Development of new supramolecular nanostructured materials based on peptide hydrogelator Ac-L-Phe-L-Phe-L-Ala-NH₂ with embedded liposomes for potential biomedical application

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In recent years design and synthesis of self-assembled nanomaterials with diversified structures and functionalities *via* fine tuning of supramolecular building blocks increased rapidly.[1] Self-assembling peptides have been widely recognized as nanomaterials with high potential for an extensive range of biomedical applications from drug delivery to tissue engineering, owing to their hydrophilic character and biocompatibility.[2,3]

Short peptide hydrogels made of two or three amino acids are of particular interest because they are cost effective and simple to prepare. It was previously shown that the newly developed self-assembling tripeptide hydrogel Ac-L-Phe-L-Phe-L-Ala-NH₂ (Ac-F-F-A-NH₂) is effective in enhancing cell proliferation and has potential for use in tissue engineering approaches.[4]

Figure 1: Chemical structure of hydrogelator Ac-L-Phe-L-Phe-L-Ala-NH₂. TEM image of hydrogel in water (2.64 mg/ml) PWK-stained. Magnification 8000x, scale bar: 1 μm.

Ac-L-Phe-L-Phe-L-Ala-NH₂ self-assembles at physiological pH without the need for any organic solvent at a minimum gelation concentration of 0.7%, w/v in saline. The tripeptide derivative contains an aromatic diphenylalanine moiety (Phe–Phe) as a minimalistic building block to drive the self-assembly of short peptides into nanostructures and hydrogels of high stability. Transmission Electron Microscopy (TEM) analysis of the nanofibrous hydrogel network has shown the presence of a mixture of fibers and straight ribbons with diameters in the range of 50–500 nm and lengths in the range of micrometers, which are stable over weeks at a very low concentration of 2.64 mg/ml (Figure 1).

The aim of the present study was preparation and characterization of supramolecular systems based on peptide hydrogelator Ac-L-Phe-L-Phe-L-Ala-NH₂ and liposomes with incorporated model proteins. The rationale behind preparation of liposomes-in-hydrogel as a delivery system is to assure sustained drug release during their prolonged presence at the administration site.

The gelling properties of the hydrogelator Ac-L-Phe-L-Phe-L-Ala-NH₂ with addition of liposomes and model proteins BSA/FITC-BSA were studied. Liposome formulations of BSA and FITC-BSA as well as empty liposomes, incorporated in the hydrogel were characterized by electron and confocal microscopy. It is known that intramolecular hydrogen bonding and noncovalent interactions between molecules of hydrogelators are responsible for the formation of the 3D gel network.[5] Therefore, we have tested the structure of the gel network after the addition of different amounts of protein and liposome.

TEM confirmed incorporation of liposomes and protein into the hydrogel (Figure 2) without disruption of the

gel network. In gel samples with built-in BSA, the gel network preserved integrity, but it was noticed that the gel fibers were thinner. Confocal microscopy proved the incorporation of liposome formulations of FITC-BSA in the hydrogel (Figure 3). It was also shown that liposomes do not impair the gel network of hydrogelator at lipid concentrations lower than the gelator concentration (Table 1).

Figure 2: TEM images of (A) liposomes in hydrogel, Pd-shadowed. Lipids:gelator = 1:2.5, (B) liposomes in hydrogel, PWK-stained. Lipids:gelator = 1:5. Magnification 10 000x, scale bar= 1 μ m, (C) BSA incorporated in the gel network (BSA:hydrogel = 1:1051), (D) liposome formulation of BSA embedded in gel network (lipids: hydrogel = 1:10), (C) and (D) PWK-stained, Magnification 16 000x. Scale bar: 1 μ m.

Table 1: Gelation of Ac-L-Phe-L-Phe-L-Ala-NH₂ after addition of liposomes.

Mass of hydrogelator (mg)	Molar ratio (lipid:gelator)	Lipid (mg)/mg gelator	Comments
3.6	1:10	0.27	Matted, white gel, quickly formed
3.0	1:5	0.55	Matted, white gel, quickly formed
2.9	1:2.5	1.09	Matted, white gel, quickly formed
3.0	1:1	2.73	The gel is not formed

Figure 3: Confocal images of liposome formulation of FITC-BSA embedded in gel network (lipids: hydrogel = 1:10), Pd-shadowed.

We showed that use of higher amounts of protein albumin (BSA)/FITC-BSA resulted in slower gel formation or inability to transition into gel. It was also demonstrated that addition of sufficiently small amount of liposome formulation of BSA and FITC-BSA in hydrogels does not impair the gel network. The described supramolecular

nanostructured material based on peptide hydrogelator Ac-L-Phe-L-Phe-L-Ala-NH₂ with embedded liposomes can be useful in various areas of application in biomedicine, including injections in situ of gelling agents for controlled release of the drug, drug delivery platforms, and tissue engineering.

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