good cytocompatibility similar to those crosslinked with citric acid.

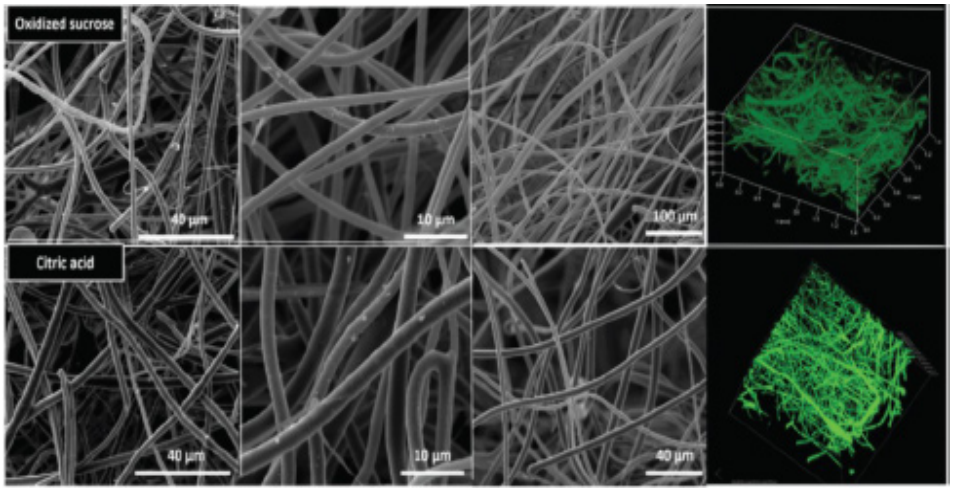

Figure 3: SEM and CLSM images of electrospunzeinscaffolds with the crosslinkings of 5 wt% oxidized sucrose and 10 wt% citric acid (Reproduced from [50]).

Efforts are being madeto improve its strength either by using chemical crosslinking agents with zein. Jiang and Yang [50] reported that the electrospunzein fibers were modified by non-toxic citric acid crosslinking catalyzed by NaOH. An up to 183% enhancement in dry tensile strength and an up to 448% improvement in wet tensile strength were generated. The cross-linked fibers were able to maintain their fibrous structure for 15 days in phosphate-buffered saline at 37◦C. Moreover, those cross-linked electrospunzein fibers showed a potential in controlled drug delivery with a 58% drug-loading efficiency and a sustained profile drug release in artificial gastric juice.Erdogan et al. [51] reported that olive leaf extract (OLE) had a potential to be used as a crosslinkerin electrospunzeinnanofibers.The study aimed to investigate crosslinking effect of OLE in addition to its role in functionalization due to its polyphenolic content. The decrease in fiber diameters and homogeneous fiber morphology could be attributed to the effects of OLE. Using OLE also provided a single preparation step for electrospinning as both zein and OLE could be processed with aqueous ethanol solution. The prepared materials were used for tissue engineering applications [51].

5. Electrospunnanofibersof Zein-based Conjugates for Biomedical Applications

Excellent biocompatibility and bioactivity are necessary requirements for a scaffold for nerve repair and regeneration. Zein was chosen as the primary material and poly(L-lysine) (PLL) was used to modify it in our study [52]. Poly(L-lysine) modified zein (ZPLL) with different PLL contents of 1.46%, 3.57%, and 6.18% was synthesized and nanofibrous membranes were prepared by electrospinning, as seen in **Figure 4**. The hydrophilicity of the membranes improved with an increase of PLL content. The biodegradability of the membranes was proved by in vitro experiments. Compared with pure zein membranes, ZPLL membranes could efficiently improve cell viability, adhesion, proliferation, and differentiation of neural stem cells. The results showed that when the PLL content was 3.57%, cell adhesion and proliferation proved to be the best and most differentiated toward mature neurons with extensive neurite formation and astrocytes rather than oligodendrocytes [52].

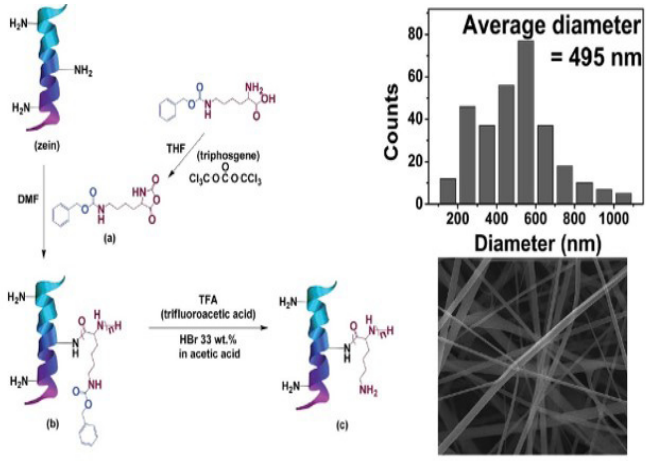

Figure 4: Syntheses of poly(L-lysine) modified zeinand electrospun poly(L-lysine) modified zeinnanofibrous membranes(Reproduced from [52]).

Critical-sized bone defects raise great challenges. Zein is of interest for bone regeneration, but it has limited ability to stimulate cell proliferation. We aimed to develop the electrospunpoly(aspartic acid)(PAsp)-modified zeinnanofibers to realize critical-sized bone defects repair [53].We developed PAsp-modified zein using click chemistry, which produces stable products by rapid reaction undermild conditions.PAsp-modified zeinnanofibrous membraneswere prepared using an electrospinning techniqueto create nanoscalefibrous membranes with interconnectingpores that mimicked the native structure of extracellular matrix.The impact of zeinnanofibers design regarding the PAspspatial arrangement anddensity mode on cell−nanofibers interaction would be furtherinvestigated to explore the ideal biomaterials in promotingosteogenesis [53].

7. Conclusions

The electrospunzein-based nanofibrous membraneshave shown great potentialfor biomedical applications. The surface chemistry, microstructure, and architectureof nanofibrous matrices significantly influence cellular adhesion,proliferation, and differentiation. The recent progress of nanofiber matrices electrospun from zein and natural polymers, zein and synthetic polymers, zein and crosslinking agentsas well aszein-based conjugateshave opened up numerous opportunitiesin tissue engineering, wound dressings and drug delivery.

8. Acknowledgments

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