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Nanostructured raspberry-like gelatin microspheres for local delivery of multiple biomolecules

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ABSTRACT

Multicompartment particles, which are particles composed of smaller building units, have gained considerable interest during the past decade to facilitate simultaneous and differential delivery of several biomolecules in various applications. Supercritical carbon dioxide (CO_2) processing is an industrial technology widely used for large-scale synthesis and processing of materials. However, the application of this technology for production of multicompartment particles from colloidal particles has not yet been explored. Here, we report the formation of raspberry-like gelatin (RLG) microparticles composed of gelatin nanoparticles as colloidal building blocks through supercritical CO_2 processing. We show that these RLG microparticles exhibit a high stability upon dispersion in aqueous media without requiring chemical cross-linking. We further demonstrate that these microparticles are cytocompatible and facilitate differential release of two different model compounds. The strategy presented here can be utilized as a cost-effective route for production of various types of multicompartment particles using colloidal particles with suitable interparticle interactions.

Statement of significance

Multicompartment particles have gained considerable interest during the past decade to facilitate simultaneous and differential delivery of multiple biomolecules in various biomedical applications. Nevertheless, common methods employed for the production of such particles are often complex and only offer small-scale production. Here, we report the formation of raspberry-like gelatin (RLG) microparticles composed of gelatin nanoparticles as colloidal building blocks through supercritical CO_2 processing. We show that these microparticles are cytocompatible and facilitate differential release of two model compounds with different molecular sizes, promising successful applications in various biomedical areas. Summarizing, this paper presents a novel strategy that can be utilized as a cost-effective route for production of various types of multicompartment particles using a wide range of colloidal building blocks.

1. Introduction

In nature, materials and organisms owe their functions to their hierarchical and complex structure [1–3]. Various studies [4] have aimed to mimic these natural structures to produce materials with advanced functionalities. The preparation of multicompartment particles has become a particularly promising biomimetic strategy during the past decade to improve the functionality [5,6]. In these particles, each individual compartment can act as a carrier for a different compound. Consequently, their multicompartmental structure can facilitate simultaneous and differential delivery of several biomolecules [7,8]. This capacity for multiple delivery of biomolecules is highly attractive for applications in pharmaceutics, cosmetics, agriculture, and food products. Various methods such as self-assembly, phase separation, and microfluidics have been employed for the production of such multicompartment particles [6]. Nevertheless, these methods are often complex and only offer small-scale production. Techniques based on the use of supercritical carbon dioxide (CO_2) have been widely exploited for large-scale

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synthesis and processing of materials [9]. These technologies have been applied for production of particles [10] and porous structures [11], and also utilized as a method for drying [12], sterilization [13] and encapsulation [14] of various compounds. Moreover, as opposed to conventional spray-drying techniques [15] which require high temperatures, supercritical CO_2 spray-drying can be performed at low temperatures allowing production of particles containing temperature-sensitive polymers and biomolecules [16,17]. Different types of biodegradable polymeric particles, such as poly lactic-co glycolic acid (PLGA), polycaprolactone (PCL), and polylactic acid (PLA), have been produced by means of supercritical CO_2 spray-drying [18]. Nevertheless, although the application of this technique for the production of particles from polymer solutions [10,18] or polymer-nanoparticle mixtures [19–21] has been widely studied, production of multicompartment particles from colloidal particles only has not been reported yet using this technique.

Here, we report the formation of raspberry-like gelatin (RLG) microparticles composed of gelatin nanoparticles as colloidal building blocks through supercritical CO_2 processing. We show that these RLG microparticles exhibit a high stability upon dispersion in aqueous media without requiring any chemical cross-linking. We investigate simultaneous incorporation of small and large model compounds, i.e. vancomycin and dextran, into the RLG microparticles and study their corresponding release kinetics. While vancomycin (MW = 1.5 kDa) used as a model therapeutic drug, dextran (MW = 20 kDa) is utilized as a model compound for growth factors. We further use two cell lines to assess the cytocompatibility of the RLG microparticles for biomedical applications. Based on these cytotoxicity and release studies, we reveal that these microparticles are cytocompatible and allow for differential release of multiple biomolecules. These results not only demonstrate the potential application of the RLG microparticles as vehicles for local delivery of multiple compounds, but can also pave the way for industrial production of various multicompartment particles using the supercritical CO_2 processing technique.

2. Experimental section

2.1. Materials

Gelatin A (from porcine skin, 300 Bloom), Glycine (≥99.0%), fluorescein isothiocyanate–dextran (FITC–Dextran; 20 kDa), vancomycin hydrochloride hydrate (potency ≥ 900 $\mu g \cdot mg^{-1}$), and alkaline phosphatase (ALP; lyophilized powder from bovine intestinal mucosa, ≥10 DEA units/mg solid) were purchased from Sigma–Aldrich. All other chemicals were of reagent grade and were used without any purification.

