Preparation and property of soluble hemostatic material with 3D knitted structure

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Abstract
To solve the problems of low mechanical properties and poor coagulation effect of soluble hemostatic dressings, a new type of soluble hemostatic fabric with three-dimensional knitted structure is proposed in this paper. Three-dimensional knitted fabrics had good extension and porous structure. This three-dimensional soluble knitted fabric can be used for skin wounds clotting and healing. This paper mainly studied the influence of structural thickness on the performance of three-dimensional knitted dressings. Characterization analysis showed that three-dimensional knitted soluble hemostatic dressings had appropriate porosity and water vapor transmission rate (WVTR), and the fabric with the maximum thickness had good elongation (70.04%) and a very low blood clotting index (BCI) (11.18%). This suggests the potential application for soluble three-dimensional knitted structure fabric on skin wound hemostasis.

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Keywords
Knitted fabric, carboxymethylation, hemostasis, 3D structure, porosity

Introduction
Uncontrolled bleeding is a major cause of death,\textsuperscript{1,2} and irregular and incompressible wounds lead to up to 30% mortality from traumatic bleeding.\textsuperscript{3,4,5,6} At present, soluble hemostatic materials that can high efficiently clot blood are urgently needed.\textsuperscript{7} These materials are generally divided into non-gauze base materials and gauze base materials.\textsuperscript{8,9} Non-gauze base materials include gels, sponges and fiber-based materials. Fiber-based materials realizes rapid hemostasis and wound healing from electrostatic spinning fiber of the shell core structure and electrostatic film of large surface area.\textsuperscript{10,11} Gels and sponges are prepared from water-soluble carboxymethyl cellulose (CMC) powder and carboxyl chitosan. The sponges have three-dimensional porous structure that can promote blood clotting,\textsuperscript{12,13} but the mechanical properties of sponge and electrostatic film are poor. On the other hand, gauze base materials have strong mechanical properties,\textsuperscript{14} it includes woven gauzes, non-woven gauzes and knitted gauzes. The water-soluble carboxymethyl gauzes are easy to process and degrade.\textsuperscript{15,16,17} However, the two-dimensional gauzes have low pores and need multiple layers to high efficiently clot blood. Methods to improve the hemostatic ability of gauzes remain to be studied.

Many scholars have studied the carboxymethyl modification of gauze. For example, Wang et al. achieved better hemostatic ability by coating carboxymethylchitosan solution and paraffin wax on both sides of carboxymethylated cotton gauze.\textsuperscript{18} This was because single-side moisture transport increased the coagulation factors concentration and initiated endogenous coagulation. This shows that the high absorbent gauze has a good effect on blood coagulation.\textsuperscript{19} In addition to the above improvements in the composite structure, there are also improvements from the yarn raw materials. ConvaTec used modified Tencel fibers to prepare soluble gauzes. Unlike cotton relied on pores to absorb blood, tencel fibers absorbs blood from the fibers. Besides, by adjusting reaction time,\textsuperscript{20} solvent composition\textsuperscript{21,22} and the mass ratio of cotton yarns, NaOH and ClCH$_2$COOH,\textsuperscript{23} Wang et al. prepared modified cotton gauzes with different carboxymethyl content and hemostatic effects. Parikh et al. used the impregnation method on the yarn dyeing machine; this method improved the uniformity of reaction and hemostatic ability of gauzes.\textsuperscript{24} These studies have improved the hemostatic ability of soluble gauze from the aspects of raw materials, modification technology and post-processing methods. But no researches focused on the fabric structure in recent years. Referring to the porous structure of sponges, the hemostatic effect of a three-dimensional porous fabrics may be better than 2D gauzes. The three-dimensional structure weaving of woven and non-woven fabrics is complicated, but three-dimensional knitted fabrics are easy to weave. As shown above, knitted fabrics with antibacterial properties, three-dimensional porosity, and certain mechanical strength have better hemostatic effects.\textsuperscript{25,26,27,28} Combined with the advantages of good mechanical properties and porous structure of 3D knitted fabrics, this paper designs three kinds of 3D knitted hemostatic fabrics with
different structure thickness. All fabrics were carboxymethyl modified to be soluble, the dissolving effect of fabrics is the same. In this paper, the tensile and compressive properties were tested to explore the effects of knitting angle and interlaced layers on the mechanical properties of fabrics. In addition, in order to explore the influence of structure thickness on hemostatic ability, the porosity, swelling ratio (SR) and liquid absorption ratio of fabrics were systematically studied.

**Experimental section**

**Materials**

Viscose filaments (600 denier/120F) were supplied by Rixin Ecological Textile Clothing Co., Ltd. Xinxiang, Henan, China. Chloroacetic acid (CA), anhydrous ethanol, sodium hydroxide (NaOH), anhydrous calcium chloride (CaCl₂) and sodium chloride (NaCl) were purchased from Sinopharm Chemical Reagent Co., Ltd. Shanghai, China. Anti-coagulant rabbit blood was purchased from Zhengzhou Jiulong Biological Products Co., Ltd. Zhengzhou, Henan, China.

