

# Drug-loaded microparticles prepared by the one-step deposition of calcium carbonate/alginate onto cotton fabrics

Huan-Ling Wu,<sup>1,3</sup> Xiao-Xiao Hou,<sup>1</sup> Christopher Branford-White,<sup>2</sup> Xiao-Zhu Sun,<sup>1</sup> Lei Tao,<sup>1</sup> Syeda Um-I-Zahra,<sup>1</sup> Li-Min Zhu<sup>1</sup>

<sup>1</sup>College of Chemistry, Chemical Engineering, and Biotechnology, Donghua University, Shanghai 201620, People's Republic of China

<sup>2</sup>Institute for Health Research and Policy, London Metropolitan University, London N7 8DB, United Kingdom <sup>3</sup>Department of Light Chemical Engineering, Yancheng Institute of Industry Technology, Yancheng 224005, People's Republic of China

Correspondence to: L.-M. Zhu (E-mail: Izhu@dhu.edu.cn)

**ABSTRACT**: Calcium carbonate  $(CaCO_3)/alginate inorganic-organic hybrid particles were synthesized and deposited on to the surface of cotton fabrics with a novel one-step procedure. The effects of the Ca<sup>2+</sup>/CO<sub>3</sub><sup>2-</sup>/alginate molar ratio on the cotton matrix were investigated. The optimization of the process resulted in a regular shaped hybrid microparticles, and scanning electron microscopy revealed that the particles were uniformly distributed on the surface of the fibers. Dynamic light scattering showed that the particles were about 2 <math>\mu$ m in diameter. Moreover, transmission electron microscopy images demonstrated that the core-shell structure of the particles existed along with CaCO<sub>3</sub> evenly enfolded into the alginate layer. An X-ray diffraction pattern displayed that the alginate/CaCO<sub>3</sub> hybrid microparticles were a mixture of calcite and vaterite crystal. Fourier transform infrared spectroscopy indicated that CaCO<sub>3</sub>/alginate hybrid particles formed *in situ* were the only deposited materials. The thermogravimetric analysis curve indicated a certain mass ratio of the alginate and CaCO<sub>3</sub> in the hybrid particles. Furthermore, the drug-loading and drug-release properties of the hybrid microparticles and the drug release could be effectively sustained. Finally, both of the microparticles and modified fabrics had good cytocompatibility. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42618.

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# INTRODUCTION

Presently, a large number of dressings are used to treat chronic wounds and infections, such as burns, chronic ulcers, and bedsores.<sup>1</sup> According to modern knowledge, the maceration or spread of the exudate to the vicinity of the wound occurs because of skin softening and constant wetting. The skin turns white and can easily get infected by bacteria and fungi; this is a prime cause of contamination.<sup>2</sup> Thus, the main objective of any dressing is to keep the wound environment uncontaminated from infectious agents and provide the appropriate conditions for healing. An ideal wound dressing should have several other features: gas permeability, durability, antibacterial efficacy, nontoxicity, and a moderate water vapor transmission rate.<sup>3–13</sup>

Successful wound treatment can only be achieved by the assurance and maintenance of hydrophilicity and an effective antimicrobial activity.<sup>14–17</sup> Many superhydrophilic synthetic materials have several disadvantages; this includes their inability to be effectively modified, which enhances the antimicrobial properties and reduces irritation to human skin, and their high production cost.<sup>18</sup> Among a variety of wound dressings, organic antibacterial materials are often less stable, particularly at high temperatures and/or pressures compared to inorganic agents.<sup>19,20</sup> Indeed, inorganic materials such as metal and metal oxides have attracted a lot of attention because of their ability to withstand harsh processing conditions.<sup>21</sup> Many heavy metals (Hg<sup>2+</sup> and Pb<sup>2+</sup>) have antimicrobial activity and also toxic properties, although some other metal ions also remain unsafe (Cu<sup>2+</sup>, Ag<sup>+</sup>, and Zn<sup>2+</sup>).<sup>22–24</sup>

