Front. Chem. China 2008, 3(2): 172–177
DOI 10.1007/s11458-008-0033-0

RESEARCH ARTICLE

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Preparation and properties of polyvinyl acetal sponge modified by chitosan

Abstract The polyvinyl acetal sponge modified by chitosan was prepared by adding chitosan/polyvinyl alcohol (PVA) solution during the acetalation reaction of PVA and formaldehyde. The effect of vesicant and chitosan to the pore morphology, water absorption ratio, water absorption rate, expansion time and mechanical properties were studied. The polyvinyl acetal sponge modified by chitosan was used as a hemostatic packing material for the injured rabbit nasal tissue. The hemostatic effect and the healing effect of the modified sponge on the nasal mucosa after nasal surgery were studied. The results indicated that the polyvinyl acetal sponge modified by chitosan has an interconnected pore structure and the wall between large pores also has small pores. The chitosan adhered on the inner surface of the pores. The increased content of vesicant led to an increase in pore diameter, in the water absorption ratio and in expansion time. However, there was only a small change in the water absorption rate and a decrease in tensile strength and compression strength were noted. With an increase in chitosan content, the pore diameter and interconnection of the sponge was reduced. Water absorption ratio, expansion time and water absorption rate decreased, but tensile strength and compression strength improved. Observation of the rabbit nasal tissue after surgical operation suggested that polyvinyl acetal sponge modified by chitosan has an anti-inflammatory, hemostatic and anti-adherent characteristic and could promote the healing and functional recovery of rabbit nasal mucosa.

Keywords chitosan, polyvinyl alcohol, hemostatic sponge, acetalation

Translated from *Journal of Jinan University* (*Natural Science & Medicine Edition*), 2007, 28(3): 283–287 [译自: 暨南大学学报(自然科学与医学版)]

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1 Introduction

Polyvinyl alcohol (PVA) [1,2], chitosan [3,4], glutin [5,6] and medical sponges are widely used as hemostatic material in neurosurgery, bowel surgery, otorhinolaryngology and gynecology surgery. They are useful for packing wounds and promoting healing. However, presently, the homemade medical sponges usually have the disadvantage of low strength with difficult extraction after operation, poor anti-adhesion and readily induces inflammatory reactions. Chitosan is a new kind of medical biodegradable material with the advantage of anti-inflammatory, hemostatic and anti-adhesive properties [5,7]. Hence, polyvinyl acetal sponge with high strength and water absorbability was taken as a modified matrix. The chitosan/PVA solution containing intertwined chitosan chains with PVA chains was added into the reacting froth during acetalation between PVA and formaldehyde. The newly added PVA chain further reacted with the surface of the formed polyvinyl acetal sponge leading to the adherence of intertwined the chitosan chain onto the surface of the sponge, endowing polyvinyl acetal sponge with anti-inflammatory, hemostatic and anti-adherent characteristic. This paper aims to study the effect of the content of vesicant and chitosan to the pore morphology, water absorption ratio, water absorption rate, expansion time and mechanical properties of the modified sponge. The modified polyvinyl acetal sponge was used as a hemostatic packing material for injured rabbit nasal tissue and the hemostatic effect after nasal surgery and the functional recovery of nasal mucosa was studied.

2 Experimental

2.1 Preparation of polyvinyl acetal sponge modified by chitosan

Chitosan (degree of polymerization = 1600 CPS, DD $\geqslant$ 85%, Shanghai Boao Biology Technology Ltd.) was dissolved in a diluted acetic acid aqueous solution to obtain 0.5 wt% chitosan aqueous solution. PVA (trademark 124, Japan Kuraray Ltd.) was dissolved in boiling water to obtain 0.5 wt% PVA aqueous solution. The above two solutions were mixed adequately according to a 1:1 volume ratio to obtain an intertwined chitosan/PVA solution.

