Colloids and Surfaces B: Biointerfaces 117 (2014) 322–329

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

A new approach for the one-step synthesis of bioactive PS *vs.* PMMA silica hybrid microspheres as potential drug delivery systems

A. Angelopoulou [a,b], E.K. Efthimiadou [a,*], N. Boukos [a], G. Kordas [a,*]

[a] *Laboratory for Sol-Gel, Advanced Materials, Physicochemical Processes, Nanotechnology & Microsystems, NCSR "Demokritos", 153 10 Ag. Paraskevi Attikis, Greece*

[b] *Department of Materials Science, School of Natural Sciences, University of Patras, 26 500 Patras, Greece*

ARTICLE INFO

Article history:
Received 14 August 2013
Received in revised form 25 February 2014
Accepted 27 February 2014
Available online 12 March 2014

Keywords:
Hybrid microspheres
Hollow silica microspheres
Bioactivity
Drug release
Hydroxyapatite

ABSTRACT

In this work, hybrid microspheres were prepared in a two-step process combining the emulsifier free-emulsion polymerization and the sol–gel coating method. In the first step, polystyrene (St) and poly(methyl methacrylate) (PMMA) microspheres were prepared as sacrificial template and in the second step a silanol shell was fabricated. The functionalized surface of the hybrid microspheres by silane analogs (APTES, TEOS) resulted in enhanced effects. The hollow microspheres were resulted either in an additional step by template dissolution and/or during the coating process. The microspheres' surface interactions and the size distribution were optimized by treatment in simulated body fluids, which resulted in the *in vitro* prediction of bioactivity. The bioassay test indicated that the induced hydroxyapatite resembled in structure to naturally occurring bone apatite. The drug doxorubicin (DOX) was used as a model entity for the evaluation of drug loading and release. The drug release study was performed in two different pH conditions, at acidic (pH = 4.5) close to cancer cell environment and at slightly basic pH (pH = 7.4) resembling the orthopedic environment. The results of the present study indicated promising hybrid microspheres for the potential application as drug delivery vehicles, for dual orthopedic functionalities in bone defects, bone inflammation, bone cancer and bone repair.

1. Introduction

Hollow microspheres with controllable size and shape have attracted enhanced technological importance due to their applications in catalysis, protection of biologically active agents, coatings on materials, and drug delivery systems [1–5]. For these applications, enhanced interest was expressed for the hybrid colloidal microspheres [6–11]. Among them, silica was principally studied owing to its high surface energy, spherical mesoporous structure and low toxicity. A wide variety of methodologies and template materials were employed for the synthesis of polymer@SiO_2 microspheres. The most conventional method for microspheres' production was based on emulsion polymerization in which a variety of polymers were used as template such as polystyrene (PS), poly(methyl methacrylate) (PMMA) and poly(methyl methacrylic acid) (PMMA) [12]. During this process, microspheres which were used as template were transferred into an aqueous/ethanol mixture solution for swelling. Following, the co-condensation of the silanol groups with tetraethoxysilane (TEOS) was carried out *via* an ammonia catalyzed sol–gel reaction on the template surface. In this way the core–shell microspheres were fabricated. Then, hollow silica microspheres were obtained generally by thermal degradation or by removing the core through selective dissolution in an appropriate solvent, such as dimethylformamide (DMF) [13,14], tetrahydrofurane (THF) [15–17] and toluene [18].

Particularly, in bone-related biomedical applications poly(methyl methacrylate) (PMMA) remained the most common bone substitute material, which was used in porous bone cements to reinforce fragile or broken bone bodies [19–23]. However, pure PMMA bone cements were characterized by poor bone bonding ability and mechanical strength [24,25]. Thus, reinforced PMMA cements were used, since they exhibited high modulus and good thermal stability, which provided substantial benefits in mechanical and thermal properties. As common reinforcing agents were used carbon, graphite, polyethylene, ultra-high molecular weight polyethylene, PMMA fibers, aramid, titanium,

* Corresponding authors at: NCSR "Demokritos", Advanced Materials, Physicochemical Processes, Nanotechnology & Microsystems, Aghia Parskevi Attikis, 15310, Athens, Greece.
E-mail addresses: elefth@chem.ims.demokritos.gr, elenefthimiadou@yahoo.com (E.K. Efthimiadou).

http://dx.doi.org/10.1016/j.colsurfb.2014.02.047
0927-7765/

bone spheres, tri-calcium phosphate, and hydroxyapatite [27–31]. Therefore, PMMA constituted a reliable material for use in silica based drug delivery vehicles with functionalities in bone disease such as defects, inflammations, and cancer.

In this work, the synthesis of hybrid sol–gel microspheres was performed *via* different organic cores. Specifically, PS@silica microspheres were prepared by a sol–gel coating process, followed by a dissolution step in a water/THF solution to decompose the organic moieties of the microspheres. The coating reaction of the PMMA@silica hybrids resulted in the one-step formation of microspheres with reduced mean diameter. The morphological characteristics (surface modification and size variations) of the above hybrids were evaluated by SEM, TEM and their structural characterization by FT-IR analysis. The comparative study of the physical properties was followed by the surface functionalization of PMMA microspheres with APTES. Hydroxyapatite formation and drug release study were also evaluated, aiming at investigating the potential bone regeneration or bone cancer therapy. The loading and release behavior of the hybrid microspheres was investigated in different treatment conditions.

2. Experimental procedure

2.1. Materials and reagents

All chemicals, which were used herein, were of analytical grade. Ammonia, anhydrous ethanol, tetra-ethoxysilane (TEOS, $Si(OC_2H_5)_4$ purity >98%), potassium persulfate (KPS), 3-amino-propyl-triethoxysilane (APTES, 99%) were purchased from Aldrich and used without further purification. Methyl methacrylate (MMA, 99%) and styrene (St, 99%) were double distilled under reduced pressure before use. Dry tetrahydrofuran (THF) was purchased by Aldrich. Doxorubicin HCl (DOX) was provided by Pharmacia & Upjohn and used as received.