Calcium carbonate (CaCO<sub>3</sub>) is a kind of nontoxic rock biomineral that has biocompatible and biodegradable properties. Moreover, CaCO<sub>3</sub> microparticles are also considered a suitable drug carrier because of their large surface area and ability to load various drugs.<sup>25–28</sup>

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Alginate is a hydrophilic polysaccharide, a biomaterial that is produced by algae, and it has found several applications in biomedical science because of its promising properties, including its biocompatibility, biodegradability, and ease of gelation. It is nontoxic and used as a moist healing material in wounding healing, drug delivery, and other tissue engineering applications. In addition, alginates have been found to be helpful in achieving successful long-lasting healing effects.<sup>29,30</sup>

Previous studies have demonstrated that the inorganic–organic hybrid structure could enhance the mechanical strength and controlled release ability of alginate beads.<sup>31–35</sup> Compared with other hybrid materials, CaCO<sub>3</sub>-based hybrid materials have shown promising potential for the development of smart drug carriers<sup>36</sup> because of their ideal pH sensitivity, biocompatibility, and biodegradability properties. The coating of cotton fabrics, for example, the pad–dry–cure method,<sup>37–42</sup> radiation, and thermal treatment<sup>43–46</sup> have been previously described. However, various issues have been raised that relate to these systems.<sup>47</sup>

In this study, we developed a simple method for preparing cotton wound dressings with antibacterial properties by immobilizing CaCO<sub>3</sub>/alginate hybrid microparticles on fabrics surface with coprecipitation. Chemical coprecipitation has been proved to be an effective and simple technique for the synthesis of many materials.<sup>48,49</sup> In this study, we outlined the generation of CaCO<sub>3</sub>/alginate hybrid microparticles and the successful *in situ* adhesive properties of alginate to cotton fabric involving a onestep process. The whole reaction process was ecofriendly, and the materials used were nontoxic.

#### **EXPERIMENTAL**

# Materials

Twill-weave, 100% cotton woven fabric (119 g/m<sup>2</sup>, warp density = 50 threads/cm, and weft density = 31 threads/cm) was purchased from Slovenian Textile Co. In the pretreatment process, the fabric was bleached in an  $H_2O_2$  bath, immersed in an NaOH solution, then neutralized with diluted HCl, washed with double-distilled water, and then dried at 60°C *in vacuo*.<sup>50</sup>

A hydrophilic polysaccharide, sodium alginate (SA; viscosity = 0.02 Pa s in a 1% aqueous solution at 20°C; Aladdin Chemistry Co., Ltd.), was used as a stabilizing agent, and a reactive organic/inorganic binder in combination with CaCO<sub>3</sub> was used to form hybrid particles. Anhydrous CaCl<sub>2</sub> (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) and anhydrous Na<sub>2</sub>CO<sub>3</sub> (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) were used as reactants. All of the solutions were prepared in double-distilled water.

Pig iliac endothelial cells (PIECs) were obtained from China Center for Typical Culture Collection (Wuhan, China). The medium for cell culture included Dulbecco's modified Eagle's medium (DMEM; Gibco), and the cells were incubated at  $37^{\circ}$ C in humidified air/5% CO<sub>2</sub>. Dimethyl sulfoxide (DMSO) and pancreatic enzyme were purchased from Sigma (St. Louis, MO). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT) was obtained from GIBCO-BRL (Gaithersburg, MD). Diclofenac sodium (DS; Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) was used as a model agent to study the active properties of the drug in the hybrid microparticles that were coated on the cotton.

# Preparation of the Alginate/CaCO<sub>3</sub> and Alginate/CaCO<sub>3</sub>/DS Microparticles

Volumes of 20 mL of SA (5 g/L) and 40 mL of Na<sub>2</sub>CO<sub>3</sub> (0.5M) were mixed and stirred for 30 min; 5 mL of CaCl<sub>2</sub>(0.1M) was then added and rapidly stirred for 5 min. The suspension was clarified by centrifugation (9000 rpm for 15 min), then precipitated, washed with water (three times), and dried at 40°C to obtain the hybrid microparticles. Various  $CO_3^{2-}/Ca^{2+}$  molar ratios (40 : 1, 50 : 1, 60 : 1, 70 : 1, and 80 : 1) were used to evaluate the effect of the particle size of the prepared hybrid material.