20 g PVA was dissolved in 200 mL boiling water and then cooled down to 50°C. 22 mL of 37 wt% aqueous formaldehyde solution, with a different content of $NaHCO_3$ vesicant as seen on Table 1, were added to the above PVA aqueous solution and the resultant mixture was agitated for 20 min at a speed of 1500 r/min. Then 20 mL of 36 wt% HCl aqueous solution was added and the resultant mixture was agitated until the maximum froth volume was reached. The resultant mixture was, again, agitated for 30 min. to stabilize the froth and the above intertwined chitosan/PVA solution was dropped into the froth under agitation, according to the composition seen on Table 1. The resultant froth then was extruded into a polypropylene mold and was cured in the mold for 5 h at 50–60°C. Thereafter, the mold was opened and the obtained sponge was washed by deionized water until the rinsed water had a pH of not lower than 7.0 and with no froth present. The resultant sponge was freeze-dried in a mold for 24 h. Finally, the pale yellow polyvinyl acetal sponges modified by chitosan with different size of pore and different content of chitosan were obtained.

2.2 Measurement of water absorption ratio and water absorption rate

The dry sponge was cut into cubes, and its original mass m_0 and volume V_0 was measured. The cuboid sponge was put into water and the time of where it was fully absorbed in water was recorded as t. The mass m_1 after full absorption in water was measured. The water absorption ratio was calculated according to formula $(m_1 - m_0)/m_0 \times 100\%$ and the water absorption rate was calculated according to formula $(m_1 - m_0)/V_0 t$.

2.3 Measurement of expansion ratio

The dry sponge was cut into cubes and pressed into sheets on a stainless steel mold at 15 MPa. The volume of the sheet sponge was measured as V_0. After full absorption in water, the volume of the cuboid sponge was measured as V_1. The expansion ratio of sponge was calculated according to formula $(V_1 - V_0)/V_0$.

2.4 Measurement of mechanical properties

The dry sponge was cut into standard samples according to GB/T 634-1996 and GB8813-1988. The tensile strength and the compression strength of the samples were tested on a Hounsfield Test Equipment (H10KS, England Hounsfield Ltd.)

2.5 Observation of pore morphology

The dry sponge was sprayed with gold and its pore structure was observed on a Scan Electronic Microscope (SEM XL-30, Netherlands PHILIPS Ltd.) at an accelerating voltage of 20 kV.

2.6 Treatment effect of injured rabbit nasal

Adult New Zealand rabbits (42 ordinary grade male New Zealand rabbit aged 1 month, with body mass between 1.5 and 2.0 kg, provided by medical laboratory animal center of Guangdong province) were used as test animals. The rabbits were anaesthetized with 3% of amobarbital sodium at 1 mg/kg, and were stabilized for the procedure to injure the nasal tissue at 1.5 cm in the inner wall of right nasal cavity until it bled. Then, the injured nasal cavity was packed with polyvinyl acetal sponge modified with chitosan and the sponge was drawn out after 24 h. The rabbit was executed by injecting 5 mL air at the auricular vein to cause embolism at 3, 7, 14 and 30 days, respectively (the executed rabbits were divided into 4 groups with 5 rabbits per group). The blood, cilia and mucosa recovery were observed on optical an microscope and scanning electron microscope (Holand PHILIPS Ltd. XL-30 SEM) and described by words. The 0.3 cm × 0.3 cm area of injured region was taken out and fixed in paraformaldehyde and dehydrated and was then embedded into wax and sliced continuously, taking one piece for every seven for HE staining. The injury and healing conditions of the rabbit nasal mucosa was observed on an optical microscope. When sampling for the SEM group, the inferior turbinate was take out together. The sample was washed 3 times and put into 2.5% of glutaraldehyde

Table 1 Composition and code of polyvinyl acetal sponges modified by chitosan with different content of vesicant and chitosan/PVA solution

code	m ($NaHCO_3$)/g	V (chitosan:PVA)/mL	code	m ($NaHCO_3$)/g	V (chitosan:PVA)/mL
No-0	0	4	No-5	4	0
No-1	2	4	No-6	4	8
No-2	4	4	No-7	4	12
No-3	8	4	No-8	4	16
No-4	12	4			

to fix for 4 h and was then made into samples using a chemical drying method. The sample surface was covered with conductive glue and sprayed with gold. The injured region, the cilia injury conditions and the amount and variety of cilia during healing were observed on SEM.

