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Multifunctional chitosan/dopamine/diatom-biosilica composite beads for rapid blood coagulation

Yanan Wang[a,1], Yangmu Fu[b,1], Jing Li[a], Yuzhi Mu[a], Xin Zhang[a], Kaichao Zhang[a], Mengqi Liang[a], Chao Feng[a,*], Xiguang Chen[a,c,**]

[a] College of Marine Life Science, Ocean University of China, 5# Yushan Road, Qingdao 266003, Shandong Province, China
[b] Department of Orthopaedics, Hainan Branch of Chinese PLA General Hospital, Sanya 572013, Hainan Province, China
[c] Qingdao national laboratory for marine science and technology, 1# Wenhai Road, Qingdao 266000, Shandong Province, China

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ABSTRACT

Uncontrollable bleeding is the main cause of death in wars and accidents. The development of emergency material for rapid hemostatic can effectively reduce bleeding-related death. The commercial hemostatic materials available in the market are difficult to meet requirements of rapid hemostasis, good biocompatibility, low cost and ease of use. In this study, we developed chitosan/dopamine/diatom-biosilica composite beads (CDDs) for rapid hemostasis with good biocompatibility. CDDs were prepared by combining chitosan with diatom-biosilica (DB) using dopamine as bio-glue. The porous internal structure of CDDs led to rapid and large amount of water absorption, which contributed to the rapid hemostasis (83 s, 22% of the control group). The hemolytic rate of CDDs was less than 5% and cell viability was above 80%, confirming its good biocompatibility. All the above results indicated that CDDs had potential to develop into safe and non-toxic hemostatic material.

1. Introduction

Uncontrollable hemorrhage caused by emergencies in daily life, natural disasters, battlefield and surgical procedures can lead to high risk of death, which was about 10% of the global death toll, causing more than 5.8 million deaths worldwide each year (Rossaint et al., 2016). There is a great demand for developing encouraging hemostatic material that can quickly stops bleeding with no bio-toxicity. Quikclot, a topical zeolite granular anti-hemorrhagic agent, controls hemorrhage by adsorbing water to increase the concentration of coagulation and promote clot formation. The exothermic reaction, high cost and lack of provisions limit its wider application (Devlin, Kircher, Kozen, Littlejohn, & Johnson, 2011; Rhee et al., 2008). Diatoms are unicellular eukaryotic algae covered with intricately detailed silica cell walls. The diatom shell (frustule), a kind of biosilica, have great potential for application in biomaterials because of its incomparable diversity of nanoporous patterns, highly ordered and hierarchical pore structure, large surface area, as well as good biocompatibility (Gordon, Losic, Tiffany, Nagy, & Sterrenburg, 2009; Leonardo, Prieto-Simón, & Campàs, 2016). Our previous study found that the negatively charged surface of diatom-biosilica (DB) could significantly promote the intrinsic blood coagulation by activating blood coagulation factor XI and XII and the cofactors HWK – kininogen and prekallikrein, resulting in efficient hemostatic performance (Feng et al., 2016). However, the potential risk of vascular embolization effect impelled us to develop a strategy for avoiding DB diffusion into normal vessels with the bloodstream.

Chitosan, a chitin deacetylated derivative, which is the only natural polysaccharide in nature (Khor & Lim, 2003). There are plentiful amino and hydroxyl groups along the chitosan chain, which makes it have a variety of biological activities (Choi, Nam, & Nah, 2016; Pellá et al., 2018). It is an ideal biopolymer with excellent biocompatibility and non-toxicity (Boucard et al., 2007). Chitosan can be degraded in vivo by several enzymes, mainly by lysozyme. The degradation products are non-toxic oligosaccharides that can be excreted or incorporated to glycosoaminoglycans and glycoproteins (Pellá et al., 2018). Moreover, the degradation products can help in repair the injured area by fibroblast proliferation (Mohandas, Deepthi, Biswas, & Jayakumar, 2018). Recently, Xu et al. prepared chitosan hydrogel by freeze-melting-neutralization method under mild conditions. Their hydrogel could be used for the delivery of biologically active growth factors and drugs that are sensitive to strong bases or high pH (Xu, Han, & Lin, 2017). Huang et al. found that chitosan sponges could shorten clotting time and blood loss,

