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In situ preparation of glycoconjugate hollow microspheres mimics the extracellular matrix via interfacial polymerization

Jing Wang, Changsheng Liu*, Ping Chi

Key Laboratory for Ultrafine Materials of Ministry of Education, and Engineering Research Center for Biomedical Materials of Ministry of Education, East China University of Science and Technology, Shanghai 200237, PR China

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ABSTRACT

A novel chitin-*graft*–polyleucine microsphere with hollow construction was prepared via ring-opening polymerization initiated with chitin based on interfacial protocol. The FT-IR and ^{1}H NMR analysis demonstrated the conjugation with regulated graft length. The study provided a facile one-step route to obtain microsphere with glycoconjugation structure. This hybrid polysaccharide–polypeptide microsphere may give promising application in drug delivery and tissue engineering.

1. Introduction

Hollow microspheres, which contain a cavity, have attracted particular attention in recent years owing to their specific applications such as carriers in drug delivery, encapsulations for artificial cells and protection of proteins, enzymes, or DNA [1–3]. Various efforts have been made to prepare both inorganic and polymeric hollow microspheres. Templating method, including the surface-grafting technique and layer-by-layer assembly, is one of the most common methods for preparing hollow spheres, but the application of this approach is limited because the process requires multiple steps in a sequential manner [4,5] whereas in most cases the inclusions of sensitive molecules that need to be encapsulated are not suitable for multiprocessing and must avoid being destroyed. Moreover, the polymerization in vesicles is apt to fail due to the intrinsic lack of compatibility or thermodynamic adjustment of the polymer and the bilayer. The other dominant approach is self-assembly of block copolymers [6–8]. In order to be acceptable as drug carrier systems, polymeric vesicles have to fulfil different requirements for biocompatibility. In view of this, copolymers with biocompatible component and controllable biodegradability are particularly attractive [9–11].

Chitin is the second abundant natural polysaccharide and is known to be non-toxic, biocompatible and enzymatically biodegradable. Furthermore, it has been an excellent starting template for biomimetic design of glycopolymer biomaterials due to its glucosamine and *N*-acetylglucosamine repeated structures, which are found ubiquitously in glycosaminoglycans within the extracellular matrix (ECM) [12,13].

Herein, we report a simple approach to prepare novel polysaccharide–polypeptide hybrid microspheres with a hollow structure. The method is based on the ring-opening polymerization of amino acid *N*-carboxyanhydride (NCA) initiated by chitin, which had been reported by Kurita et al. [14,15]. Nevertheless, the previous study focused on the synthesis of the copolymer. The graft production had comparatively poor solubility in most of the common solvents, which hampered its further process and applications. Up to date, little work was done using this glycopeptide material to develop novel architectures. In this study, through designed interfacial polymerization protocol, the hollow microspheres could be prepared *in situ*. Owing to the gelating in the process, the particles are stable. To the best of our knowledge, there have been no reports of one-pot polymerization to prepare glycopeptide microspheres via an interfacial strategy under mild reaction conditions.

2. Experimental

2.1. Materials and reagents

All reagents used were available from commercial sources. Chitosan was obtained form Shanghai Kabo Co. Ltd. (China) with average molecular weight (Mw) of 1.5×10^5. The degree of deacety-

* Corresponding author. Tel.: +86 21 64251308.
E-mail address: feifeiwangj@yahoo.com.cn (C. Liu).

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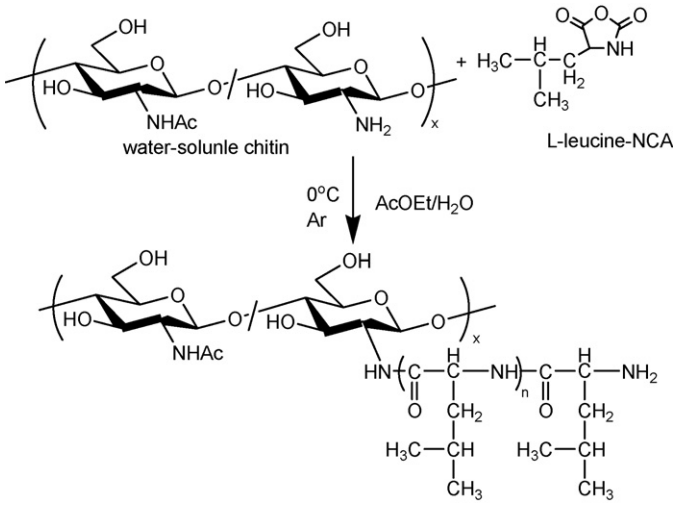

Fig. 1. Schematic illustration to show the reaction between water-soluble chitin and L-leucine *N*-carboxyanhydride (NCA).

lation (DD) was determined as 92% by ^{1}H NMR. L-Leucine was purchased from Huamei Biochemical Co. Tetrahydrofuran (THF) and *n*-hexane were distilled from sodium/benzophenone in an inert atmosphere to remove water before using. Ethyl acetate was distilled from P_2O_5. All other solvents were of analytical grade and used without further purification.