2.2. Synthesis of gelatin nanoparticles

Spherical gelatin nanoparticles were synthesized by a two-step desolvation method [22]. As the first step, 50 g of gelatin powder was dissolved in 1 L of MilliQ water at 50 °C. Thereafter, 1 L of acetone was added to the gelatin solution and the mixture was left for 1 h at room temperature to precipitate high molecular-weight gelatin. Afterward, the supernatant was discarded, the gelatin precipitates were dissolved in MilliQ water and lyophilized until further usage. For the second step, 3.75 g of the freeze-dried gelatin was dissolved in 75 mL of MilliQ water and the pH of solution was set to 2.5 using hydrochloric acid (5 M). Thereafter, 225 mL of acetone was added to the gelatin solution at 40 °C using a syringe pump with a dripping rate of 12 mL/min during vigorous stirring of the solution at 1000 rpm. In order to stabilize the gelatin nanoparticles by cross-linking, an excessive amount of 25 wt% glutaraldehyde solution (2 mL) was added to the gelatin dispersion at room temperature and left to react for ~16 h while stirring at 600 rpm. Thereafter, 300 mL of a 100 mM aqueous solution of glycine was added to the gelatin dispersion to block unreacted aldehyde groups. After 1 h of stirring, the dispersion was filtered with a nylon mesh (100 μm mesh size). Subsequently, the dispersion was washed with distilled water through four centrifugation (at 5000 rpm for 1 h) and redispersion steps. At last, the nanoparticles were dispersed in a 1:3 (V:V) mixture of acetone:water at a concentration of 8 mg/ml, and stored at 4 °C for further processing.

2.3. Production of RLG microspheres

A supercritical fluid spray-drying process [17] was used for processing of the suspensions of gelatin nanoparticles. In this process, supercritical CO_2 was used as an antisolvent for the gelatin nanoparticles and extraction medium for the solution. Considering the low miscibility of supercritical CO_2 and water, acetone was included in the solution to enhance the drying process. A scheme of the experimental setup has been illustrated in Fig. 1. The process was carried out by spraying of the suspension of gelatin nanoparticles into a high pressure vessel through the inner tube of a coaxial nozzle at a flow rate of 0.5 ml/min. Prior to the spraying of the suspension, the vessel was pressurized with CO_2 to 150 bar and its temperature was set to 40 °C. During the process, supercritical CO_2 was injected into the vessel at a flow rate of 30 kg/h through the outer tube of the coaxial nozzle. The pressure and temperature of the vessel were kept at 150 bar and 40 °C, respectively, and the produced particles were collected on top of a paper filter mounted at the bottom of the vessel. After spraying the suspension, the vessel was flushed for 30 min with fresh CO_2 to remove any remaining solvent and slowly depressurized to atmospheric pressure before opening and collecting the particles. Samples were stored at 4 °C in the dark before and after processing.

2.4. Incorporation of biomolecules into RLG microspheres

In order to incorporate vancomycin and FITC-dextran into the RLG microparticles, these compounds were dissolved in the suspension of gelatin nanoparticles prior to supercritical CO_2

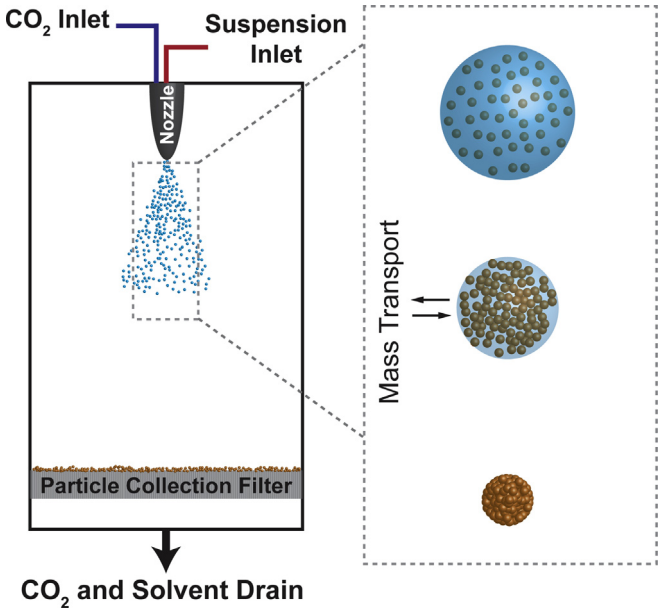

Fig. 1. Schematic illustration of the production setup and formation mechanism of the RLG microparticles.