Materials Science and Engineering C 77 (2017) 293–299

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Agar/gelatin bilayer gel matrix fabricated by simple thermo-responsive sol-gel transition method

Yifeng Wang *, Meng Dong, Mengmeng Guo, Xia Wang, Jing Zhou, Jian Lei, Chuanhang Guo, Chaoran Qin

School of Material Science and Engineering, Wuhan University of Technology, 122 Luoshi Road, Wuhan 430070, China

ARTICLE INFO

Article history:
Received 16 December 2016
Received in revised form 25 March 2017
Accepted 26 March 2017
Available online 27 March 2017

Keywords:
Gelatin
Agar
Biopolymer
Gel matrix
Thermally responsive sol-gel transition

ABSTRACT

We present a simple and environmentally-friendly method to generate an agar/gelatin bilayer gel matrix for further biomedical applications. In this method, the thermally responsive sol-gel transitions of agar and gelatin combined with the different transition temperatures are exquisitely employed to fabricate the agar/gelatin bilayer gel matrix and achieve separate loading for various materials (e.g., drugs, fluorescent materials, and nanoparticles). Importantly, the resulting bilayer gel matrix provides two different biopolymer environments (a polysaccharide environment vs a protein environment) with a well-defined border, which allows the loaded materials in different layers to retain their original properties (e.g., magnetism and fluorescence) and reduce mutual interference. In addition, the loaded materials in the bilayer gel matrix exhibit an interesting release behavior under the control of thermal stimuli. Consequently, the resulting agar/gelatin bilayer gel matrix is a promising candidate for biomedical applications in drug delivery, controlled release, fluorescence labeling, and bio-imaging.

1. Introduction

Currently, various gel matrices (e.g., beads and microspheres) have drawn considerable attention due to their potential applications in drug delivery, tissue engineering and drug release [1–4]. In particular, biopolymer-based gel matrices are considered to be attractive delivery systems in the biomedical field because of their favorable properties such as biocompatibility, biodegradability, and non-toxicity [5–9]. For instance, Poulain et al. employed a coacervation method to prepare microspheres based on inulin for controlled release of serine protease inhibitors [10].

Gelatin (a protein derived from collagen) exhibits a number of advantages such as biocompatibility, non-toxicity and low cost, and it is an excellent candidate for numerous biomedical applications [11–15]. It is worth mentioning that gelatin has thermally responsive sol-gel transition property. Specifically, when temperature of a gelatin solution is lowered below room temperature, protein coils convert to triple helices and a 3D network gel is progressively generated. On the other hand, when the temperature of the gel is raised above approximately 30 °C, a reverse transition of helix to coil takes place and the gel converts to a liquid [16–18]. The thermally responsive property of gelatin has been enlisted to develop gelatin-based microgels for drug delivery that enable a thermally triggered drug release [19]. For example, Curcio et al. produced a grafted thermo-responsive gelatin microsphere as a delivery system for drug release [20].

Agar is a natural polysaccharide extracted from seaweed, and it has also been widely used in the biomedical field for its good biocompatibility and non-toxicity. Interestingly, agar also shows the thermally responsive sol-gel transition property similar to gelatin. It has been reported that agar molecules exist as random coils when dissolved in water at high temperature (above 85 °C), but they form double helices and subsequently become a gel network at low temperature (30–40 °C) [21–25]. With respect to agar's biomedical applications, Kang et al. developed an agar bead which could be used as a carrier for oral delivery of a therapeutic agent [26].

It should be noted that the combined protein/polysaccharide system is promising for application in the biomedical field due to their biocompatibility, biodegradability, and non-toxicity [27]. Particularly, several researchers have paid attention to building combined matrices of agar and gelatin for biomedical applications. Lam et al. reported that formaldehyde-free agar/gelatin microcapsules containing berberine HCl and gallic acid could be used as a microencapsulation system for drug deliveries [21], and they also developed a green gelatin/agar microencapsulation system to improve potential antifungal activities of *Phyllanthus urinaria* [28]. Wakhet et al. studied agar/gelatin based cohydrogels, emulgels and bigels as vehicles for controlled drug release [29]. Consequently, the above-mentioned researches indicate that the agar/gelatin matrices have promising applications in the biomedical field, which deserves our further studies on this direction. However, to date little attention has been paid to utilizing the thermally responsive sol-gel transitions of agar and gelatin as well as their different transition temperatures to fabricate a combined agar/gelatin gel matrix to perform further biomedical applications.