2.2. Synthesis of chitin-graft–polypeptide microsphere

Water-soluble chitin (WSC) was prepared according to the method reported [16]. L-Leucine NCA was synthesized by treating amino acid with triphosgene in THF at 50 °C, and recrystallized from THF/hexane [17].

The graft copolymerization of α-amino NCA onto chitin was carried out heterogeneously (Fig. 1). In a typical run, a given amount of the L-leucine NCA dissolved in dried ethyl acetate, together with span-80 as the foreign emulsifier at final concentration of 1 mg/ml, was subjected to the reaction with WSC at 0 °C under argon for 2 h. After that, the mixture was poured into large amount of DMF (DMF/H_2O = 10, v/v). The resulting grafting copolymer was filtered and washed with DMF/water for purification. The precipitate was then washed with acetone in grades and collected after lyophilization.

2.3. Cryo-breaking of hollow microspheres

Cryo-breaking technique was carried out under ultrasonification in liquid nitrogen medium [18] to obtain the broken vesicle, and details are as follows. Microspheres dispersed in DMF were frozen by immersing into liquid nitrogen medium and then partially melted by naturally exposing to the air. At this point, the microspheres were placed into an ultrasonification chamber, and the tip of the ultrasonicator was let to touch at the solid/liquid interface. The process was lasted for ca. 45–50 s. After that, the sample was ready for subsequent lyophilization.

2.4. Measurements

^{1}H NMR spectra of graft copolymers were obtained with an Avance 500 MHz spectrometer (Bruker Co., Germany) at room temperature, using CF_3COOD as solvent. The Fourier-transform infrared transmission spectra were recorded using an attenuated total reflection (ATR) method in AVATAR360 spectrometer (Nicolet, USA). Scanning electron microscopy (SEM) images were conducted on a field emission scanning electron microscope (JSM-6360LV, JEOL, Japan) with an acceleration voltage of 15 kV. The microparticles were lightly sputter coated a thin layer of Au/Pd prior to the observation. Optical microscope observation was performed on an Inverted Microscope (TE2000-U, Nicon Co., Japan).

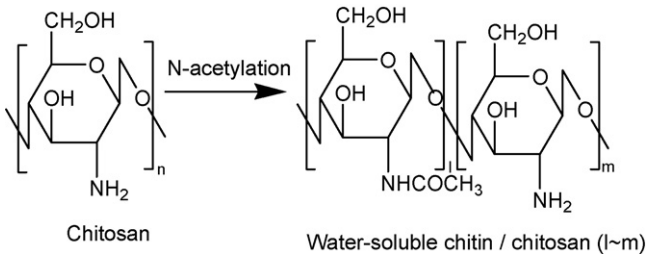

Fig. 2. Synthetic routes of partially deacetylated WSC from chitosan.

3. Results and discussion

3.1. Formation of glycoconjugate microsphere

Kurita et al. [14,15] have showed the possibility of preparing chitin–polypeptide complexes by the graft copolymerization of amino acid *N*-carboxyanhydride onto chitin. Ma and coworkers [19] either reported the synthesis of chitosan-*g*-poly(L-leucine). But up to now, only sparse studies have been carried out on the applications in biomedical field using this kind of polysaccharide–polypeptide hydrid materials. The comparative dissatisfactory solubility of the graft production might be the main obstacle for further process and application.

In this paper, the heterogeneous graft conditions and the insolvable graft productions were made use of. The solubility of chitosan was remarkably poorer because of its high crystallinity. Through acetylation (Fig. 2), partially deacetylated chitin with degree of deacetylation close to 0.50 could dissolve in water smoothly. This suggested the feasibility of preparing microsphere concurrently in the copolymerization.

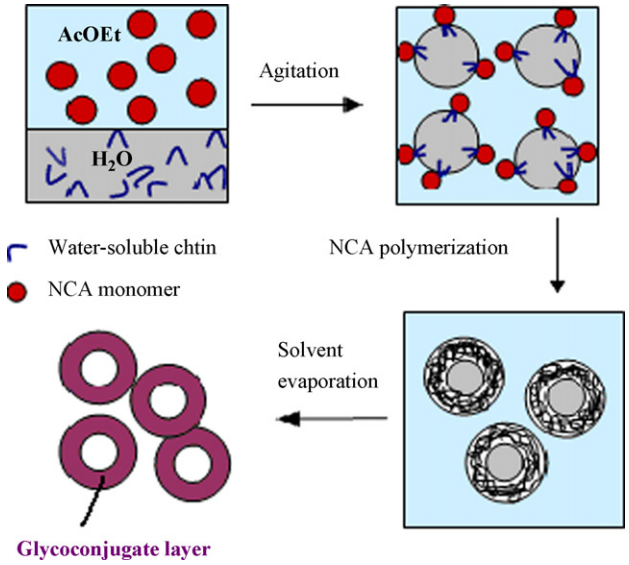

Fig. 3. Schematic procedure for the *in situ* synthesis of glycoconjugate hollow microspheres via interfacial polymerization approach.