Preparation and characterization of novel silica-butyrylchitosan hybrid biomaterials*

A. P. ZHU, Z. ZHANG, J. SHEN
Department of Polymer Science and Engineering, Nanjing University, Nanjing, 210093 People's Republic of China

Novel silica-butyrylchitosan hybrid biomaterials were produced by a sol–gel technique, using butyrylchitosan as the organic species incorporated into the silicon alkoxide (TEOS) based network. 3-acryloxypropyl trimethoxysilane (MPTMS) was used effectively to combine the organic and inorganic species to form uniform hybrid biomaterials. All the samples made were in the form of thin, flexible films with transparent clarity. The blood-clotting and platelet adhesion assay confirmed that these hybrid biomaterials displayed potential good blood compatibility.

1. Introduction

The development of the new materials with both organic and inorganic structures is of great interest with respect to the achievement of obtaining the special property materials, and the sol–gel process has provided new opportunities for making such materials [1–3].

Chitosan [2-amino-2-deoxy-D-glucan] is a unique polysaccharide derived from chitin [4] and has been widely used in various biomedical applications due to its biocompatibility, low toxicity and biodegradability [5]. Some derivatives of chitosan such as sulfated and acylated chitosan have anticoagulant properties [6–8]. Many selectively modified chitosan products have shown unique biological activities and physicochemical properties [9–10], which make these chitosan derivatives be important in various biomedical and pharmaceutical applications [11].

Three-dimensional structures formed by crosslinking sulfation and acylated chitosan are useful for anti-coagulant coatings of medical devices, for it was recognized that a crosslinkable coating would offer additional advantages over the existing systems in terms of film stability and the possibility of anchoring the polymer to the substrate [12]. Sol–gel technique can be used to prepare an organic/inorganic hybrid crosslinking network.

In this paper, innovative silica-butyrylchitosan hybrid biomaterials were fabricated by the sol–gel technique. We selected o-butyrylchitosan as the organic species, for o-butyrylchitosan reacts with 3-acryloxypropyl trimethoxysilane (MPTMS) to synthesize MPTMS-o-butyrylchitosan. MPTMS-o-butyryichitosan can be easily incorporated into the TEOS based network by hydrolysis and condensation of methoxysilyl-groups in the BCS-MPTMS with TEOS.

The purpose of the current research was to prepare uniform silica-butyrylchitosan hybrid biomaterials and investigate their blood compatibility.

2. Materials and methods

2.1. Materials

Chitosan powder was obtained from Lianyungang Biologicals Inc (China). The degree of deacetylation was 90% determined by infrared spectroscopy. Methanol (AR), methanesulphonic acid (AR), acetone (AR), butyric anhydride (AR), (3-acryloxypropyl) trimethoxymethylsilane (AR), tetraethyloxysilane (TEOS) were used as purchased.

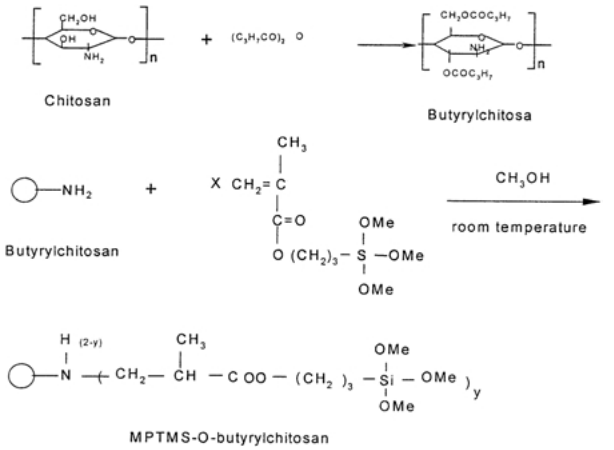
2.2. Preparation of butyrylchitosan

The butyrylchitosan was synthesized according to the method reported by Stuart Grant *et al*. [13]. In short, chitosan powder (2.1 g) was added to methanesulphonic acid (11 ml) and the mixture was stirred at 0 °C for 15 min to produce an homogeneous solution. Butyric anhydride (20 cm^3) was added drop-wise and the total mixture stirred at a temperature between 0 °C and 5 °C for 2 h. The resulting gel was stored at − 15 °C overnight. The thawed product was precipitated by pouring into acetone (300 ml), filtered and extracted for 18 h with acetone, and then the butyrylchitosan obtained was dried in vacuum.