3 Results and discussion

3.1 Effect of the content of vesicant and chitosan on the mechanical properties of polyvinyl acetal sponge modified by chitosan

The larger the water absorption ratio of medical hemostatic sponges, the stronger is the ability to absorb and hold body fluids. This will facilitate the operation and the recovery of the wound after operation. The larger the expansion ratio and water absorption rate, the stronger the press of the sponge to the packed wound which will highlight the hemostatic effect. The expansion ratio and water absorption ratio of modified sponge increased with the increased content of $NaHCO_3$ vesicant (Fig. 1a). The more the content of vesicant, the more the amount of foam during frothing process which leads to the increased pore ratio per unit volume of sponge which absorbs and holds more water. Due to the increased pore ratio per unit volume of sponge, the pressed sponge per unit volume decreased resulting in the decreased expansion ratio of the modified sponge which represented the expansion extent from pressed volume to the origin volume. But at a high vesicant content, the water absorption rate varied a little with the increased content of vesicant (see Table 2). This was attributed to the fact that at close water absorption ratios, the water absorption rate of sponge depended on its hydrophilicity which in turn was related to the degree of acetalization of sponge. Because of the same reaction conditions, the degree of acetalization was basically the same, leading to the close water absorption rate. The content of vesicant also affected the mechanical properties of modified sponges (see Fig. 1b). With the increased content of vesicant, the tensile strength and compression strength of the modified sponge was gradually reduced. With vesicant levels above 8 g, the tensile strength and compression strength only had small changes. This was attributed to the increased content of vesicant which decreased the solid content per unit sectional area which in turn decreased the strength of modified sponge.

Due to the addition of intertwined chitosan/PVA solution during the acetalization of polyvinyl acetal sponge, with further acetalization, the new added PVA reacted with the surface of the formed froth, leading to adherence of chitosan onto the inter surface of sponge. With increased content of chitosan solution, the adhered chitosan increased, which in turn changed the physical properties of the resultant sponge. It can be seen from Fig. 2 that with increased amount of chitosan/PVA solution, the water absorption ratio and expansion ratio decreased and the water absorption rate was even reduced by an order (see Table 2). This was attributed to the fact that chitosan

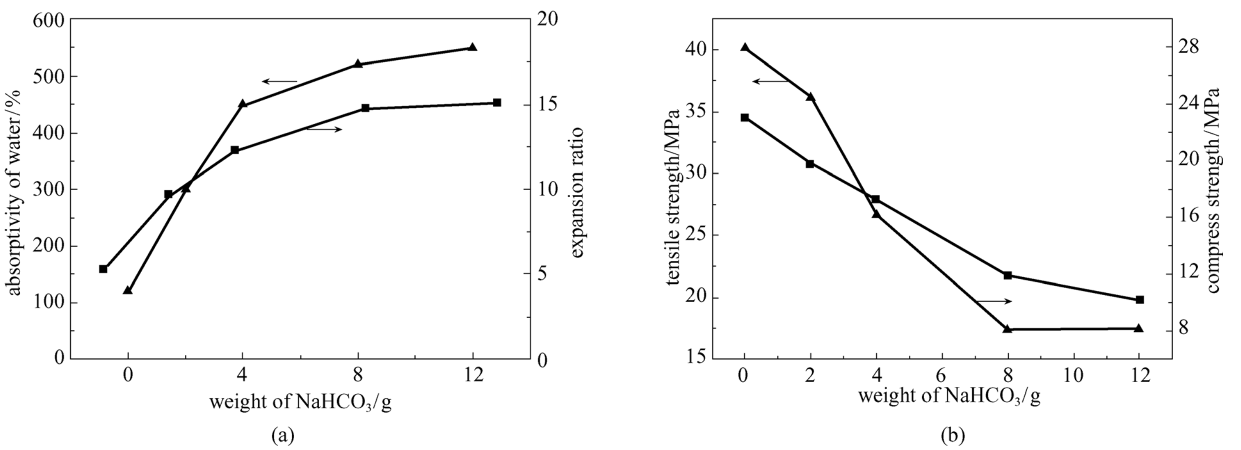


Fig. 1 Variety of physical properties of the polyvinyl acetal sponge modified by chitosan with the content of $NaHCO_3$ vesicant