Together with the previous solution, 20 mL of SA solution and 40 mL of  $Na_2CO_3$  solution were mixed and stirred for 30 min. At the same time, DS (0.025, 0.05, 0.075, and 0.1 mg) was dissolved in a 5 mL CaCl<sub>2</sub> solution and stirred for 10 min. Alginate/CaCO<sub>3</sub>/DS microparticles were prepared by the rapid addition of CaCl<sub>2</sub> and DS in the mixture of  $Na_2CO_3$  SA. This cocktail was stirred, centrifuged, washed, and dried as done previously. This preparation was regarded as the drug-loaded hybrid particles.

#### Preparation of Cotton Fabrics Coated with Alginate/CaCO<sub>3</sub>

The modification of the cotton fabric was performed in a onestep process. Cotton ( $6 \times 6 \text{ cm}^2$  pieces), 20 mL of SA (5 g/L), and 40 mL of Na<sub>2</sub>CO<sub>3</sub> were mixed for 30 min at room temperature. A volume of 5 mL of CaCl<sub>2</sub> (0.1*M*) was added, continuously stirred for 5 min, and then dried at 120°C. In this way, drug-loaded cotton fabrics were prepared.

# *In Vitro* Drug Release of Cotton Fabrics Coated with Alginate/CaCO<sub>3</sub>/DS

Cotton coated with alginate/CaCO<sub>3</sub>/DS was placed in 100 mL of phosphate buffer (pH 6.4) and shaken in a water bath. The temperature was kept at 37°C, and the oscillation rate set at 110rpm. A volume of 4 mL of release media were removed at regular intervals, and the volume was kept constant by the addition of fresh phosphate buffer. The amount of released drug was measured with an ultraviolet spectrophotometer (UV-2102, Unico Instrument Co., Ltd., Shanghai, China). The amount of drug release was analyzed through the standard curve of DS.

# Evaluation of the In Vitro Cytocompatibility

**Cytotoxicity Evaluation of the Microparticles.** PIECs in complete medium [DMEM containing 10% fetal bovine serum (FBS), 100 U/mL penicillin/streptomycin; volume = 180  $\mu$ L] were seeded directly in a 96-well plate (1 × 10<sup>4</sup> cells/well) and put in an incubator for 24 h (37°C, 5% CO<sub>2</sub>). Then, 20  $\mu$ L of the freshly prepared solutions of the alginate/CaCO<sub>3</sub> and alginate/CaCO<sub>3</sub>/DS hybrid microparticles at a given concentration were added to each well. PBS buffers (20  $\mu$ L) were added to the control group. All of the PIECs were incubated in a incubator (37°C, 5% CO<sub>2</sub>) for 24 h. Then, the culture plates were taken out of the incubator. The used DMEM in every well was replaced by 180  $\mu$ L of DMEM and 20  $\mu$ L of MTT solutions. After incubation for 4 h, 200  $\mu$ L of DMSO was added to each well and shaken for 30 min at room temperature. The optical density (OD) values of the purple solution were measured at



Table I. Sizes of the Hybrid Microparticles with Different Compositions

| Composition                | CO <sub>3</sub> <sup>2-</sup> /Ca <sup>2+</sup><br>(mol/mol) | SA (mL) | Size (µm) |
|----------------------------|--|---------|-----------|
| CaCO <sub>3</sub>          | 40   | 0       | 2.4143    |
| $Alginate/CaCO_3$          | 40   | 10      | 1.7783    |
| $Alginate/CaCO_3$          | 50   | 10      | 1.5899    |
| $Alginate/CaCO_3$          | 60   | 10      | 1.2145    |
| $Alginate/CaCO_3$          | 70   | 10      | 0.9862    |
| $Alginate/CaCO_3$          | 40   | 20      | 1.9023    |
| $Alginate/CaCO_3$          | 40   | 30      | 2.0241    |
| Alginate/CaCO <sub>3</sub> | 40   | 40      | 2.2702    |

570 nm with a microplate reader (Multiskan, ThermoFisher). Finally, cell viability could be calculated by the OD values.

