Materials Science and Engineering C 63 (2016) 18–29

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Preparation of porous carboxymethyl chitosan grafted poly (acrylic acid) superabsorbent by solvent precipitation and its application as a hemostatic wound dressing

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ARTICLE INFO

Article history:
Received 16 November 2015
Received in revised form 9 February 2016
Accepted 17 February 2016
Available online 22 February 2016

Keywords:
Chitosan
Superabsorbent polymer
Modification
Solvent precipitation method
Hemostatic wound dressing

ABSTRACT

The volume phase transition of a hydrogel initiated by shrinking may result in complex patterns on its surface. Based on this unique property of hydrogel, we have developed a novel solvent precipitation method to prepare a kind of novel superabsorbent polymers with excellent hemostatic properties. A porous carboxymethyl chitosan grafted poly (acrylic acid) (CMCTS-g-PAA) superabsorbent polymer was prepared by precipitating CMCTS-g-PAA hydrogel with ethanol. Its potential application in hemostatic wound dressing was investigated. The results indicate that the modified superabsorbent polymer is non-cytotoxic. It showed a high swelling capacity and better hemostatic performance in the treatments of hemorrhage model of ear artery, arteria cruralis and spleen of the New Zealand white rabbit than the unmodified polymer and other commonly used clinic wound dressings. The hemostatic mechanism of the porous CMCTS-g-PAA polymer was also discussed.

1. Introduction

Uncontrolled hemorrhage is the leading cause of death on the battlefield, civilian trauma, large-scale disaster site and contingency operations [1,2]. Significant blood loss may lead to shock, hypothermia, coagulopathy, acidosis and late "second-hit" mortality that occurs through the development of sepsis and multiple organ failure [3,4]. The efficient hemostatic agents and dressings can promote coagulation, arrest ongoing hemorrhage, and thus effectively prevent death [5].

Among the currently available wound dressings, chitosan and zeolite are the most commonly used for the severe hemorrhage of artery. Chitosan is a marine polymer that can be extracted from the shells of various shellfishes including crabs and prawns. It has versatile properties, such as biocompatibility, biodegradability, excellent hemostatic behavior and promoting wound healing [6]. However, the hemostasis of chitosan is significantly affected by its structure [7]. It has been reported that only chitosan with specific structures can be used as a hemostatic material. Zeolite is an inorganic compound widely used for severe hemorrhages due to its porous structure and excellent absorbency. However, its side effects, such as significant thermal tissue injury limit its application [8].

Superabsorbent polymers and hydrogels have attracted considerable attentions due to their high swelling capacity and potential hemostatic ability to prevent accumulation of exudates [9]. The superabsorbent polymers prepared from chitosan and its derivatives are promising hemostatic materials with good absorption behaviors and potential hemostatic ability [10]. In the previous reports, we prepared several superabsorbent polymers by graft copolymerizarion and crosslinking reaction of chitosan and its derivatives [11–13].

It can be predicted that the porous chitosan superabsorbent polymers and hydrogels would have high swelling capacities and could be used as a promising hemostatic material with excellent comprehensive properties. In addition, the biocompatibility of chitosan avoids the disadvantages of zeolites. Their structures and properties can be easily controlled by adjusting reaction conditions. However, the currently available techniques for the preparation of porous superabsorbent polymers with pore-forming agents or emulsion template method are complicated with low efficiencies. The pore-forming agents or emulsifier is easy to be remained in the polymer networks, leading to toxic products [14,15].

Hydrogel undergoes volume phase transition with the changes of its external environment, such as temperature and solvent composition [16,17]. It has been reported that complex structures, such as grain,

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http://dx.doi.org/10.1016/j.msec.2016.02.048
0928-4931/

bubble and bamboo-like patterns, can be formed on the surface of a dehydrated hydrogel during the phase transition [18]. The occurrence of these characteristic conformations depends on the phase transition rate [19,20]. Tanaka et al. [21], Tokita et al. [22] and Li et al. [23] elucidated the physical basis underlying the formation and evolution of the pattern through different aspects.

We have previously prepared carboxymethyl chitosan grafted poly (acrylic acid) (CMCTS-g-PAA) superabsorbent polymer by graft copolymerization of acrylic acid on the skeleton of carboxymethyl chitosan, followed by crosslinking reaction [24]. Later, we found that the swollen CMCTS-g-PAA hydrogel underwent discontinuous phase transition in ethanol/water mixture solution with the increase of ethanol concentration [25]. In the present work, based on these unique properties of CMCTS-g-PAA hydrogel, a novel physical solvent precipitation method was developed to prepare modified CMCTS-g-PAA superabsorbent polymer. The well swollen superabsorbent polymer was precipitated with ethanol to form porous superabsorbent polymer under the contraction stress of the hydrogel. Its biocompatibility and hemostatic property were investigated. The results indicate that the toxic residual monomer in polymer was leached during precipitation and the swelling rate and hemostatic property of the superabsorbent was significantly promoted.

Table 1
The swelling kinetic parameters of the modified CMCTS-g-PAA superabsorbent polymer.