2.3. Preparation of the 3-acryloxypropyl trimethoxysilane-butyrylchitosan (BCS-MPTMS)

The synthesis reaction of BCS-MPTMS is shown in Scheme 1. Methoxysilyl-groups were introduced into butyrylchitosan by Michael addition of (3-acryloxy-

*National Key Foundational Research & Development Project of China, G 1999064705.



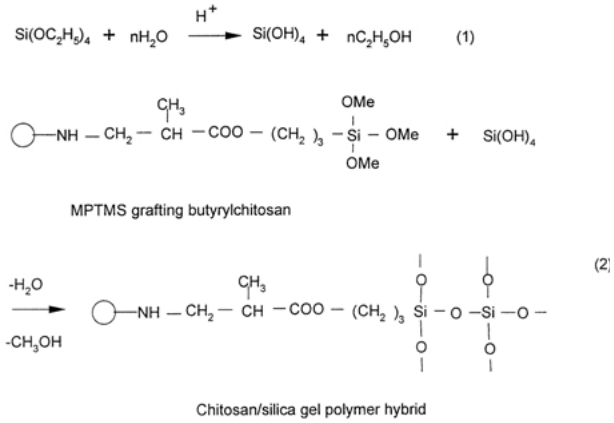
Scheme 1 Synthesis reaction of butyrylchitosan and BCS-MPTMS.

propyl) trimethoxymethylsilane to the amino groups of butyrylchitosan. BCS-MPTMS was prepared in a homogeneous system: into a 100 ml flask, 0.9 g butyrylchitosan, 10.0 ml of methanol and 0.3 g MPTMS were added, the flask was sealed, and the mixture was stirred with a magnetic stirrer at 30 °C for 20 h. The reaction mixture was concentrated, the thawed product was precipitated by pouring into acetone, filtered and extracted with acetone in a Soxhlet apparatus for 24 h, then was dried in vacuum.

2.4. Preparation of hybrid films

Silica-butyrylchitosan hybrid film was produced by sol–gel process: hydrolysis and condensation of methoxysilyl-groups in the BCS-MPTMS and TMSO. The routes of preparation of silica-butyrylchitosan hybrid matrices are shown in Scheme 2, from which it can be found that MPTMS cannot only react with butyrylchitosan but also condense with TEOS. As a result, MPTMS makes butyrylchitosan combine with silica matrix, which benefits to form uniform organic/inorginic hybrid material. The silanol condensation reaction can be easily accomplished either by exposure of the coating to atmospheric moisture, or by control of water addition, either in the form of vapor (for example, in a controlled humidity chamber), or as a liquid into a solution.

The samples were prepared as follows: in 50 ml flasks, BCS-MPTMS 3.5 g, TEOS 4.8 g, hydrochloric acid (0.1611 mol/l) 0.9 g and methanol 100 ml were added



Scheme 2 The route of preparation of silica-butyrylchitosan hybrid biomaterial.

in order and the mixture was stirred with a magnetic stirrer for 30 min to prepare the clear and transparent precursor solution.

Silica-butyrylchitosan films can be conveniently casted from the above precursor solution on 10 cm diameter Petri dishes. The Petri dishes were weighed accurately and stored at room temperature to constant mass (for several days to complete sol–gel reaction), film thickness can be predetermined by adjusting the thickness of the casting solution. The transparent film was placed in the oven, the temperature raised slowly to 80 °C and maintained at this temperature for 2 h to obtain the hybrid film.