Cytotoxicity Evaluation of the Untreated and Modified Fabrics. Three groups of untreated and modified cotton fabrics were located in 24-well plates, and another group without fabric was set as a control. Then, the culture plates were sterilized by an alcohol steam for 4 h, and a PBS solution was used to wash the residual alcohol. After being soaked by DMEM, all of the culture plates were put in an incubator for 24 h (37°C, 5% CO<sub>2</sub>). After that, 200  $\mu$ L of the PIEC suspension with a cells density of 1.0  $\times$ 10<sup>4</sup> cells/mL was seeded to each well with DMEM (containing 10% FBS) and then incubated in an incubator (37°C, 5% CO<sub>2</sub>). The time points of the test were set as 1, 3, and 5 days. At each point, the culture plates were taken out of the incubator. The used DMEM in every well was replaced by 360  $\mu$ L of DMEM and 40  $\mu$ L of MTT solutions. After incubation for 4 h, 400  $\mu$ L of DMSO was added to each well, and the plates were shaken for 30 min at room temperature. Afterward, the solutions in each well were transformed into 96-well plates. Finally, the OD values of the purple solution were measured at 570 nm with a microplate reader (Multiskan, ThermoFisher).

# Characterization with Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), X-ray Diffraction (XRD), Fourier Transform Infrared (FTIR), and Thermogravimetric Analysis (TGA)

The size distribution of the microparticles was determined with a laser light scattering (LLS) system (BI-200SM, Brookhaven) combined static laser scattering and DLS. The measured particle sizes were the average diameters.

The morphology of the microparticles and modified fabrics were characterized with a JSM-5600LV scanning electron microscope (JEOL, Tokyo, Japan).

To prepare the TEM samples, a drop of the diluted microparticles was placed onto a 400-mesh copper grid and dried at room temperature. The samples were viewed at 80 kV with a JEM-2100 electron microscope (JEOL, Ltd., Japan).

XRD measurements were performed on a Bruker D&Advance X-ray powder diffractometer with a graphite monochromatized Cu K $\alpha$  ( $\lambda = 0.15406$  nm). A scanning rate of 0.05°/s was applied to record the pattern in the 2 $\theta$  range of 10–70°.

The FTIR spectra of the untreated and chemically modified cotton fabrics were recorded on a Nicolet-Nexus 670 FTIR spectrometer (Nicolet Instrument Corp., Madison, WI) in the wavenumber range 500–4000 cm<sup>-1</sup>; the spectra were collected at a  $2\text{-cm}^{-1}$  resolution with 128 scans by the preparation of KBr pellets with a 3 : 100 sample-to-KBr ratio.

Thermogravimetric data were recorded on a TG209F1 thermogravimetric analyzer (TA Instruments Co., Delaware) from 20 to 900°C at a heating rate of 20°C/min under a nitrogen atmosphere.

# **RESULTS AND DISCUSSION**

# Size of the Hybrid Microparticles

To obtain spherical CaCO<sub>3</sub> microparticles, SA, a negatively charged polysaccharide, was added to the reaction mixture during the preparation to control the crystallization and growth behavior of the CaCO<sub>3</sub> particles. The size of microparticles was measured with DLS. In Table I, it is clearly demonstrated that the size of the microparticles was between 1 and 2.5µm, and alginate/CaCO3 is smaller than CaCO3 particles. This indicated that alginate modification decreased particle size and could attribute to SA acting as a stabilizing agent that retards the growth of the hybrid macroparticles. And, it also showed that microparticles with a  $CO_3^{2-}/Ca^{2+}$  ratio of 70/1 are smaller in size compared to  $CO_3^{2^-}/Ca^{2^+}$  (ratio of 50/1). Thus it appears that the alginate/CaCO3 microparticles may be more condensed due to the higher  $CO_3^{2-}$  content, so resulting is a decrease in size. The same phenomenon could explain the effect of different concentrations of alginate on the size of the hybrid microparticles. The higher the content of the alginate was, the smaller the condensed structure of the hybrid material was.<sup>51</sup>

# SEM

Chemically modified alginate/ $CaCO_3$  hybrid microparticles and untreated cotton fabric samples were analyzed with a JSM-5600LV scanning electron microscope. The SEM images are shown in Figure 1.



