Thermoresponsive Self-Assembled Tropoelastin Nano/Micro-Particles for Protein Delivery

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ABSTRACT SUMMARY

In this work, self-assembled nano/micro-particles based on tropoelastin derived from human adipose tissue were developed as vehicles for protein delivery. Human tropoelastin showed sol-to-particle transition with lower critical solution temperature (LCST) of 25-40 °C in aqueous milieu. The self-assembled tropoelastin nano/micro-particles are able to encapsulate significant amounts of insulin by simple mixing at low temperature in water and heating up to body temperature. These thermoresponsive tropoelastin nano/micro-particles, which were derived from human adipose tissue, have great potential as a smart protein/drug delivery system.

INTRODUCTION

Polymer nano/micro-particles are under increasing scrutiny as vehicles for the encapsulation and sustained release of small molecules and proteins for drug delivery, tissue engineering and other biomedical applications. In particular, natural polymers are suitable materials as nano/micro-particles for clinical application due to their versatile traits, including biocompatibility, biodegradability and low immunogenicity. The structural protein elastin is one of the main components of the extracellular matrix (ECM), which provides structural integrity to the tissues and organs of the body. This high cross-linked and therefore insoluble protein is the essential element of elastic fibers, which account for elasticity to tissue of lung, ligament, skin and arteries. Not only elastin but also its precursor material, tropoelastin, have inspired already for many years. The most interesting characteristic of tropoelastin is its ability to self-assemble under physiological conditions, thereby leading to the development of new.

We describe here the fabrication of insulin-loaded nano/micro-particles using tropoelastin derived from human adipose tissue. Tropoelastin undergoes reversible, temperature-dependent aggregation of the hydrophobic blocks in an aqueous solution, affording protein/drug loaded nano/micro-particles physiologically relevant conditions.

EXPERIMENTAL METHODS

Human tropoelastin was obtained from adipose tissue through two successive major steps: i) extraction of ECM by pulverization, centrifugation, alkaline and alcohol treatment; ii) isolation of tropoelastin from ECM by hydrolysis in oxalic acid at 90 °C. Briefly, raw ECM was treated with NaCl solution and then heated in an autoclave to remove the impurities. The residues were hydrolyzed in oxalic acid at 90 °C. This process was repeated several times until the residues were completely hydrolyzed. The supernatant was dialyzed and lyophilized by freeze-drying. The particle formation and dynamic behavior of purified human adipose-derived tropoelastin were characterized by UV-Vis spectroscopy (at 286 nm), dynamic light scattering, and scanning electron microscopy (SEM).

For visualization of insulin loading, tropoelastin and insulin were labeled with FITC and FPR648 fluorescence dye, respectively. Insulin (0.04 mg/1 mg tropoelastin) was added to 7.5 mg/mL of tropoelastin solution and then the mixture was heated at 37 °C, resulting insulin-loaded nano/microparticles. The morphological examination of the nan/micro-particles was performed by SEM and confocal microscopy.

RESULTS AND DISCUSSION

Dynamic light scattering measurements revealed a significant dependence of apparent particle size on temperature and tropoelastin concentration (Fig. 1). Upon heating, the tropoelastin self-assembled into nano/micro-particles with mean diameters ranging from 400-800 nm, depending on tropoelastin concentration and temperature.

Most important, tropoelastin is thermally responsive. Below a critical temperature (~ 10 °C), tropoelastin are soluble, when temperature reached over 30 °C, the tropoelastin undergoes a solution to particle phase transition. In this study, the optimal concentration of tropoelastin (7.5 mg/mL) was
determined to have a transition temperature just above normal body temperature (around 37 °C) for use as an injectable protein delivery system.

The nanoparticles had uniform spherical shape with hollow or multicore inner structure and showed high loading efficiency of insulin (approximately 93%). Overall, the reversible, temperature-dependent, sol-to-particle transition behavior of human tropoelastin may provide a new exciting opportunity for use in a range of drug delivery and tissue engineering applications.

CONCLUSION

The self-assembled nanoparticles based on natural elastin derived from human adipose tissue were developed as vehicles for protein delivery. Tropoelastin can be self-assembled into spherical nano/micro-particles of various sizes at body temperature and nano/micro-particles with multicore structure exhibited a high protein loading efficiency. Overall, as the simple and efficient approach, the tropoelastin nano/micro-particles provides a smart platform for designing advanced delivery carriers for use in a range of therapeutic and tissue engineering applications.

REFERENCES

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