2.5. Characterization and measurements

FT-IR analyses were performed on a Nicolet 170sx spectrometer. The thermal properties of the hybrid films were measured by TGA on V1.1B TA Inst 2100. Samples of 3–9 mg were heated to 800 °C at a heating rate of 10 °C/min. The surface topography was analyzed by SEM on X-650 scanning electron microanalyzer. Rectangular test samples (0.5 cm × 2.5 cm) were cut from silica-butyrychitosan hybrid films and strained to failure on an Instron-4466.

2.6. Blood-clotting assay

A blood-clotting assay was developed in order to assess the effectiveness of the hybrid coatings in delaying the blood-clotting process. When platelets are contacted to the glass surface, factor XII will adhere to the surface and be activated, thereby initiating the clotting cascade via the intrinsic pathway. For this assay, 75 mm × 12 mm glass tubes were used, sonicated in DCM and dried prior to use.

In order to coat a glass tube, the tube was filled with the precursor solution in the sol–gel process, left for 1 min before the solution was poured out of the tube, whilst rotating the tube between the fingers. The tube was then placed on a spiramix to allow the hybrid coating to dry evenly within the tube at room temperature for several days and to be treated at 80 °C for 2 h. Ten samples of each film were prepared in each case and compared to 10 uncoated controls.

Fresh frozen platelet-rich plasma (100 µl) was added to each sample tube and allowed to stand for 2–3 min at 37 °C before the addition of 100 µl of 0.025 M $CaCl_2$ solution (also at 37 °C), at which point a stopwatch was started. The mouth of the tube was then sealed tightly with parafilm and the tube tilted backwards and forwards whilst still partially submerged in the water bath to maintain the temperature at 37 °C. The time was noted at the point when a fibrin clot was visible.

2.7. Platelet adhesion

Platelet contact SEM studies were also carried out on the samples in order to check activated platelets, fibrin clots etc. PET strips (30 mm × 9 mm) were cleaned thoroughly by wiping with DCM and then dipped into the precursor solution of the sol–gel process. They were allowed to dry at room temperature for several days and

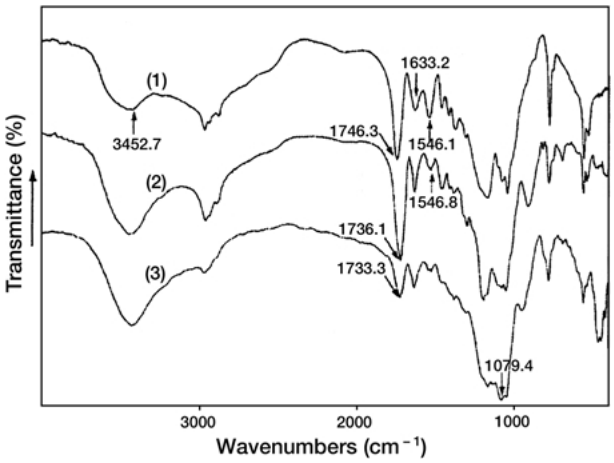


Figure 1 FT-IR spectra of (1) butyrylchitosan, (2) MPTMS-o-butyrylchitosan and (3) silica-butyrylchitosan hybrid film.

were treated at 80 °C temperature for 2 h to obtain PET strips coated with thin hybrid film. The PET strips were contacted with 4 ml platelet-rich plasma for 3 h. After washing in PBS they were fixed using 2% gluteraldehyde solution for 30 min, washed again and then sequentially immersed into 50%, 60%, 70%, 80%, 90% and 100% ethanol solution and dried in a desiccator. It was sputtered-coated with gold before being imaged by SEM.

3. Result and discussion

3.1. Hybrid films preparation and structure characterization

Fig. 1 shows the FT-IR spectra of butyrylchitosan, BCS-MPTMS and silica-butyrylchitosan hybrid biomaterials. In the FT-IR spectrum of butyrylchitosan (Fig. 1(1)), three new absorption bands at 1741, 779 and 1207 cm^{-1} are attributed to υ (C═O), w (CH_2) and υ (C—O) respectively, which are characteristic absorptions of butyrylated group. The absorption intensity of amino group band at (1950 cm^{-1}) is still intense. This result indicates that acylation takes place on the hydroxyl group of chitosan, which confirms o-acylation [11].

