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Construction of versatile multilayered composite nanoparticles from a customized nanogel template

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ABSTRACT

We present a highly adaptable design platform for multi-responsive, multilayered composite nanoparticles (MC-NPs) with fine-tunable functional layers. A flexible disulfide-linked nanogel template is obtained by a controlled *in-situ* gelation method, enabling a high degree of control over each successive layer. From this template, we optimize "smart" biomaterials with biofunctional surfaces, tunable drug release kinetics, and magnetic or pH-responsive functionality, fabricated into MC-NPs for targeted drug release and periosteum-mimetic structures for controlled rhBMP-2 release towards bone tissue formation *in-vivo*. Such a versatile platform for the design of MC-NPs is a powerful tool that shows considerable therapeutic potential in clinical fields such as oncology and orthopedics.

1. Introduction

Multilayered composite nanoparticles (MC-NPs) are gaining a significant interest in the pharmaceutical industry with applications ranging from controlled drug delivery to theranostic imaging [1–8]. The fabrication process typically involves layer-by-layer deposition of biologically functional surfaces onto silica, gold, or polystyrene templates, thereby leading to versatile and tunable compositions of NPs. These multilayered structures allow unprecedented control over shell-mediated biological interactions, demonstrated in the landmark work of Richtering et al. in the fabrication of stimuli-responsive microgels with unique core/shell behavior [9,10]. However, reported fabrication methods of MC-NPs to date, including condensation from vapor, chemical coating, and solid-state processes, offer little control over the properties of individual layers so that morphology, elasticity, and particle size cannot be independently adjusted for each layer [11–14]. The key challenge in the design flexibility and complexity of MC-NPs is the difficulty of creating a versatile template with easily adjustable properties.

Recently, we reported a controlled *in-situ* gelation method for fabrication of hydrogels and hydrogel particles [15,16]. Adjustment of the gelation time followed by seed emulsion allowed a high degree of control over each layer, yielding a biocompatible multilayered nanogels with tunable size, swelling capacity, and degree of crosslinking. Inspired from the facile fabrication of customized multilayered nanogels, we hypothesized that this general approach could be applied towards the production of multi-responsive MC-NPs. In this work, we first establish a versatile design platform for MC-NPs, where fabrication criteria included various stimuli-responsive layers, biofunctional surfaces, and adjustment of gelation times. With these fundamental understandings, we next specifically design "smart" MC-NPs responsive to magnetic and pH stimuli, and explore further applications as controlled-release rhBMP-2 carriers in periosteum-mimetic structures for bone

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tissue formation *in-vivo* (Fig. 1).

2. Materials and methods

2.1. Materials

1-(2-aminoethyl)piperazine (AEPZ, Aldrich), N,N'-bis(acryloyl) cystamine (BAC, Fluka), poly(ethylene glycol) methyl ether (Mn: 750, Aldrich), span80 (J&K) and tween80 (J&K) were purchased and used without further purification. α-Amino-ω-methoxy-poly(-ethylene glycol) was prepared from poly(ethylene glycol) methyl ether according to literature [17]. L-lactide and glycolide were purchased from PURAC (the Netherlands) and purified by recrystallization in ethyl acetate (Beijing Tonghua fine chemicals company) twice. Poly(lactic-*co*-glycolic acid) (PLGA) was prepared by ring-opening polymerization of L-lactide and glycolide under 65 Pa in sealed glass ampoules at 180 °C for 20 h in the presence of stannous octoate as catalyst (0.05 wt%). The raw PLGA (50/50) (85,000) (Molecular weights were determined by GPCmax VE-2001 gel-permeation chromatography) was purified by dissolving in chloroform and re-precipitation from ethanol, followed by drying in vacuum at room temperature for 48 h. The 1,4-dioxane and other reagents were obtained from Beijing Chemical Reagents Company, China and directly used without further treatment. The 1,4-dioxane used in here was not chromatographically pure. All other chemicals were purchased from Sigma-Aldrich.

2.2. Synthesis of hyperbranched poly(BAC2-AEPZ1)-PEG (BAP)

The hyperbranched poly(BAC2-AEPZ1)-PEG (BAP) was synthesized by a one-pot, two-step Michael addition polymerization [15]. In detail, BAC (3.0 mmol) was dissolved in 10 mL of methanol at room temperature. AEPZ (1.5 mmol) was added dropwise to the solution while stirring, followed by rinsing with 2 mL of methanol. The mixture was stirred at 50 °C for about 6 days. 2.3 mmol of α-amino-ω-methoxy-PEG (Mn = 750) was added stirred at 60 °C for one week to seal terminal vinyl groups. The product was precipitated from the reaction using 200 mL of diethyl ether under vigorous stirring. The polymer was collected and purified by re-precipitation from a methanol solution into 100 mL of acetone containing 5 mL of 37% concentration HCl followed by drying under vacuum at 50 °C for 24 h. A water soluble hyperbranched poly(-amido amine), poly(BAC2-AEPZ1)-PEG (BAP), was obtained as depicted in Fig. 2.