**Figure 1.** SEM images of the (a) alginate/ $CaCO_3$  hybrid microparticles, (b) untreated cotton fabrics, (c) chemically modified cotton fabrics, and (d) chemically modified cotton fabrics at a higher magnification.



Figure 2. TEM images of the alginate/CaCO<sub>3</sub> hybrid microparticles.

The SEM images in Figure 1 demonstrate the dimensions and morphology of alginate/CaCO3 and these chemically modified cotton fabrics. Figure 1(a) demonstrates that the hybrid microparticles were in a regular spherelike form with a size about 2.0  $\mu$ m, whereas Figure 1(c) reveals that the surface roughness was greater than that of the untreated fabric material because of the coated microparticles, as shown in Figure 1(b-d). Together with those shown in Figure 1(c), the spherical alginate/CaCO<sub>3</sub> particles were evenly distributed on the fiber surface, probably even in the presence of a layer of alginate on the surface of the cotton fabric [Figure 1(d)]. As a biocompatible macromolecule, the added alginate played an important role. It could not only help to get spherical microparticles with a narrow size distribution through its control over the crystallization process but could also be beneficial to maintain the biocompatibility of the microparticles.<sup>52,53</sup> In addition, as alginate is composed of anionic and hydrophilic polysaccharides, it has been broadly applied in the pharmaceutical area to increase the entrapment efficiency and promote sustained drug release. Through these observations, we concluded that the existence of alginate was beneficial for fabric performance, even though its form was in microparticles or covered on the surface of the fabric.

# TEM

The TEM images of the alginate/CaCO<sub>3</sub> particles are shown (Figure 2).

The hybrid microparticles [Figure 2(a)] containing less alginate appeared to be more condensed [Figure 2(b)], and this was in agreement with the SEM observations. From these images, the more detailed structure of the alginate/CaCO<sub>3</sub> microparticles was analyzed. We concluded that alginate and CaCO<sub>3</sub> uniformly formed inorganic–organic hybrid particles; this may have favored drug loading and sustained release because of the porous and loose structure of the microparticles. Figure 2(c,d) presents a small part of the outer surface of the microparticles. The image in Figure 2(d) was taken after that of the particles in Figure 2(c), which were irradiated. As shown by a comparison of the two images, the alginate/CaCO<sub>3</sub> microparticles in Figure 2(d) were lighter than those in Figure 2(c). We assumed that these microparticles lost the alginate, which had been melted by irradiation.

# XRD

The change on the crystal shape and the dispersion state of  $CaCO_3$  after the addition of SA in the hybrid microspheres was assessed by XRD, as shown in Figure 3.

As shown in Figure 3, the diffraction peaks were consistent with the corresponding standard diagram of  $CaCO_3$ . The XRD patterns showed that the product was mostly composed of the calcite phase; we observed that the (012), (104), (006), (100), (202), (024), (018), (116), and (122) facets of the  $CaCO_3$  calcite. Additionally, the products also might include a small quantity of vaterite from the corresponding diffraction peaks of the (100), (101), (102), (110), and (104) facets of the  $CaCO_3$  vaterite. These results imply that the alginate/ $CaCO_3$  hybrid microparticles were a mixture of calcite and vaterite crystal and displayed good crystallinity from the peaks. The calcite is the main component because the peak intensity of calcite is much more stronger than that of the vaterite.

Furthermore, we concluded that the alginate had an important impact on the crystal form of the CaCO<sub>3</sub> phase.<sup>54,55</sup>

#### FTIR Spectroscopy

To further support the coating mechanism, the FTIR spectra of the untreated and chemically modified cotton fabrics are given (Figure 4).