Table 2 Water absorption rate of polyvinyl acetal sponge modified by chitosan

code	water absorption rate/($g\cdot cm^{-3}\cdot s^{-1}$)	code	water absorption rate/($g\cdot cm^{-3}\cdot s^{-1}$)
No-0	0.105	No-5	0.395
No-1	0.295	No-6	0.080
No-2	0.385	No-7	0.057
No-3	0.415	No-8	0.030
No-4	0.425		

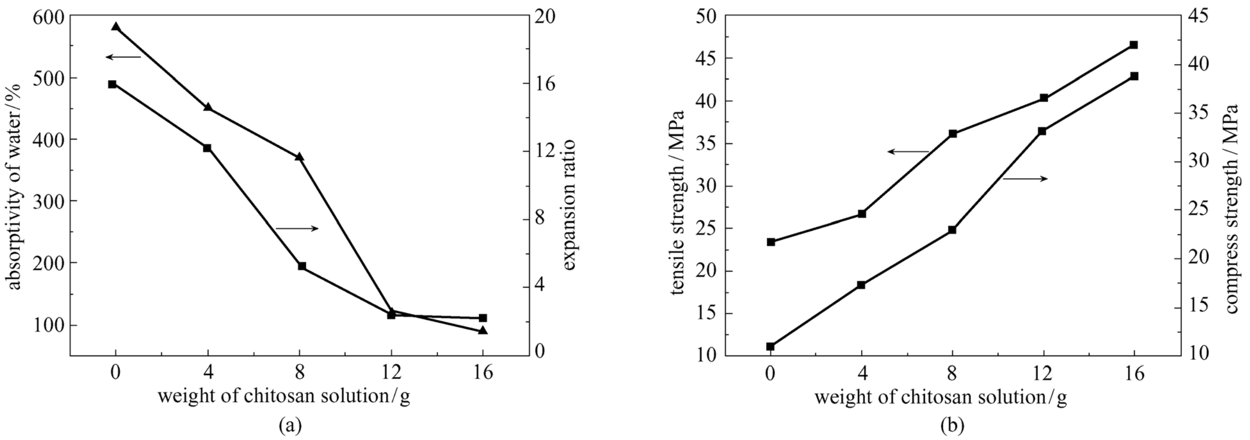


Fig. 2 Variety of physical properties of the polyvinyl acetal sponge modified by chitosan with the content of chitosan solution

adhered to the pore surface during the acetalization and shrunk the pore volume which in turn reduced the contact surface of the hydrophilic group of the sponge with water and depressed the water absorption ability of the sponge. At the same time, with the increased content of chitosan in the pore, the solid content per unit sectional area of sponge increased, which in turn improved the tensile strength and compression strength.

