

Construction and Properties of Zein-based Cell Supports and Scaffolds

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ABSTRACT

Zein, a protein abundant in corn, is capable of self-assembly and may form a variety of structural morphologies. Zein micro-fibers and sponges were prepared from colloidal suspensions. They had good tensile strength and elastic properties and biocompatible surfaces. Zein surfaces were able to induce cell adhesion at specific locations thus allowing for spatial control of cell growth. Zein constructs of enhanced capabilities may lead to effective tissue engineering leading to cartilage replacement and the treatment of bone injuries.

Keywords: zein, microstructure, fibers, porous, controlled-release

1 INTRODUCTION

Zein, a major protein of corn, is insoluble in water but readily dispersed in alcohol-water mixtures. Zein has an amphiphilic character due to its unusual amino acid sequence which contains over 50% hydrophobic residues.[1] Matsushima and coworkers[2] proposed a structural model where the α -helical segments were aligned in an antiparallel fashion forming a prism of $13*1.2*3$ nm in dimension. α -Helices were connected at each end by glutamine-rich bridges. Accordingly, the prism sides, formed by the outer surface of the helices, were hydrophobic, while the top and bottom surfaces of the prism, containing the glutamine-rich bridges, were hydrophilic.[3] Wang and coworkers[4] employed surface plasmon resonance (SPR) to study the hydrophobic/hydrophilic character of zein surfaces. They concluded that the zein molecule contains sharply defined hydrophobic and hydrophilic domains at its surface.

Amphiphilicity is a main driving force for

self-assembly.[5] Self-assembly gives rise to the lyotropic ordered phases, cubic, hexagonal, gyroid and lamellar.[6] Zein is capable to self-assemble to a variety of structural morphologies. Wang and coworkers[7] observed the formation of spheres, bicontinuous, and lamellar structures from zein in aqueous ethanol solutions. Lai and coworkers[3] observed that zein adsorbed fatty acids forming periodic structures at the nanoscale. Wang and coworkers[4] reported the formation of zein nanoscale tubes by self-assembly.

Fibers and sponges are two kinds of structures that have been widely used in the development of cell supports and scaffolds for tissue engineering. Slavik and coworkers[8] have developed a molding technique to produce fibrous chitosan scaffolds, which mimick the structures found in extracellular matrix of natural tissues, to support attachment and proliferation of cells. Griffon and coworkers[9] studied the chitosan sponge and found that increasing interconnectivity and pore size of the sponge improved chondrocyte proliferation and metabolic activity.

Zein is a new kind of biomaterials for tissue engineering. Zein biocompatibility was tested by Wang and coworkers.[10] They found that fibroblast cells were able to respond to differences in surface chemistry, as adopted by zein applied on different substrates. Tu and coworkers[11] have developed zein as cell supports and scaffolds for bone regeneration. They reported that complexes of zein scaffolds and rabbit MSCs could undergo ectopic bone formation in the thigh muscle pouches of nude mice. Wang and coworkers[12] added stearic acid and oleic acid to zein to improve its mechanical properties, since the inherent lack of strength associate with porosity is a common limitation in the development of porous scaffolds. The objective of this

study was to investigate the formation of fibrous and sponge structures of zein for construction of cell supports and scaffolds.

2 MATERIALS AND METHODS

2.1 Materials

Zein was obtained from Showa Sanyo Co. Ltd. (Tokyo, Japan). Ethanol (190 proof, USP) was from Decon Labs, Inc. (King of Prussia, PA).

2.2 Scanning Electron Microscopy (SEM)

Zein (0.20 -150 mg/ml) was dissolved in ethanol-water mixtures (30% - 95% (v/v)). Samples of solutions (10ml) were placed on aluminum dishes (Fisher Scientific, Pittsburgh, PA) to undergo EISA. Dried samples were gold coated (300Å) by an Emitech K575 sputter coater (Ashford, UK) to help improve electrical conductivity of sample surfaces. SEM images were obtained with a JEOL 6060LV General Purpose SEM (Peabody, MA).

2.3 Focused Ion Beam (FIB) Microscopy

FEI dual beam 235 FIB (Hillsboro, OR) is a combination of a high resolution field emission SEM and a scanning metal ion beam microscope. Ga ion beam was used by the FIB to remove the material on the sample surface in a controlled pattern.