**Preparation of knitted fabric**

The thickness (0.18–0.22 mm), tensile properties (stress 6–8Mpa, strain 15%–18%) and blood clotting index (BCI) (about 30% after 5min) of Beijing Tykesman absorbable hemostatic gauze were used as commercial controls. On the same time, the thickness (5–10 mm) and water absorption (not less than 35 times the original weight) of absorbable gelatine sponge were used as other commercial controls.

Six groups of viscose yarns were fed together into the CMS 530 HP multi gauge Stoll flat knitting machine (E7), (Germany). In this paper, three fabrics are named T1, T2 and T3 respectively. The fabrics are based on rib structure, and different proportions of loops and floats form different interlaced layers. The number of interlaced layers are represented by rows. The structure of a row of T1 fabric is two front needle bar loops and floats spanning two stitches, a course is composed of two rows. The structure of a row of T2 fabric is two front needle bar loops and floats spanning four stitches, a course is composed of four rows. The structure of a row of T3 fabric is two front needle bar loops and floats spanning six stitches, a course is composed of six rows. In order to avoid the excessive length of the floats affecting knitting, T3 fabric adjusts the loop position within the range of the floats.

The fabric thickness was measured by the digital fabric thickness meter (YG141LA, Ningbo Textile Instrument Factory, China) for 10 times and the average value and standard variation are obtained. The fabric thickness measured by vernier caliper was consistent with that measured by digital fabric thickness meter. The thicknesses of T1, T2 and T3 fabrics were 4.25 ± 0.02 mm, 5.37 ± 0.04 mm and 7.75 ± 0.02 mm, respectively. The knitting angle of the fabric was measured. The thickness of the fabrics was within the range of sponge. Basic parameters of fabrics were shown in Table 1. Images of fabrics were shown in Figure 1.
Preparation of soluble knitted fabric

Six gram of sodium hydroxide powder was added to 30 mL deionized water (20% W/V) to prepare the sodium hydroxide solution, 1.5 g of fabric was added to this solution, the mixture A was reacted in the SHA-B water bath constant temperature oscillator (120 r/ min) at 75°C for 20 min (Changzhou, China). The alkalized pretreated fabric was

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**Table 1. Basic parameters of three fabrics.**

| Sample | Density (5 cm) | Thickness (mm) | Rows (course) | Knitting angle | Structure of a row |
|--------|----------------|----------------|---------------|----------------|--------------------|
| T1     | 24             | 20             | 4.25 ± 0.02   | 2              | ![Diagram](image1)  |
| T2     | 25             | 20             | 5.37 ± 0.04   | 4              | ![Diagram](image2)  |
| T3     | 26             | 20             | 7.75 ± 0.02   | 6              | ![Diagram](image3)  |

**Figure 1.** Images of fabrics. Real images of three fabrics before (a) and after (b) carboxymethylation, (c) three-dimensional simulation models of three fabrics.

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obtained. Meanwhile, a mixture B of sodium hydroxide solution (4.2 g NaOH, 5.25 mL deionized water, 30 mL anhydrous ethanol) and chloroacetic acid solution (7.5 g CA, 7.5 g anhydrous ethanol) was prepared. Then, the alkalized pretreated fabric and the mixture B were reacted at 75°C for 3 h in a SHJ-4D digital display thermostatic magnetic stirring water bath (Jintan Youlian Instrument Research Institute, Changzhou, Jiangsu). The rotor speed was 2000 r/min. The drying condition was 50°C for 1 h. The specific fabric modification process was shown in Figure 2.

**Characterization**

Scanning electron microscope and FT-IR. The internal microstructure of soluble hemostatic fabrics was observed by a scanning electron microscope (SEM, Su1510, Hitachi, Japan). Prior to the testing, the fabrics (square shape, 10 mm width, and 10 mm length, thickness: 4.25/5.37/7.75 mm) were secured on a metal base with conductive glue. The test voltage was 5 kV, the images were obtained with a magnification of ×1600, 2100×, 55× and 700×. Infrared spectra refer to the identification of material molecules, Fourier-transform infrared (FTIR) spectra was acquired using a spectrometer (Nicolet is10, Thermo Fisher Scientific (China) Co., Ltd, USA) over the range of 500–4000 cm⁻¹. The scanning resolution was 0.05 cm⁻¹ and the scan number was 32.

Porosity. The liquid displacement method was used to determine the porosity of the soluble hemostatic fabrics. The fabrics (square shape, 10 mm width, and 10 mm length, thickness: 4.25/5.37/7.75 mm) were immersed into 10 mL of anhydrous ethanol to reach a saturation point. Gently wiping the ethanol off the surface with a filter paper and immediately weighed (W₁). Each fabric was recorded for three times. The porosity was calculated according to equation (1)

\[
P (\%) = \frac{W₁ - W₀}{\rho₀V₀} \times 100
\]

(1)

Where P is the porosity, where W₀ and W₁ are the weight of fabrics before and after immersion, V₀ is the initial volume of the fabrics, and ρ₀ is the density of the anhydrous ethanol (0.79 g/mL).
**Water vapor transmission rate.** The Water vapor transmission rate (WVTR) of the fabrics was measured according to the American Society for Testing and Materials (ASTM) E96–00 procedure. All fabrics were placed at room temperature of 20°C. The fabrics (circular shape, diameter: 1.1 cm, thickness: 4.25/5.37/7.75 mm) were placed tightly over the mouth of volumetric flask, the flask contained 10 mL of deionized water and was sealed edge to prevent water vapor losing through the mouth except the fabric. Each fabric was recorded for three times. The WVTR (g/m²/day) was calculated with the following equation (2):

$$\text{WVTR} = \frac{W_i - W_t}{A} \text{g/m}^2/\text{day}$$  \hspace{1cm} (2)

Where A is the area of the volumetric flask mouth (m²), where Wi and Wt are the weight of the bottle containing water(g) before and after 24 h respectively.