			σ_0/E (g/g)	τ_0 (min)	k_i (g/min)	t_c (min)
Particle size (μm)	315	Unmodified	723.9	7.16	53.67	35.85
		Modified	811.4	7.44	66.36	28.64
	165	Unmodified	792.6	4.53	116.24	22.87
		Modified	945.6	4.49	139.45	21.42
	115	Unmodified	719.7	7.03	53.78	31.64
		Modified	999.6	5.01	137.47	23.39
	88	Unmodified	812.3	6.44	71.57	35.29
		Modified	963.2	3.01	177.13	14.77
Concentration of the polymer swelled (m/m)		0.95%	840.0	7.52	68.31	32.12
		0.77%	831.1	6.24	85.27	28.28
		0.65%	836.8	5.02	96.74	21.44
		0.56%	878.8	3.04	160.70	12.88
		0.49%	832.7	3.13	116.89	15.10
Swelling time before treatment (h)		1	843.5	7.58	54.62	33.05
		6	878.8	3.04	160.70	12.88
		12	860.6	6.34	66.97	28.35
		24	854.2	6.83	64.60	27.80
		48	872.3	6.31	68.03	27.53

Note: E is Young's modulus, which indicates the ability of the material to resist deformation. σ_0/E is the equilibrium deformation at $t \to \infty$, which is the equilibrium swelling ratio for superabsorbent polymer. τ_0 is the relaxation time (delayed time), imply the resistance of polymer to water penetration. k_i is the slope of the line from time zero to the time reaching 70% equilibrium swelling ratio. t_c is the time when the curve deviates from the equilibrium swelling value.

2. Materials and methods

2.1. Materials

CMCTS-g-PAA superabsorbent polymer was prepared as described previously [24]. Chromatographic grade 3-(N-morpholino) propyl sulfonic acid (MOPS) was supplied by Amersco Co., Ltd. (USA). Isotonic saline solution was purchased from No. 5 Pharmaceutical Factory of Haerbing, China. Phenol, ethanol and dimethyl sulfoxide (DMSO) were analytical grade. Bovine serum and DMEM culture medium were purchased from HyClone Co., Ltd. (USA). 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) was purchased from Amersco Co., Ltd. (USA). Trypsin was supplied by Beijing North TZ-Biotech Develop, Co., Ltd. (Beijing, China). Penicillin and streptomycin were purchased from North China Pharmaceutical Co., Ltd. (Shanghai, China). Absorbable gelatin sponge was from Nanjing Jinling Pharmaceutical Co., Ltd. (Nanjing, China). Calcium alginate and starch were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). Baiyao powder was purchased from Jinxiang Pharmacy (Beijing, China). Sterile hemostatic gauze was supplied by Qianjiang Kingphar Medical Material Co., Ltd. (Qianjiang, China).

2.2. Preparation of porous CMCTS-g-PAA superabsorbent polymer by solvent precipitation method

The conditions for the modification of CMCTS-g-PAA by solvent precipitation are listed in Table 1. Briefly, CMCTS-g-PAA superabsorbent polymers with average particle sizes of 315 μm, 165 μm, 115 μm and 88 μm were respectively suspended in 150 mL distilled water and heated at 25 °C in a water bath for a certain period of time to swell. Ethanol was added into the suspension to quickly precipitate the polymer. The precipitate was filtered and dried under vacuum at 60 °C to obtain the modified CMCTS-g-PAA superabsorbent polymer.

2.3. Scanning electron microscopy (SEM)

The surface morphologies of the superabsorbent polymers before and after the modification was were imaged with a scanning electron microscope (QUANTA200, Philips-FEI Co., the Netherlands). The samples were coated with Au prior to imaging.

2.4. Determination of the swelling property of superabsorbent polymers

Superabsorbent polymer (0.100 g) was placed into a sieve pouch. The pouch was then immersed in distilled water. After being swelled for a suitable period, the pouch was taken out and the excess water was removed. The swollen polymer was accurately weighed. Then the swelling ratio (Q, g/g) was calculated by:

$$Q = (m_2 - m_1)/m_1 \tag{1}$$

where m_1 is the weight of the dry superabsorbent polymer, and m_2 is the weight of the swollen superabsorbent polymer. For each sample, the experiment was repeated for three times and the average values were used.

The swelling kinetics of the superabsorbent polymer was simulated using the Voigt model as previously reported [26].

2.5. Determination of the acrylic acid residues in superabsorbent polymers

Acrylic acid residue in the superabsorbent was analyzed with an Agilent 1100 series HPLC (Agilent Technologies, USA) equipped with G1312A binary pump, G1314A wavelength adjustable UV detector and Agilent chemical workstation. The column was ZORBAX Eclipse XDB-C18 (5 μ, 150 × 4.6 mm). The flow rate of the mobile phase (0.02 M MOPS aqueous solution with, pH = 5.20) was set to 0.8 mL/min. The detection wavelength was 210 nm and the injection volume was 20 μL. A standard calibration curve was established with 50, 20, 10, 5, 2, 1 and 0.5 μg/mL acrylic acid standards. The linear regression equation of the calibration curve was:

$$y = 1.9203 + 51.8228x \tag{2}$$

where x is the concentration (μg/mL) of acrylic acid and y is the peak area. The linear correlation coefficient (R) was 0.99995.

Dried superabsorbent polymer (0.100 g) was accurately weighed and added to 10 mL 0.9% isotonic saline solution, shaken on an oscillator at room temperature for 1 h and filtered. The filtrate was collected and analyzed with the HPLC system under the conditions as described above to determine the residual acrylic acid in the superabsorbent polymer.