In the FT-IR spectrum of BCS-MPTMS (Fig. 1(2)), it can be seen that there is a decrease in the intensity of the amino groups compared to that of butyrylchitosan, while

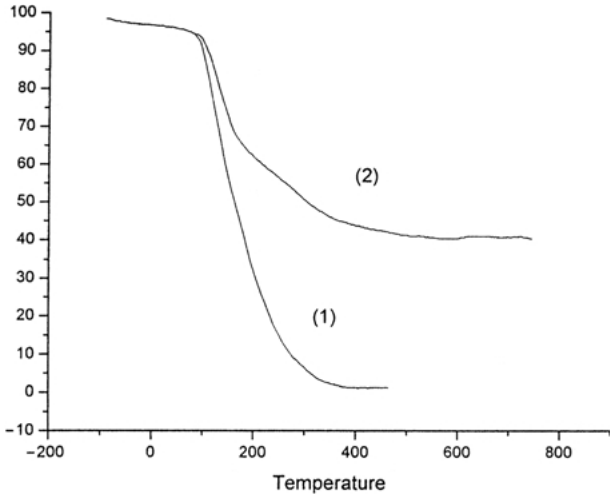


Figure 2 TGA thermogram of weight loss as a function of temperature, (1) butyrylchitosan (2) silica-butyrylchitosan hybrid.

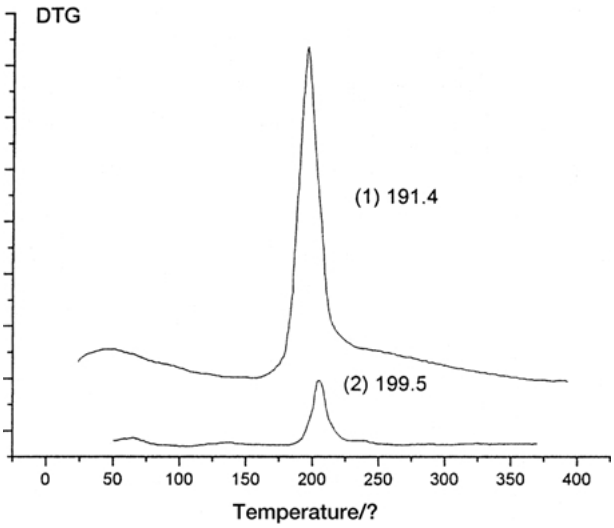


Figure 3 DTG curves of butyrylchitosan and silica-buttyrylchitosan hybrid, (1) butyrylchitosan (2) silica-buttyrylchitosan hybrid.

the intensity of carbonyl group in ester group (1736 cm^{-1}) increases which is due to the bonding of MPTMS to the amino groups of butyrylchitosan.

From Fig. 1(3), the FT-IR spectrum of the film of silica-butyrylchitosan hybrid, it can be seen that the new wide absorption band at 1079.4 cm^{-1} appears, which is the characteristic of Si–O–Si. This result confirms that network structure has formed by hydrolysis and condensation of methoxysilyl-end-groups.

TGA and differential thermogravimetric (DTG) curves for butyrylchitosan and its hybrid film are shown in Figs. 2 and 3, from which it can be seen that the onset of the thermal decomposition of hybrid butyrylchitosan film shifted significantly toward higher temperature than that of butyrylchitosan and the decomposition velocity of hybrid butyrylchitosan was much lower than that of butyrylchitosan, these results indicated indirectly that the crosslinked networks had