* Corresponding author.
E-mail address: yifengwang@whut.edu.cn (Y. Wang).

http://dx.doi.org/10.1016/j.msec.2017.03.254
0928-4931/

Here, we attempt to develop a novel, simple and environmentally-friendly method to generate a "green" polysaccharide/protein gel matrix for biomedical applications. In particular, the thermally responsive sol-gel transitions of agar and gelatin combined with their different transition temperatures are exquisitely used for generating the gel matrix, then achieving further applications in separate loading. It is noteworthy that using this method we can fabricate the gel matrix with two different biopolymer environments (a polysaccharide environment vs a protein environment). In contrast to a single-layer matrix, this type of bilayer matrix offers a convenient means to respectively load diverse materials (e.g., drugs, nanoparticles, and fluorescent materials) in different layers, which is beneficial to retaining the properties (e.g., magnetism, and fluorescence) of loaded materials and reducing mutual interference. Interestingly, the release switch of loaded materials in the different layers can be triggered under the control of thermal stimuli, thus the developed agar/gelatin bilayer gel matrix provides attractive applications in drug delivery, controlled release, bio-labeling, and bio-imaging.

2. Material and methods

2.1. Chemicals and materials

Gelatin (from porcine skin), agar (1300 g/cm^2 gel-strength), sodium salicylate, aniline blue, methyl orange, methyl red and rhodamine B were purchased from Sinopharm Chemical Reagent Co., Ltd., China. Other chemicals were obtained from commercial resources in China. All chemicals were of analytical grade and were not purified before use.

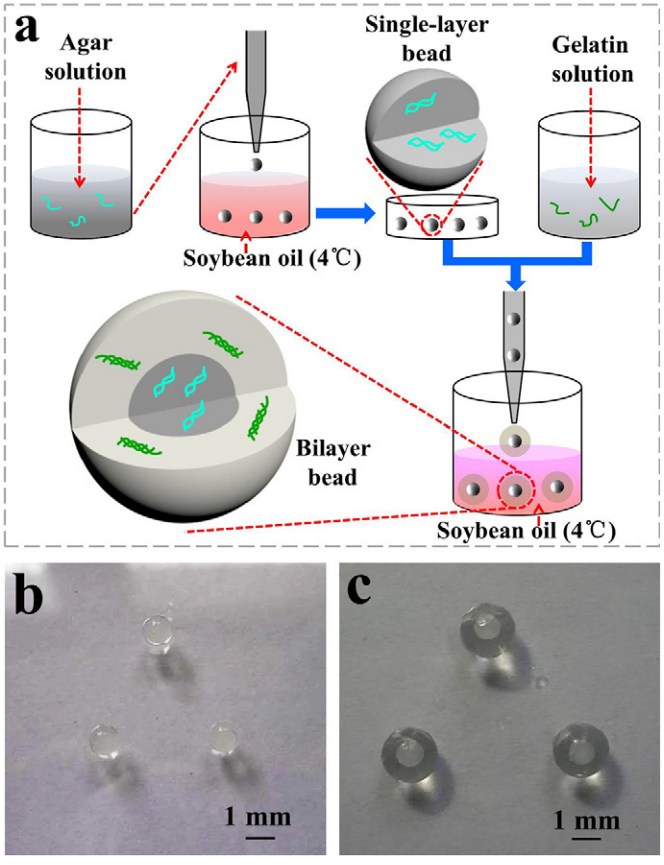

Fig. 1. (a) Schematic illustration of preparation process of the agar/gelatin bilayer gel matrix. (b) Photograph of single-layer agar beads. (c) Photograph of agar/gelatin bilayer beads.

