Enzymatically Triggered Microcapsules For Water-soluble Drug Delivery Applications

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Statement of Purpose: Matrix Metalloproteinases (MMPs) are enzymes that play an important role in many physiological and pathological processes (1-4). In the current project, we are interested in MMP enzyme-triggered drug delivery. The objective of the current study is to design a gelatin-based microcapsule system which can release water-soluble drugs at elevated levels of MMP-9 but retain most of the drug at low physiological levels of MMP-9. Since levels of MMP-9 and MMP-2 enzymes (gelatinases) are elevated in several diseases such as arthritis, bacterial infection, breast cancer, sepsis, HIV infection, retinopathy, and skin wounds, this triggered drug delivery system is useful as a novel therapy with the benefits of higher efficacy, low toxicity, and less chance of drug resistance.

Methods:

PLGA Nanoparticle Synthesis

We have synthesized PLGA-based nanoparticles containing both a water-soluble drug (vancomycin.HCl, Sigma) and a dye (rhodamine 6G fluorescent dye, Sigma) using our developed method (5). Briefly, 100 mg of PLGA and PLGA-PEG polymer were dissolved in 6 mL of methylene chloride. 100 µL of the water phase, containing dye or drug, was emulsified in the above solution and then further dispersed in 1% sodium cholate solution. Subsequently, this double emulsion solution was diluted in 0.5 % sodium cholate solution and evaporated for 1 hr to remove the solvent.

PLGA-Gelatin Microcapsules Synthesis

The above PLGA nanoparticles were dispersed in mineral oil for complex coacervation in gelatin using the established procedure. Briefly, the gelatin is dissolved in deionized water at 52°C. Mineral oil preloaded with the PLGA nanoparticles is homogenized into the gelatin solution to form droplets of less than 20 µm in diameter. After addition of polyphosphates and proper pH adjustment, the reaction mixture is cooled to room temperature to allow gelatin shells to form around the oil droplets. The microcapsules were washed with water and lyophilized to form a dry powder for drug release.

Characterization and drug release

Both an optical microscope and a fluorescence microscope were used to image these hybrid PLGA-Gelatin microcapsules. The basic release medium consisted of 150 mM NaCl, 50 mM Tris-HCL, 10 mM $CaCl_2$ and 0.05% Brij-35 (without and with 100 ng/mL of MMP-9). PLGA-gelatin microcapsules were incubated in the above release media at 37°C for 1 hr., the released vancomycin was measured by UV-Vis spectroscopy (Lambda) by monitoring the absorbance at 280 nm.

Results: Optical images clearly demonstrate that PLGA nanoparticle aggregates were encapsulated inside gelatin shells (Figure 1A). Since the cores contain PLGA nanoparticles incorporating a water-soluble fluorescent dye, the core is visible by fluorescent microscope (Figure 1B). An overlay of optical and fluorescence images (Figure 1C) clearly demonstrates that the PLGA nanoparticles can be loaded inside the gelatin microcapsules. These hybrid gelatin-PLGA particles released higher amounts of vancomycin in the presence of the MMP-9 (100 ng/mL) enzyme compared to those without MMP-9 after incubation at 37°C for 24 hrs. (Figure 1D). These preliminary results demonstrate that it is feasible to deliver water-soluble drugs using a modified complex coacervation method.

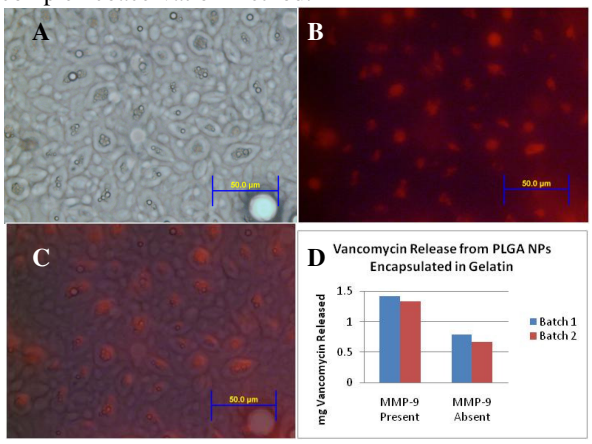

Figure 1. A) Optical image of PLGA nanoparticle-containing gelatin microcapsules without any crosslinking (bar = 50µm). B) Fluorescence image of microcapsules, showing presence of fluorescent dye. C) Overlay of optical and fluorescent images, showing that dye has been encapsulated within the particles. D) Vancomycin release data in the presence and absence of MMP-9.

Conclusions: Water soluble drugs such as vancomycin can be loaded inside PLGA nanoparticles. These nanoparticles can be encapsulated inside gelatin microcapsules. In the presence of elevated levels of MMP-9, gelatin capsules degraded, resulting in drug release from the PLGA nanoparticles. With further optimization of the drug release profile and encapsulation efficiency, this MMP-9 enzyme triggered drug delivery system has great therapeutic potential for a variety of diseases.

References:

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