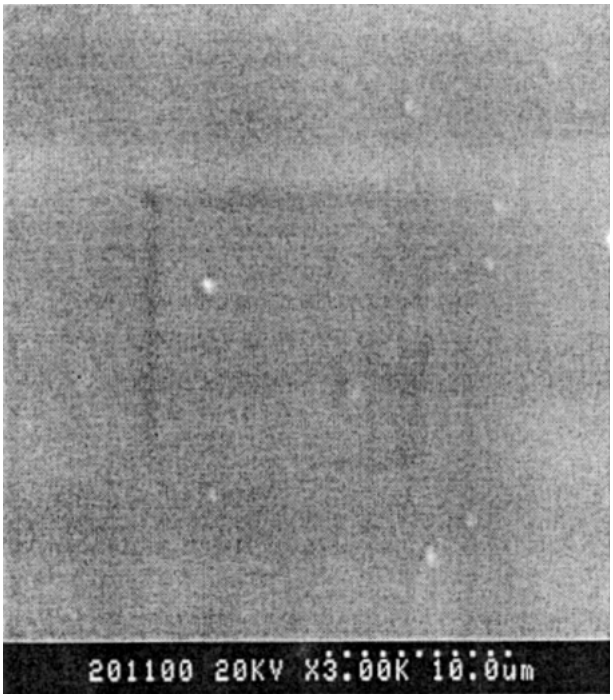
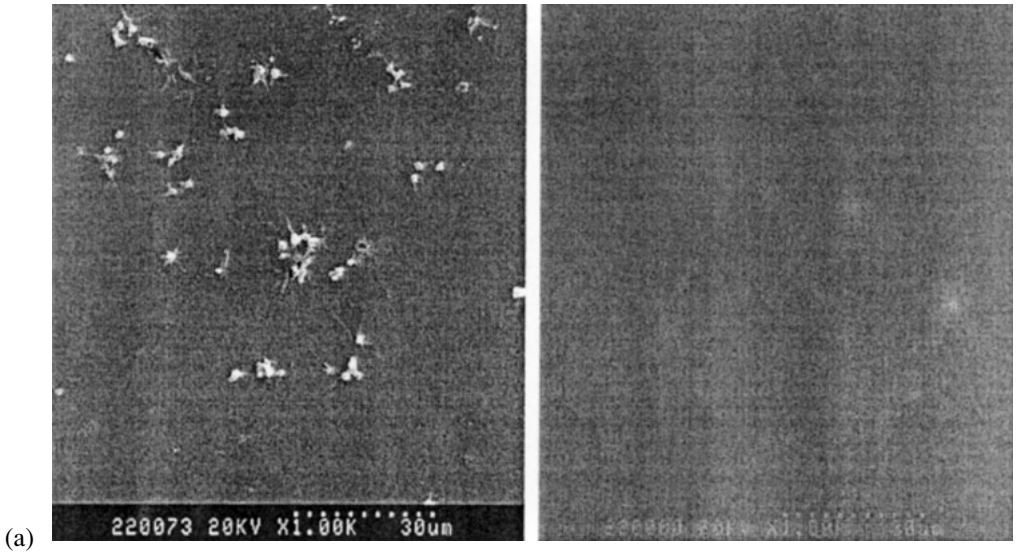


Figure 4 Scanning electron micrographs of hybrid surface.



(a) (b)

Figure 5 SEM images of the strips after being contacted with platelet-rich plasma (a) uncoated strip (b) PET strip coated with silica-butyrylchitosan.

formed by hydrolysis and condensation of methoxysilyl-groups in the BCS-MPTMS. It was the formation of the crosslinking network that imposed restrictions on the molecule movement of butyrylchitosan and made the thermal decomposition temperature of butyrylchitosan increase.

3.1.1. SEM analysis

It can be detected from Fig. 4 that silica is uniformly distributed in the butyrylchitosan matrix, and there is no obvious interface between the two phases. These results suggest that the butyrylchitosan and the silica matrix are well dispersed.

3.1.2. Mechanical properties

Mechanical properties of butyrylchitosan and its hybrid films are shown in Table I, it can be found that the tensile elongation of silica-butyrylchitosan hybrid film was higher than that of butyrylchitosan while the modulus of the hybrid film was lower than that of butyryichitosan. From the analysis result of mechanical properties, it can be concluded that the flexibility of butyrylchitosan have increased when it has been crosslinked into a network structure. This increased flexibility may attribute to enhance the antithombogenicity of this hybrid material coating, for an idea antithombogenic coating should have good flexibility [12].