2.2. Preparation of agar/gelatin bilayer gel matrix

After a series of comparison experiments, the most suitable solution concentrations and the optimized preparation conditions were chosen in the following experiments. Initially, an agar solution (5% w/v) was prepared by adding 5.0 g of agar to 100 mL distilled water with stirring for 30 min at 95 °C. Next, a specific volume (e.g., 50 μL) of the agar solution was added dropwise to a cold soybean oil (4 °C) using a pipette. The droplets of the agar solution were incubated in the cold soybean oil for about 20 min until they formed stable single-layer agar beads. In addition, these agar beads were carefully cleaned using distilled water and ethanol respectively, then stored in a 4 °C refrigerator.

Subsequently, a gelatin solution (10% w/v) was prepared by adding 10.0 g of gelatin to 100 mL distilled water with stirring for 30 min at 40 °C, then the above single-layer agar beads were mixed in the gelatin solution. Next, the mixture (containing agar beads) were added dropwise to a cold soybean oil (4 °C) using a pipette, and the droplets were allowed to be incubated in the cold soybean oil for about 20 min until they converted into stable agar/gelatin bilayer beads. Finally, these agar/gelatin bilayer gel beads were cleaned using distilled water and ethanol respectively, and stored in the 4 °C refrigerator.

2.3. Loading studies

We first loaded magnetic Fe_3O_4 nanoparticles in the inner layer of agar/gelatin bilayer gel matrix. The magnetic Fe_3O_4 nanoparticles well dispersed in water were prepared using a previously reported method [30], then these Fe_3O_4 nanoparticles were dispersed in the agar solution (5% w/v) with continuously stirring at 95 °C. Next, the agar solution containing Fe_3O_4 nanoparticles was added dropwise to the cold soybean oil (4 °C), and the droplets were incubated in the cold soybean oil until they converted to stable single-layer gel beads, then these single-layer beads were cleaned using distilled water and ethanol respectively. In addition, the single-layer beads containing Fe_3O_4 nanoparticles were mixed in the gelatin solution (10% w/v), and the mixture was added dropwise to the cold soybean oil (4 °C), then the droplets were incubated in the soybean oil to form bilayer gel beads containing Fe_3O_4 nanoparticles in the inner layer. Finally, these bilayer beads were cleaned using distilled water and ethanol respectively, and stored in the 4 °C refrigerator. The surface morphology of the agar/gelatin bilayer bead containing Fe_3O_4 nanoparticles was observed by a scanning electron microscopy (Ultra Plus, Zeiss, Germany). The swelling ratio of agar/gelatin bilayer bead was measured in PBS solution (pH = 7.4) by a previously reported method [31,32].

Furthermore, we separately loaded two different fluorescent materials (rhodamine B and carbon dots) in the different layers of agar/gelatin bilayer gel matrix. The carbon dots well dispersed in water were produced by our previously reported approach [33]. Initially, rhodamine B was dissolved in the agar solution (5% w/v), and the carbon dots were dispersed in the gelatin solution (10% w/v). Then, bilayer gel beads containing rhodamine B (in the inner agar layer) and carbon dots (in the outer gelatin layer) were produced by the above-mentioned method. Using the same method, we hierarchically loaded different dyes (aniline blue and methyl orange) in the different layers of bilayer beads (aniline blue in the inner agar layer, and methyl orange in the outer gelatin layer).

2.4. Release studies

We further investigated the release behavior of the agar/gelatin bilayer gel matrix loaded with different materials under the control of thermal stimuli. In the first release experiment, Fe_3O_4 nanoparticles were dispersed in the agar solution (5% w/v) while rhodamine B was dissolved in the gelatin solution (10% w/v), then the bilayer beads containing Fe_3O_4 nanoparticles (in the inner agar layer) and rhodamine B (in the outer gelatin layer) were generated by the above method.