* Corresponding author.
** Corresponding author at: College of Marine Life Science, Ocean University of China, 5# Yushan Road, Qingdao 266003, Shandong Province, China.
E-mail addresses: fengchao@ouc.edu.cn (C. Feng), xgchen@ouc.edu.cn (X. Chen).
[1] Co –first author: Yanan Wang and Yangmu Fu contributed equally to this work.

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Table 1
Comparison of different chitosan-based hemostatic materials.

Product name	Material	Form	Manufacturer	Reference
HemCon	Chitosan acetate salt	Bandage	Hemorrhage Control Technologies, Inc. Oregon, USA	Granville-Chapman, Jacobs, and Midwinter, (2011)
Celox	Chitosan compounds	Granules	MedTrade Products Ltd. Crewe, England	Devlin et al. (2011); Granville-Chapman et al. (2011)
Celox Gauze	Chitosan	Gause	MedTrade Product, Crew, UK	Littlejohn, Bennett, & Drew (2015)
Chito Gauze	Chitosan	Gause	HemCon Medical Technologies, Portland, OR	Littlejohn et al. (2015); Te Grotenhuis, van Grunsven, Heutz, and Tan (2016)
Chitoflex	Chitosan	Roll of chitosan	HemCon Medical Technologies, Portland, OR	Devlin et al. (2011); Granville-Chapman et al. (2011)

and exhibited excellent degradability (Huang et al., 2015). Previous studies have shown that the electrostatic interaction between the positive charge on the surface of chitosan and the negative charge on the surface of blood cells is the main reason for the hemostatic activity of chitosan (Pusateri et al., 2006). This interaction has no effect on coagulation factors and cannot cause changes in aPTT and PT (Feng et al., 2016). In past decades, various chitosan-based hemostatic products have been marketed. For example, HemCon bandage (HemCon Medical Technologies, Inc., Portland, OR), a topical chitosan wafer controls hemostasis by adhering to tissues and sealing the injury. Celox (Medtrade Biopolymers, Crewe, UK, distributed in the United States by SAM Products, Portland, OR) was a chitosan granule that promotes hemostasis through adsorption and dehydration (Gegel et al., 2010). However, the hemostasis effect of single chitosan materials is weakness due to its limited indication range and instability therapeutic effect. Combination of chitosan and inorganic hemostatic agents is considered as an alternative strategy for improving the hemostatic effect of materials (Table 1).

Mussel-inspired chemistry relies mainly on the adhesion of dopamine to various materials and surfaces. 3, 4-dihydroxy-L-phenylalanine (DOPA) is considered to be the key component of mussel adhesive proteins (Waite, Andersen, Jewhurst, & Sun, 2005). Dopamine is a DOPA derivative whose molecular structure is similar to that of DOPA. It has been reported that dopamine could oxidize spontaneously to polydopamine under aerobic and alkaline conditions. As famous "bioglue", polydopamine can be deposited on almost all types of organic and inorganic materials and form functional coatings on the surface of these materials (Qiu, Yang, & Xu, 2018). More importantly, there are many functional groups on polydopamine, such as catechol, amines and imine. These functional groups can be used as reactive groups for further functionalization and to design and obtain ideal functional materials.