2.2. Preparation of core–shell hollow Silica microspheres

2.2.1. Synthesis of PS@Silica microspheres

The preparation of anionic polystyrene microspheres, was followed at surfactant-free emulsion polymerization using the anionic initiator KPS, as described by Goodwin [26]. Briefly, PS spheres (of about 350 ± 20 nm diameter), were obtained using an analogy of styrene/water/KPS:1/10/3 (ml/ml/mg) at 70 °C under N_2. After 24 h, a conversion of 88% was accomplished. Then, the suspension was centrifuged at 6000 rpm for 5 min at 10 °C, for several times. The final reaction, was followed in an analogy of ethanol/water:9/1 (ml/ml) mixture at 50 °C, by hydrolyzing TEOS in the presence of 100 mg anionic PS spheres. Following, 2.4 ml aq. NH_3 30% were added and within 5 min 0.6 ml TEOS. At the neutral pH used the silica species possessed a slight negative charge. Therefore, the anionic PS spheres ensured the rapid capture of the silica species. This process ensured the formation of a smooth coating preventing the fabrication of silica byproducts, since the hydrolysis was a rapid process. After 2 h, 2.4 ml aq. NH_3 30% were added and the reaction was accomplished in 2 h. The final coated microspheres were obtained by centrifugation of the mixture (2000 rpm for 5 min at 10 °C) several times.

The PS core was removed by treating the hybrid microspheres in a mixture solution of water/THF:50/0.5 (ml/ml), at room temperature. After 48 h, the suspension was centrifuged at 1500 rpm for 10 min at 10 °C, and the microspheres were transferred to water for two extra centrifugation/dispersal steps.

2.2.2. Synthesis and functionalization of PMMA@Silica microspheres

For the preparation of anionic poly(methyl methacrylate) (PMMA) microspheres, surfactant-free emulsion polymerization was followed using the anionic initiator KPS. The obtained PMMA spheres (of about 320 ± 20 nm diameter), were prepared using a mixture of MMA/water/KPS:1/0.0075/1 (ml/ml/mg) solution at 70 °C. After 24 h, a conversion of about 92% was accomplished and the suspension was centrifuged at 8000 rpm for 5 min at 10 °C. The isolated solid was resuspended *via* ultra-sonic bath and re-centrifuged for three times. The coating reaction with TEOS was followed in a mixture solution of water/ethanol:9/1 (ml/ml) at 55 °C, by hydrolyzing TEOS in the presence of 100 mg anionic PMMA spheres and aq. NH_3 30%. Since, TEOS hydrolysis was a rapid process, in order to ensure the formation of a smooth coating and to prevent the formation of secondary silica spheres, excessive ammonia solution of 3.0 ml was used. The reaction was followed for 24 h and PMMA/silica microspheres were obtained by subsequent steps of centrifugation at 8000 rpm for 5 min at 10 °C following the above mentioned procedure. Two mechanisms were performed for PMMA dissolution, the first was during the silica layer formation by ammonia and the second was after treating the core–shell microspheres with THF.

Moreover, the functionalization with APTES, was accomplished through a coating reaction in a water/ethanol:15/50 (ml/ml) solution at room temperature, in the presence of 150 mg PMMA (or PMMA-silica) spheres for 24 h. The functionalized microspheres were obtained by centrifugation at 8000 rpm for 5 min at 10 °C. According to literature [46], APTES in basic aqueous solutions (where pH > 7.0) appeared in six- or five-membered intramolecular rings. Here, APTES presence increased the pH of the solution at about ~11 resulting in ring formations, which suppressed condensation of APTES. Moreover, the morphology of the amino-modified silica hybrid microspheres was thought to be primarily controlled by the hydrolysis of the ethoxide groups (-Si-OEt) of TEOS, which resulted in silanols (—Si—OH) terminal functional groups and their subsequent condensation. The potential brush morphology occupied by APTES molecules on the surface of the functionalized hybrid microspheres, was presented in Scheme S1 A (see in the SI). Probably, the APTES brush molecules enclosed water molecules or possessed many water molecules in the outside of the bend. This effect was probably attributed to the alkaline pH of the solution, in which practically all amino groups were neutral (—NH_2) and the silanol groups (—Si—OH) were in the anionic form Si—O^- [43].

2.3. Instrumentation

The morphology and average size of the microspheres before and after core removal and after SBF treatment was determined by scanning electron microscopy (SEM), using a Zeiss35 VP microscope with field emission electron gun (resolution 1.7 nm) 30 kV, coupled with an energy dispersive X-ray analyzer (EDX) for elements analysis, using a Philips Quanta Inspect (FEI Company, Eindhoven, the Netherlands) microscope with a W (tungsten) filament 25 kV. For SEM analysis, the samples were gold coated to reduce charging effect. The microspheres were characterized by Fourier transform infrared spectroscopy (FT-IR) using a Perkin Elmer Precisely Spectrum 100 Spectrometer equipped with an attenuated total reflectance (ATR). Infrared transmission spectra (FT-IR) were recorded from 400 to 4000 cm^{-1}.

Hybrid microspheres were studied before and after incubation in SBF solution by X-ray powder diffraction (XRD) and the patterns were recorded from 10° to 70° 2θ at a scan rate 0.02°/min. To estimate the crystallite size, the peak broadening of XRD reflection was used based on Scherrer's formula [48]: $X_s = (0.9\lambda)/(\mathrm{FWHM}\cos\theta)$,