Sustained Protein Delivery System Using Core/shell Nanoparticles

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Introduction

A number of investigations have been devoted to understand the formation of core/shell particles [1-2] and much interest has been focused on developing the core/shell particles as an effective means to encapsulate a large number of materials ranging from low molecular weight organic molecules to biological macromolecules. [3] In our previous report, the core/shell nanoparticles with a lecithin lipid core were prepared and characterized as a delivery system for liphophilic drug.[4-5] Further study has been performed to evaluate the core/shell nanoparticles as a protein delivery system. VEGF was used as a model drug, which has been considered as a potential treatment for stroke due to its angiogenic and direct neuroprotective action. To achieve an optimum therapeutic effect, the sustained release of VEGF is highly required and new types of drug delivery systems should be designed. In this study, the core/shell nanoparticles were designed (see Figure 1) and evaluated for efficient protein delivery system.

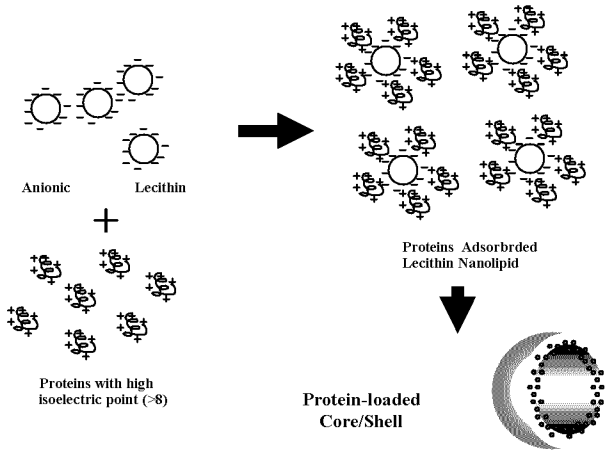

Figure 1. Schematic Description of Protein-Loaded Core/Shell Nanoparticles.

Experimental

Preparation of core/shell nanoparticles. The lipid phase used as a core was composed of 40 weight % aqueous solution of lecithin from soy bean oil with the form of nanoparticles, which were prepared by the sonication using a probe type ultrasonic wave homogenizer (Branson Sonifier Model 185). The weighed amounts of lipid phase, VEGF, trehalose (cryoprotectant, 5 weight % of total weight of dried nanoparticles) and F-127 aqueous solution were mixed and subjected to the freeze-drying to induce the formation of polymeric shell on the surface of protein drug-loaded lipid core.

In vitro VEGF release from core/shell nanoparticles. 10 mg of VEGF-loaded nanoparticles were suspended in 0.2 ml of phosphate buffered solution (PBS) and this solution was subsequently put into a dialysis tubes. The dialysis tube was placed into 15 ml of PBS containing 2.0 mM sodium azide and 0.1 % (w/v) bovine serum albumin (BSA) and kept in reciprocal shaking water bath (Jeio Tech., Korea) at 37 °C and 35 rpm. At each time point, the whole medium was taken and replaced with the fresh release medium. The amount of released VEGF into the release medium was determined by ELISA analysis (Molecular Devices, USA).

Results and discussion

Aqueous concentrated lecithin mixtures show typical lamellar liquid crystalline behavior and the individual lamellae tend to form a spherical supramolecular structure. In our study, the average diameter of lecithin nanolipids was 65.4 nm and the surface charge from zeta potential measurement was −50.9 mV indicating that lecithin nanolipids can form an ionic complex with positively charged molecules. Based on this characteristics, the complex formation between lecithin and protein drug can be induced by choosing a proper protein with high isoelectric point (>8) and protein-loaded core/shell nanoparticles can be obtained based on the preparation method of core/shell nanoparticles in our previous study.[4-5] For efficient loading of model protein drugs, the complex formation was induced between lecithin and VEGF (the isoelectric point: pH 8.4) in the F-127 aqueous solution containing trehalose and freeze-dried to form the polymeric shell.

After freeze-drying of VEGF/lecithin nanolipid complex in the F-127 aqueous solution containing trehalose, the obtained powder was resuspended in PBS and characterized with particle size analyzer. With the formation of polymeric shell, the size of nanoparticle was increased up to 275.3 nm and negative charge of lecithin nanolipid was shielded (-17.18 mV). Cry-TEM picture clearly demonstrates the formation of core/shell nanoparticles as shown in Figure 2

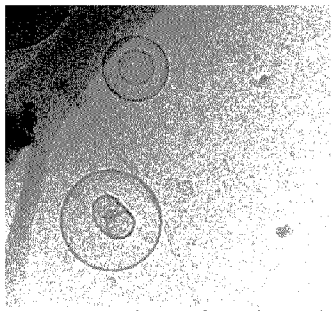

Figure 2. Cryo TEM Picture of Protein-Loaded Core/Shell Nanoparticles.

The release of VEGF from the nanoparticles was observed as a function of the concentration of F-127 aqueous solution. In the case of nanolipid without polymeric shell, the significant burst was observed, releasing about 90 % of the initial loading amount during 2-week period. With the formation of core/shell nanoparticles, the sustained release pattern was observed, releasing about 80 % of the initial loading amount during 42-day period.

The core/shell nanoparticles in this study is intended to improve blood flow in the ischemic area in the body through the formation of new blood vessels, a process known as angiogenesis with delivering the angiogenic protein, VEGF. In this approach, the core/shell nanoparticles will be directly injected into the ischemic area by injection catheter and this will enables the sustained and controlled release of VEGF in the area of the ischemic area with low blood flow. The feasibility of this approach will be reported later.

Conclusions

With the formation of nanoparticles with the core/shell structure, the stability of lecithin nanolipids was significantly increased and the sustained release patterns were achieved with model protein drugs such as lysozyme and VEGF. The core/shell nanoparticles in this study can be utilized to improve blood flow in the ischemic area in the body through the formation of new blood vessels, a process known as angiogenesis with delivering the angiogenic protein, VEGF using injection catheter.

References

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