2.3. Preparation of loose and compact nanogels

The inverse mini-emulsion method was adopted for producing loose and compact nanogels (Fig. 3). In detail, decane was selected as the organic continuous phase, and a mixture of span80/tween80 (0.49 g/0.51 g) with a weight ratio of 49: 51 was used as surfactant. The organic mixture containing decane and surfactants was formulated with the decane/surfactant weight ratio of 19 g: 1 g. The aqueous solution was prepared by dissolving 10 mg of BAP in 25 μL of deionized water, and basified using 7.8 μL of 5 M Sodium hydroxide (NaOH). Then 32.8 μL of the aqueous solution was immediately added into 25 mL of the organic mixture, stirring at 700 rpm to yield a stable mini-emulsion. The emulsion was injected with 2 mL of deionized water to lower the pH and terminate gelation after a predetermined time ranging from 4 to 10 h. The emulsion was centrifuged at 1000 rpm for 1 min, and the upper surfactant and decane layers were discarded. The remaining liquid was dialyzed by deionized water to obtain pure, surfactant-free nanogels.

2.4. Preparation of nanogel/silica NPs

The template method was utilized for producing loose and compact double-layered nanoparticles. To prepare the template, decane was selected as the organic continuous phase, and a mixture of span80/tween80 (0.49 g/0.51 g) was used as surfactant. The organic mixture containing decane and surfactants was formulated with the decane/surfactant weight ratio of 19:1. The aqueous solution was prepared by dissolving 10 mg of BAP in 25 μL of deionized water, and basified using 7.8 μL of 5 M NaOH. Then 32.8 μL of the aqueous solution was immediately added into 25 mL of the organic mixture, stirring at 700 rpm to yield a stable mini-emulsion. The emulsion was injected with 2 mL of deionized water to lower the pH and terminate gelation after a predetermined

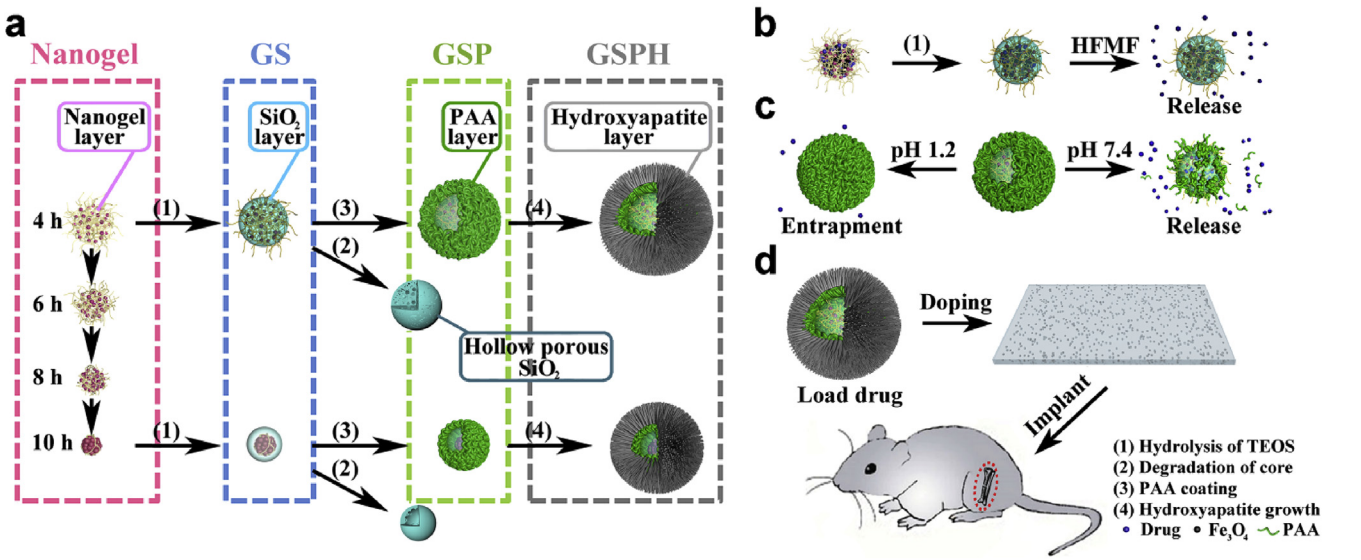

Fig. 1. The fabrication process of various multilayered composite nanoparticles. a) Controlled formation of the nanogel core, silica shell-nanogel core NPs (GS), pH-responsive poly-(acrylic acid) (PAA)-silica-nanogel NPs (GSP), and hydroxyapatite (HA) coated PAA-silica-nanogel NPs (GSPH). b) Magnetic-responsive release produced by magnetic silica-nanogel NPs under high-frequency alternating magnetic fields (HFMF). c) pH-responsive release of PAA-silica-nanogel NPs. d) *In-vivo* translation of multilayered composite nanoparticles toward periosteum-mimetic biomaterials for bone repair.

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