In this study, chitosan/dopamine/DB beads (CDDs) were developed for hemorrhage control using simple alkalization precipitation method. Chitosan has been proven to have excellent biodegradability; dopamine has weak biodegradability, but its excellent biocompatibility made it widely used in the field of biomedicine; diatom is a kind of natural algae, some studies have found that it has weak cytotoxicity, we had coated it in the porous structure inside the beads, which could not only protect its porous structure, but also reduce the cytotoxicity. CDDs concentrated the advantages of the three materials and exhibited excellent hemostatic properties and biocompatibility. Moreover, the big size of CDDs (more than 1 mm) could effectively prevent small particles from entering the blood vessels and causing congestion.

2. Experimental section

2.1. Materials

Chitosan (deacetylation degree of 85%, provided by Sinopharm Chemical Reagent Co., Ltd., and molecular weight of 1390 kDa, measured by viscosity method), dopamine, acetic acid, NaOH, KBr, PBS (0.02 M, pH = 7.4), saline solution were purchased from Sinopharm Chemical Reagent Co., Ltd. DMEM medium, Cell Counting Kit-8 (CCK-8) and fetal bovine serum were supplied by Solarbio (Beijing, China). *Coscinodiscus* sp. (CCAP 1013/11) was supplied by Key Laboratory of Marine Genetics and Breeding, Ministry of Education, Ocean University of China. New Zealand White Rabbits were purchased from Lukang Pharmaceutical Co., Ltd (Qingdao, China), which were cared for and treated in accordance with the National Research Council's Guide for the care and use of laboratory animals, and the native laws and institutional guidelines, as per the State Scientific and Technological Commission of the People's Republic of China Statement No. 2: Laboratory Animal Management Regulations (edition 2011). All reagents and solvents are used as-is without further purification.

2.2. Preparation of composite CDDs

CDDs were prepared by alkalization precipitation method using chitosan-dopamine mixed solution and DB. The diatoms (*Coscinodiscus* sp. (CCAP 1013/11)) were cultured in f/2 medium and alternating light and dark every 12 h. After 5 d incubation (logarithmic phase), the diatom cells were collected by filtration and washed three times with deionized water. Each 200 mg diatoms was added to the mixture of 50 ml H_2O_2 (30% v/v) and 50 ml HCl (2 mol/L) for 24 h. The frustule was then collected in a filtered way and washed more than 10 times with deionized water to ensure that the organic residue was removed completely. The purified frustule was dried in vacuum at 35 °C for 24 h to get DB (Feng et al., 2016). 2 g chitosan was added to 100 ml (1% v/v) acetic acid solution and 2% chitosan solution was obtained, 200 mg dopamine and 200 mg DB were added into the chitosan solution and stirred well. The mixture solution was added to a syringe of 1 ml and pushed into 30% (w/v) NaOH solution at a distance of 15–20 cm slowly and gradually to get wet CDDs. After stirring gently for 1 h with rotor, the NaOH solution was rinsed with running deionized water. The wet CDDs were lyophilized to get dry CDDs.

Chitosan beads (Cs), chitosan/dopamine composite beads (CDs) were prepared as control. The preparation of CDs was the similar as that of CDDs by using chitosan-dopamine mixed solution. And Cs was obtained by using chitosan solution without dopamine and DB.

2.3. Characterization of CDDs

50 wet beads were randomly selected to measure their particle size (S_{wet}) with vernier caliper, the particle size of dry beads (S_{dry}) was measured by same method. The shrinkage rate was calculated by the following equation:

$$\text{Shrinkage Ratio (\%)} = (S_{wet} - S_{dry}) / S_{wet} \times 100 \quad (1)$$

The surface and cross-sectional morphologies of the beads were imaged with a scanning electron microscope (QUANTA200, Philips-FEI Co., the Netherlands).

The structure of beads was analyzed on a NEXUE470 Fourier Transform Infrared Spectrophotometer (Nicolet, Madison, USA). A pure KBr particle was used for background scanning before the spectra of the samples were measured. The beads and KBr were grinded together in the mortar and the spectra were measured over the range of 4000 to 400 cm^{-1}, with a 4 cm^{-1} resolution and an accumulation of 20 scans.