3 RESULTS AND DISCUSSION

3.1 Structure Characterization

Zein-based cell supports and scaffolds were prepared by evaporation induced self-assembly (EISA) method. Various microstructures of zein, which include fibers and sponges, were obtained by varying the concentrations of zein in aqueous ethanol solvent and choosing different surfactants.

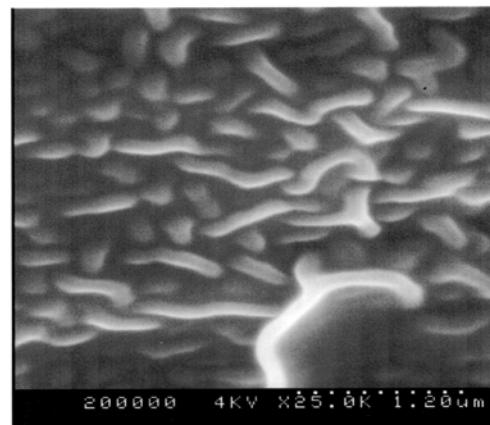


Figure 1. SEM image of zein fibers obtained by EISA from an 80% ethanol solution containing zein and oleic acid.

Figure 1 shows a SEM image of zein fibers obtained by EISA from an 80% ethanol solution containing zein and oleic acid. The melting and compression of the soft fibers shown in the image were due to the high liquid surface tension [8]. Before melting and condensed, the fibers, which were 0.5-1.5μm in length, interconnected and made a porous 3D network with room available for cell seeding and culturing.

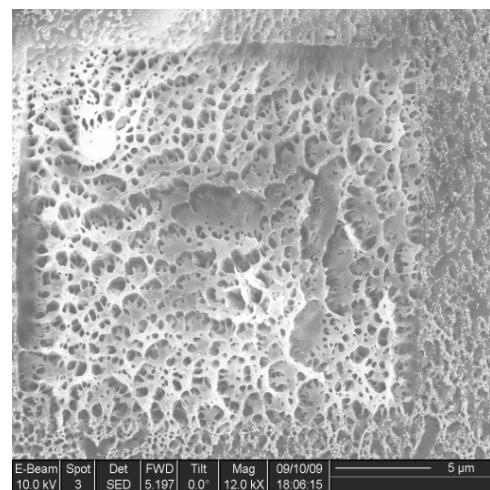


Figure 2. SEM image of zein sponge obtained by EISA from an 80% ethanol solution containing zein, acetic acid and triglycerides.

Figure 2 shows a SEM image of zein sponge obtained by EISA from an 80% ethanol solution containing zein, acetic acid and triglycerides. The surface material was removed by FIB to show the interior structure. Zein sponge

pores ranged from tens to hundreds of nanometers in size. The size of the pores can be controlled by the concentration as well as the selection of the surfactants. Controlled pore size has advantage on the cell proliferation and activity.[9]

Figure 3 shows a SEM image of zein fibers obtained by EISA from an 80% ethanol solution containing zein and acetic acid. The fibers, 2-5 μ m in size, were much larger than the fibers in Figure 1. They also melted together leaving larger pores for cell seeding. The interconnection and networking varied with the size and shape of the fibers, which was related to the concentration and selection of the mixture of surfactants.

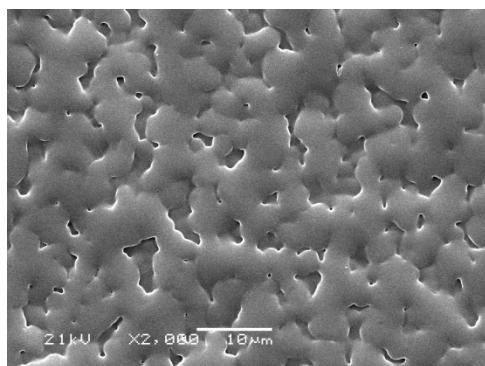


Figure 3. SEM image of zein fibers obtained by EISA from an 80% ethanol solution containing zein and acetic acid.

4 CONCLUSIONS

Zein is an amphiphilic protein capable of self-assembly into fibers and sponges. The formation of fibers and sponges was dependent on zein concentration and surfactant selection. SEM and FIB images showed the structure of fibers and sponges obtained by EISA from a solution of zein and surfactants. The size and shape of zein fibers and sponges may be controlled by the selection and concentration of the surfactant mixture to comply with biomedical requirements. Future work will involve seeding and culturing the scaffolds with cells such as bone marrow cells and chondrocytes. The performance of zein scaffolds will be evaluated by biomedical and biological techniques.

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