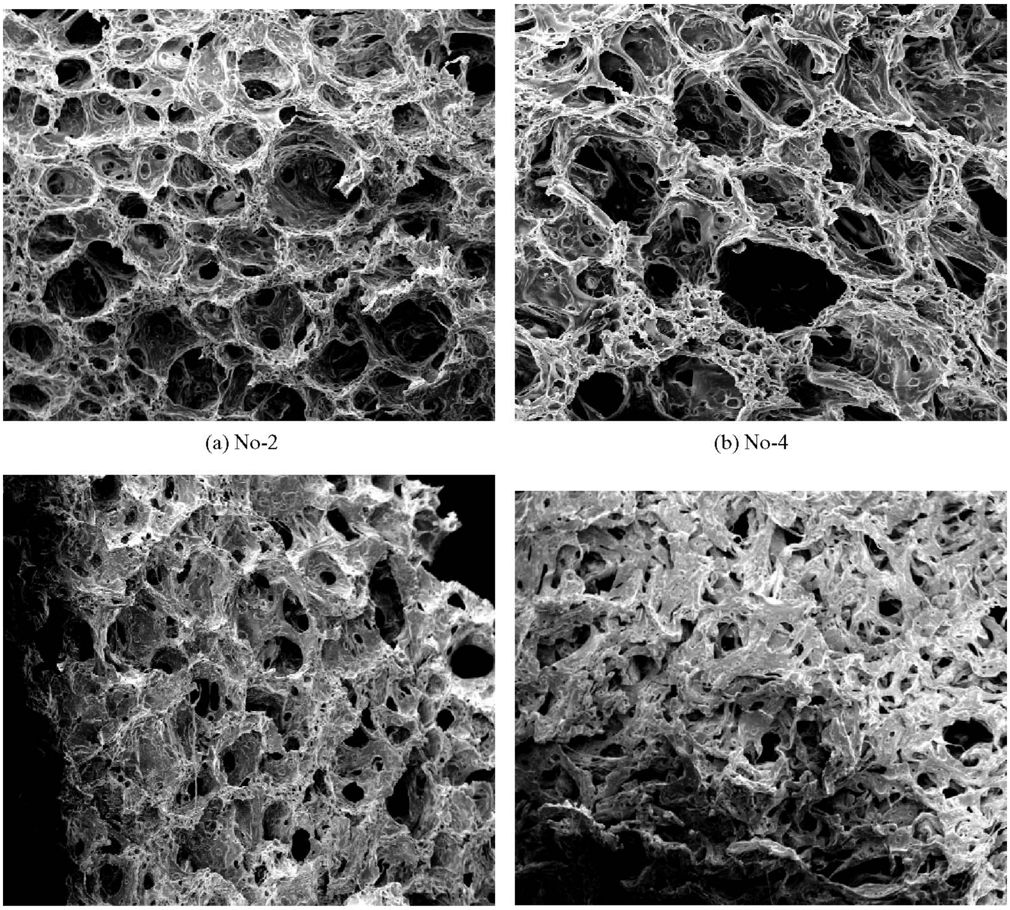
The increased vesicant facilitated the increase of in the water absorption ability of the sponge, but went against the mechanical properties of the sponge. The increased content of chitosan/PVA solution facilitated the improvement of chitosan adherence, therapeutic effect and mechanical properties, but went against the water absorption ability and hemostatic packing effect. Hence, it is necessary to find the equilibrium content between the vesicant and chitosan/PVA solution. This study found that all other constituents being equal, the modified sponge with the addition of 4 g of vesicant and 8 mL of chitosan/PVA solution had the better integrated properties (No-6 sample).

3.2 Pore structure of polyvinyl acetal sponge modified with chitosan

Figure 3 shows the SEM pictures of polyvinyl acetal sponge modified with chitosan with different compositions magnified by 100 times. From Fig. 3(a) and Fig. 3 (b), we can see that with a low content of chitosan/PVA solution (see No-2 sample and No-4 sample), the pores in the modified sponge are large and uniform and the pores connect with each other. There are small connected pores on the wall of large pores. The connected pores' structure and the hydrophilicity of the polyvinyl acetal resin induced the high water absorbability and dilatability of these two sponge samples. Comparing Fig. 3(a) and Fig. 3(b), it can be seen that increasing the vesicant content from 4 g of the No-2 sample to 12 g of the No-4 sample, the pore size was markedly enlarge, but the pore wall only has small changes. On the same resin content and pore wall thickness, the larger the pore size, the more space the froth would take. Hence, the larger water absorption rate and expansion rate were observed in the No-4 sample than in the No-2 sample. Comparing Fig. 3(a), Fig. 3(b) and Fig. 3(c), it can be seen that with the same vesicant content, with an increased content of chitosan/PVA solution, the pore size of sponge shrunk, and the wall between pores thickened. The connected pore size on the large pore wall was also reduced. It can also be seen that too much chitosan destroyed the pore structure which is in favor of water absorption of the sponge leading to the marked reduction in water absorption ratio and expansion ratio. Moreover, the increased content of chitosan resulted in a thickened pore wall which in turn increased the resin that can sustain stress leading to the improved tensile strength and compression strength. Direct observation of SEM shows that the polyvinyl acetal sponge prepared by adding 4 g vesicant and 8 mL chitosan/PVA solution had a more satisfactory pore structure.