Both spectra presented characteristic bands of cellulose where the alginate/CaCO<sub>3</sub>-coated fabric showed additional sharp single bands at 1031 and 1418 cm<sup>-1</sup>; these were attributed to the C—OH vibrational band and  $CO_3^{2-}$  antisymmetric stretching vibration band. Another sharp band at 3442 cm<sup>-1</sup> was due to the —OH vibrational band. Thus, these results confirm that alginate/CaCO<sub>3</sub> hybrid microparticles existed, and their coating was a physical phenomenon regardless of the chemical interaction of groups.



Figure 3. XRD patterns of the alginate/CaCO3 hybrid microparticles.





**Figure 4.** FTIR spectra of the untreated (black line) and chemically modified cotton fabrics (red line). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

# TGA

TGA was used to determine whether the samples contained alginate. Depending on the content of SA, we identified the two varying TGA curves of alginate/CaCO<sub>3</sub> shown in Figure 5, where the remaining sample weight (percentage) is plotted as a function of the temperature. Samples 1 and 2 contained 15 and 10 mg of alginate, respectively. All other reaction conditions remained unchanged.

From the results of TGA, we observed that both of the curves showed two weight loss stages. One was probably due to the loss of biopolymer up to about 500°C. A subsequent loss in mass was observed at approximately 600°C; this was similar to the case of the pure CaCO<sub>3</sub> sample. TGA data for alginate and CaCO<sub>3</sub> were reported previously, where the weight loss between 150 and 500°C contributed to the decomposition of alginate;<sup>46</sup> that above 600°C was due to the decomposition of CaCO<sub>3</sub> into CaO and CO<sub>2</sub>. This was consistent with our observations. In addition, a distinct difference between the two samples was observed. The curve of sample 1 showed more loss of weight;



**Figure 5.** Thermogravimetric curves of the alginate/CaCO<sub>3</sub> hybrid microparticles containing different amounts of SA. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]



Figure 6. Air permeability rate of the different cotton fabrics.

this indicated the greater content of alginate than compared with that in sample 2. Further study confirmed that the alginate was involved in the  $CaCO_3$  particles, and the particles were  $CaCO_3$ /alginate hybrids.

#### Air Permeability

The air permeability is an important property of textiles; it influences the flow of vapor and air from an internal to an external environment. Because of this background, we considered the air permeability of the textiles. The permeability of the chemically modified alginate/CaCO<sub>3</sub> and untreated cotton fabric samples were evaluated with a digital-type main permeability tester (YG461E, Ningbo Textile Instrument Factory, Zhejiang, China).The pressure was set at 200 Pa with fabrics squares  $(11 \times 11 \text{ cm}^2)$ , and the results are shown (Figure 6).

The sample numbers on the *x* axis were the (1) untreated cotton fabrics, (2) CaCO<sub>3</sub>-coated cotton fabrics without alginate, (3)–(6) alginate/CaCO<sub>3</sub>-coated cotton fabrics (0.05 g of alginate,  $CO_3^{2-}/Ca^{2+}$  molar ratios = 40, 50, 60, and 70, respectively), and (7)–(9) alginate/CaCO<sub>3</sub>-coated cotton fabrics (molar ratio of  $CO_3^{2-}/Ca^{2+}$  = 40, alginate amounts = 0.1, 0.15, and 0.2 g, respectively).

Compared to the untreated and chemically modified cotton fabrics, we found that the air permeability of the coated cotton fabrics decreased. These results verify the presence of the coated layers on the cotton fabrics. Moreover, we also noted that the air permeability of the fabric decreased with increasing alginate; this implied that an alginate-enriched membrane was formed on the surface of the cotton fabrics. This was in agreement with the data from SEM. For the modified samples, the difference in the air permeability was marginal. Although the air permeability of the chemically modified samples decreased compared to that of the untreated cotton fabric, it appeared not to affect the properties of the fabrics when used as wound dressings.

#### **Tensile Tests**

The tensile mechanical properties of the alginate/CaCO<sub>3</sub>-coated and untreated cotton fabrics were studied (MTS 2/N). The strain rate was set to 100 mm/min, and the sample size was

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**Figure 7.** Tensile curve of the alginate/CaCO<sub>3</sub> and untreated cotton fabrics. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

 $200 \times 50 \text{ mm}^2$ . The samples were tested in the warp direction, and the results are shown (Figure 7).

