Tuning the Bioactive and Mechanical Properties of Self-assembled Nanofiber Networks For Synthetic ECM Mimics

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Introduction

Natural tissues consist of nanofibrillar proteins which self-assemble into complicated hierarchical structures. Nanostructured tissue engineering is the new strategy to mimic structural characteristics, biological complexity, and self-assembly processes at the nanoscale level to create high order organization closely resembling natural tissues. We have developed a tunable self-assembled nanofiber network which mimics several key properties of ECM. The nanofiber network achieves its complicated hierarchical structure by self-assembly of functional peptide amphiphiles (PAs). The nanofiber network interacted strongly with encapsulated cells and regulated their cellular behaviors by acting like the natural extracellular matrix (ECM).

Materials and Methods

Synthesis of PA

Functional amino acid sequences (cell-mediated enzymes sensitive sequences with or without cell adhesive sequences, RGDS or RDG) were synthesized by using standard Fmoc-chemistry on Advanced Chemtech Apex 396 peptide synthesizer. Alkylation was obtained by reacting the N terminus of the peptides with Palmitic acid. PAs were purified in reverse phase HPLC, and analyzed by MALDI-TOF mass spectrometry.

Self-assembly into nanofiber networks

2 wt% of PA stock solution was prepared in DI water, and adjusted the pH to 7.4 by adding 1 M NaOH. 50 μl of PA solution was placed in 12 well silicon FlexiPerm attached on glass cover slide. 50 μl of DI water containing 0.1 M $CaCl_2$ (pH 7.4) was added to induce the formation of self-assembled nanofiber networks. Molar ratio of PA and calcium ions (Mr = mole of calcium ions/mole of PA) was changed from 0 to 4.

Characterization of nanofiber networks

Biodegradability of PAs and their blends was investigated by simulated *in vitro* system using collagenase in order to determine whether biodegradability could be controlled by compositions of PAs. The viscoelastic properties of nanofiber networks were investigated using Rheometry. The structural conformation of peptide amphiphiles was investigated using circular dichroism (CD).

Cell encapsulation in nanofiber networks

Rat maxillary incisor pulp cells and Human glioma cells were encapsulated in nanofiber networks. 50 μl of 2 wt% of PA DI water was placed in 12 well silicon FlexiPerm attached on glass cover slide. Cells were suspended in Dulbecco's Modified Eagle's Medium (DMEM) at concentration of 10 million cells/mL, and appropriate amount of 0.1 M $CaCl_2$ (pH 7.4) was added to immediately. 50 μl of cell suspension solution was added to PA solution and gently mixed. After 1 hr of incubation, additional medium was added.

Results and Discussion

Successful synthesis and purification of PA was confirmed with MALDI-TOF. CD spectra revealed that PA in aqueous solution (Mr=0) formed β-sheet structures and also maintained this structure even in formation of nanofiber networks (Mr=1 and 2). Introduction of calcium ion into PA solution leads to self-assembly of PA into nanofiber networks at physiological conditions. As shown in Figure 1a, 1wt% of PA was prepared in aqueous solution at neutral pH without calcium ions and visualized by cryo-TEM. The image showed that they looked like amorphous aggregations and some of them formed large oblong structures. However, the addition of calcium ions resulted in self-assembly by eliminating repulsive forces of negative charge and leaded to the growth into long nanofibers with length of several micrometers (Fig. 1b). The nanofiber networks were proteolytically degraded in one month by collagenase. TEM images showed that nanofibers were broken into micelles aggregations or denatured ribbon shaped nanofibers (Fig.1c). The viscoelastic properties of nanofiber networks were found to be dependent on calcium concentrations and the length of PAs. Cell viability was evaluated as more than 90% after 3 days of encapsulation. Cells were also encapsulated in nanofiber networks with various concentrations of cell adhesive sequences. Cell spreading was dependent on the concentrations. Consequently, the self-assembly of PAs, growth of nanofibers, and viscoelastic properties of the nanofiber networks could be tailored by modifications of the PA and addition of divalent ions. Importantly, the length of the PA was found to dramatically affect the native properties of the nanofiber network so that the bioactivity, cell-mediated degradation, and mechanical properties could be controlled.

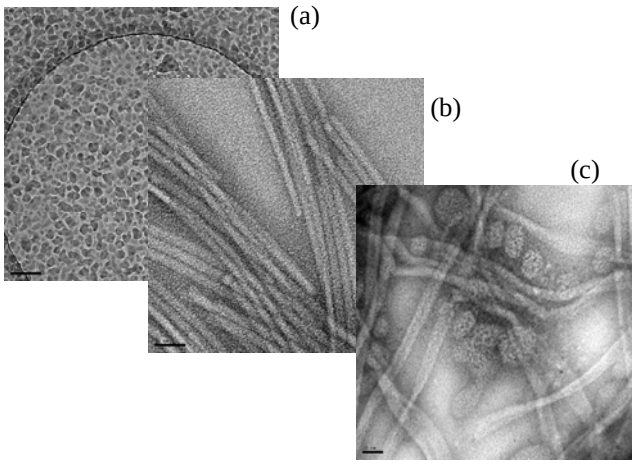

Figure 1. TEM images of self-assembled nanofiber networks (a) Cryo-TEM images without calcium ions, (b) TEM images after addition of calcium ions, and (c) TEM images after 3 weeks of proteolytic degradation. Scale bar: 200 nm for (a) and 20 nm for (b and c)

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