3.3 Treatment effect of injured rabbit nasal tissue

Before systematic animal experiments, we carried some simple comparative experiments. The results indicated that homemade glutin sponge could not be integrally drawn out from the injured rabbit nasal after a 24 h packing due to its low strength. The homemade pure polyvinyl acetal sponge adhered with the wound and injured the rabbit nasal tissue. Only the polyvinyl acetal sponge modified by chitosan of this study could be drawn out from the nasal smoothly without any adherence. Hence, this study only took polyvinyl acetal sponge modified by chitosan (No-6 sample) as packing material for systematic investigation. In Fig. 4 and the hemostatic effect of the polyvinyl acetal sponge modified by chitosan and the recovery of mucosa function can be seen. When observed on an optical microscope



(a) No-2 (b) No-4

(c) No-6 (d) No-8

Fig. 3 SEM pictures of the polyvinyl acetal sponge modified by chitosan with different composition (× 100)

after HE staining (see Fig. 4a and Fig. 4 b), it was found that on the condition of immediate administration after injury, the rabbit nasal mucosa had many neutrophilic granulocytes and macrophages (see Fig. 4a), but the polyvinyl acetal sponge modified by chitosan could be drawn out from the nasal smoothly without adherence after being packed for 24 h. On the 14 d after treatment, the amount of neutrophilic granulocytes and macrophages were markedly reduced, indicating that the inflammation was effectively restrained on the injured region (see Fig. 4b). It should be noted that regular cilia of rabbit nasal tissue is long and detailed and is arranged in order. From the SEM pictures of rabbit nasal tissue after injury and immediate administration, the cilia of the injured region basically disappeared (see Fig. 4c). On the 14 d after treatment operation (see Fig. 4d), the injured region grew lots of cilia and the cilia recovered to regular structure indicating that polyvinyl acetal sponge modified by chitosan could promote the healing of the wound and the recovery of nasal mucosa function. Furthermore, this study also recorded the recovery state after 3 d, 7 d, 14 d, and 30 d of treatment operation. After the 3 d of treatment, the epidermis recovered to the original thickness, but the cells were disorderly in arrangement, neutrophilic granulocytes and macrophages were observed. After the 7 d of treatment, the inflammatory cell number was markedly reduced and no cilia were observed. After the 14 d of treatment, the epidermis and cilia recovered to regular its structure, but the arrangement of cilia were more disorderly than the normal cilia arrangement. The cilia was shorter than the regular structure and arranged out of order. After the 30 d of treatment, the cilia recovered to its normal structure. The experimental results adequately proved that the polyvinyl acetal sponge modified by chitosan had good anti-inflammatory, hemostatic, non-adherent characteristics with ease of use and was suitable for clinical applications.

4 Conclusions

Polyvinyl acetal sponge with high strength and water absorbability was taken as the modified matrix. The chitosan/PVA solution containing intertwined chitosan chain with PVA chain was added into the reacting froth during