The tensile force for the alginate/CaCO<sub>3</sub>-coated sample was 20% greater than that of the untreated cotton fabric. This was due to the three-dimensional (3D) mesh structure of the alginate film formed on the surface of the cotton fabric; this seemed to enhance the mechanical strength of the single yarn and also improve the holding force among the similar fiber. We concluded that the chemical modification of the cotton fabric had a positive effect on the mechanical properties of the yarns.

# *In Vitro* Drug-Release Curve of the Cotton Fabrics Coated with Alginate/CaCO<sub>3</sub>/DS

As we know, polysaccharides are highly hydrolyzed in water. Drugs with low molecular weights encapsulated in the polysaccharides can be diffused out quickly and easily; this leads to a low encapsulation efficiency and a fast drug-release rate for water-soluble drugs with low molecular weights. CaCO<sub>3</sub>-based hybrid systems can enhance the encapsulation efficiency and control the drug-release rate, according to previous studies.



Figure 8. *In vitro* drug-release curve of the cotton fabrics coated with alginate/CaCO<sub>3</sub>/DS microparticles.



Figure 9. Cell viability rate of the PIECs after the addition of microparticles for 24 h. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 8 shows the release properties of the hybrid microparticles at 37°C in PBS. A sustained release pattern of the drug from the microparticles was observed (ca. 60% after 7 h). Because of the hydrophilicity of the alginate chains, the initial burst release was observed. The alginate/CaCO<sub>3</sub> hybrid microparticles have many pores and provide a strong drug-loading capability regardless of their surface charge, as the drug could be loaded into the hybrid microparticles because of the capillary force.<sup>25</sup> Additionally, alginate could swell in the phosphate buffer, and as a result, the release of the water-soluble drug could be efficiently sustained for a specific time period.

# Evaluation of the In Vitro Cytocompatibility

To evaluate the cytotoxicity of the system consisting of the alginate, cotton fabrics, and  $CaCO_3$ , we used PIECs, and the results are given (Figures 9 and 10).

In Figure 9, it is clearly evident that both alginate/CaCO<sub>3</sub> and alginate/CaCO<sub>3</sub>/DS had no apparent cytotoxicity effects, as an 80% PIEC viability was observed after incubation for 24 h. However, compared to the control, the PIEC viability was lower



Figure 10. Cell proliferation of the PIECs on different cotton fabrics. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

with increasing concentration of the hybrid microparticles. In this study, we considered that the PIEC viability decreased as the concentration of the hybrid microparticles increased.

The results of cell proliferation on fabrics are shown in Figure 10. All of the fabrics manifested good cytocompatibility, although PIECs proliferated with different rates on different substrates. PIECs proliferated on the untreated cotton fabric with the highest rate; this demonstrated that the 100% cotton fabrics had the best cytocompatibility. The MTT absorbance of the cotton fabrics modified with different microparticles was lower than that of the control group; this signified that cell proliferation was inhibited as the deposition of microparticles, although it was mentioned that the microparticles had no apparent cytotoxicity effects, as previously discussed. The inhibition rate of the drug-loaded microparticles was a little higher than that of the drug-unloaded ones; this indicated that the loaded drug slightly inhibited the proliferation of the PIECs.

### CONCLUSIONS

In this study, we discussed the successful preparation of alginate/CaCO<sub>3</sub> inorganic–organic microparticles and uniformly deposited them on the surface of a cotton fabric with a onestep coprecipitation method. The process was simple, convenient, and environmentally friendly. Both physical and chemical characterization studies have shown that the alginate/CaCO<sub>3</sub> hybrid was well dispersed on the surface without changing the properties of the fabric. In addition, the drug-loading and drugrelease properties of the hybrid microspheres were studied, and the results show that the water-soluble DS could be effectively loaded in the hybrid microparticles and the drug release could be effectively sustained. Furthermore, both the microparticles and modified fabrics showed good cytocompatibility.

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