TABLE I Mechanical properties of butyrylchitosan and its hybrids

Sample	Strain at break (%)	Modulus (Young)(MPa)
Butyrylchitosan	2.6	957
Silica-butyrylchitosan hybrid	6.6	270

3.2. Anticoagulant property characterization

The data in Table II show a marked increase in clotting time for the samples coated with silica-butyrylchitosan.

TABLE II Clotting times for uncoated and coated sample tubes

Sample coating ($n = 10$)	Clotting time (s)
Uncoated	205.3
Coated with silica-butyrylchitosan	456.7

This result indicated that silica-butyrylchitosan hybrid had antithombogenic properties. This may be because cross-linked silica-butyrylchitosan hybrid contains silyl groups and there is a possibility of interaction among these groups and the glass surface, and because a crosslinkable coating can offer additional advantages over the exising systems in terms of coating stability and the possibility of anchoring the polymer to the substrate.

Scanning electron micrographs of the strips after being contacted with platelet are shown in Fig. 5, The uncoated surface is covered with cellular matter, but there is almost no sign of any cellular matter on the coated PET strip. This collection of *in vitro* data suggests that the silica-butyrylchitosan can effectively inhibit the platelet attachment under the test conditions.

This obtained antithombogenic effect may be because that the silyl groups in the silica-butyryichitosan hybrid are easy to bind to the substrate and because that the hybrid materials obtained can combine the butyryl-chitosan merit with that of inorganic silica. However, the true reason is under investigation.

4. Conclusion

With MPTMS as a crosslinking agent, innovative silica-butyrylchitosan hybrid biomaterials were produced by sol–gel technique, using o-butyrylchitosan as the organic species to be incorporated into the TEOS based network.

The silica-butyrylchitosan hybrid films are clear, transparent, colorless with good mechanical properties and show uniform structure in the hybrid surface. These hybrid network materials displayed good blood compatibility under *in vitro* test conditions.

References

1. CHEN HONGMEI, TIAN XIAOMING and ZOU HAN *Immobilization Biotechnol.* **26** (1998) 431.
2. H.-H. HUANG, B. ORLER and G. L. WIKES *Macromolecules [J]* **20** (1987) 1322.
3. I. YUSUKE, I. HIDEAKI, N. KENSUKE and C. YOSHIKI *ibid.* **33** (2000) 4343.
4. R. A. A. MUZZARELLI, C. JEUNIAX and G. W. GOODAY editors. ''Chitin in Nature and Technology'' (Plenum Press, New York, 1986).
5. J.-K. FRANCIS SUH and HOWARD W. T. MATTHEW, *Biomaterials* **21** (2000) 2589.
6. E. G. MALETTE, H. J. EUIGLEYM Jr., R. D. GAINES, N. D. JOHNSON and W. G. RAINER *Ann. Thorac. Surg.* **35** (1993) 55.
7. SHIGEHIRO HIRANO, MIN ZHANG, MASUO NAKAGAWA and TERUO MIYATA *Biomaterials* **21** (2000) 997.
8. S. HIRANO and Y. NOISHIKI *J. Biomed. Mater. Res.* **19** (1985) 413.
9. N. KUBOTA, N. TATSUMOTO, T. SANO and K. TOYA *Carbohydrate Res.* **324** (2000) 268.
10. S. HIRANO, Y. TANAKA, M. HASEGAWA, K. TOBETTO and A. NISHIOKA *Carbohydrate Res.* **137** (1985) 205.
11. K. Y. LEE, W. S. HA and W. H. PARK *Biomaterials* **16** (1995) 1211.
12. A. L. LEWIS, Z. L. GUMMING, H. H. GOREISH, L. C. KIRKWOOD and L. A. TOLHURST *ibid.* **22** (2001) 99.
13. S. GRANT, H. S. BLAIR and G. MCKAY *Polymer Commun.* **29** (1988) 342.

Received 13 December 2001
and accepted 12 June 2002