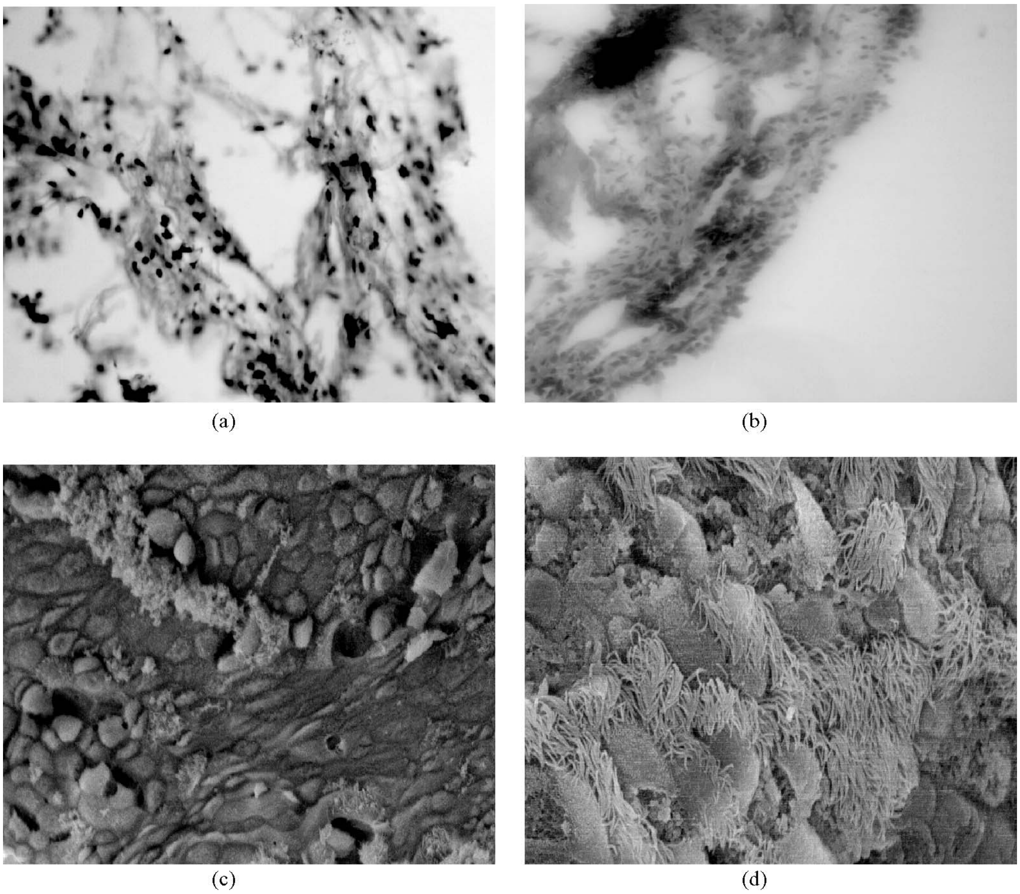


Fig. 4 Observation by Optical microscope and SEM of the injured nasal mucosa packed by No-6 sponge sample modified by chitosan immediately and 14 days of treatment operation
(a) Optical microscope picture of injured rabbit nasal mucosa after HE staining and immediate administration (× 200);
(b) Optical microscope picture of injured rabbit nasal mucosa after HE staining and 14 days of treatment operation (× 200) ;
(c) SEM picture of injured rabbit nasal mucosa after immediate administration (× 4000);
(d) SEM picture of injured rabbit nasal mucosa after 14 days of treatment operation (× 4000)

acetalation between PVA and formaldehyde and the newly added PVA chain further reacted with the surface of the formed polyvinyl acetal sponge, leading to adherence of intertwined chitosan chain onto the inter surface of sponge and endowing polyvinyl acetal sponge with anti-inflammatory, hemostatic and anti-adherent characteristics. The content of vesicant and chitosan had a significant effect on the pore morphology, water absorption ratio, expansion ratio and mechanical properties. When 4 g of vesicant and 8 mL of chitosan/PVA solution were added, the polyvinyl acetal sponge modified by chitosan exhibited the pore structure and mechanical properties that was suitable for the clinical use. When used as a hemostatic packing material for injured rabbit nasal tissue, this modified polyvinyl acetal sponge showed good anti-inflammatory, hemostatic and anti-adherent characteristics and was easy to use. It can promote the healing of the nasal mucosa.

Acknowledgements The work was supported by the National Natural Science Fundation of China (Grant No. 20504018).

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