**Swelling ratio and dissolution time.** A gravimetric method was performed to measure the SR of fabrics. The fabrics (square shape, 10 mm width, and 10 mm length, thickness: 4.25/5.37/7.75 mm) were put in the culture dish (about 6.7 mm in inner diameter) with 10 mL of deionized water. At regular time intervals, the fabrics were weighed with the help of a ruler. 0.1 g of fabric were put into 20 mL deionized water, then the dissolution time was observed. Each fabric was recorded for three times. The SR ratio was calculated according to equation (3):

$$\text{SR} = \frac{W_t - W_a}{W_a} \times 100\%$$  \hspace{1cm} (3)

Where Wa and Wt are the initial and wet weights of the fabrics (g) respectively.

**Water/saline/blood-absorption value measurements.** 0.5 g of fabric were put into three liquids (4 mL) for just 10min to ensure it does not dissolve. 8.298 g of NaCl and 0.278 g of anhydrous CaCl₂ were dissolved into 1 L of deionized water to prepare a composite salt solution. The blood was anticoagulant rabbit blood. Fabrics immersed for 10 min and directly weighed, each fabric was recorded for three times. The absorption value was calculated using the following equation (4):

$$\text{Absorption value} = \frac{W_b - W_a}{W_a}$$  \hspace{1cm} (4)

Where Wa and Wb are the initial and wet weights of the fabrics (g) respectively.

**Mechanical properties.** The tensile and compression test were conducted through MTS Exceed E43 (MTS Systems (China) Co., Ltd., Guangzhou, Guangdong, China) at a relative humidity of 80% and a temperature of 25°C. In a certain humidity environment, this condition was to simulate the actual situation of the fabric on the skin surface. The tensile fabrics were 70 mm in length × 10 mm in width × 4.25/5.37/7.75 mm in thickness, the gauge length was 4 mm, the pre-tension was 1 N, the fabrics were stretched at a constant speed of 10 mm/min until it broke. The tensile strength, elongation at break and stress-strain were measured. For compression test, the fabrics were 20 mm in length × 20 mm in width × 4.25/5.37/7.75 mm in...
thickness, fabrics were compressed up to 80% of their original thickness at the displacement rate of 0.5 mm/min. The stress (MPa) - strain (%) were recorded. The average value of five measurements was recorded for each sample.

**Blood-clotting time assay.** The clotting time of fabrics was measured by Behrens method.\(^{31}\) 100 mg of fabric were placed in a 5 mL centrifuge tube on a 24-well needle bar and preheated at 37°C for 5 min. Then 1 mL of rabbit anticoagulant blood and 0.2 mL 0.2 mol/L of calcium chloride solution were added into the centrifuge tube. Tilting the tube every 15 s until blood was no longer flowing, and recorded the clotting time. The blank control group only added blood. The commercial gauze in the control group was cut to similar size to the three fabrics, overlaying multiple layers of gauze until they weigh 100 mg. Average values of three measurements were recorded for each sample.

**Whole-blood clotting in vitro.** Blood Clotting index is the percentage of unclotted blood cells and can be used to measure the hemostatic effect, it was carried out according to the previous literature.\(^{32}\) 100 mg of fabric were placed in a beaker and preheated at 37°C for 5 min. At the same temperature, 0.1 mL of rabbit anticoagulant blood and 0.02 mL of calcium chloride solution were dropped on the surface of the fabrics and reacted for 1 min, 2 min, 3 min, 4 min and 5 min, respectively. Then 25 mL deionized water was added into the beaker and reacted 5 min. Control group was added 0.1 mL rabbit anticoagulant blood and 25 mL of deionized water. The commercial gauze of the control group was cut to the same size as the three fabrics, overlaying multiple layers of gauze until they weigh 100 mg. Finally, the supernatant was collected and determined by UV-visible spectrophotometer (Puxi General Instrument Co., Ltd.) TU-1901 UV-visible spectrophotometer (wavelength 540 nm). Beijing, China). The BCI was calculated according to equation (5):

\[
\text{BCI} (\%) = \frac{I_a}{I_0} \times 100\% \tag{5}
\]

Where \(I_a\) is the absorbance of sample, \(I_0\) is the absorbance of the blank control group, each sample was tested for 3 times and the average value was taken.

Experiment according to the above scheme was tested again in a culture dish (about 6.7 mm in inner diameter). The color of the water represented the degree of blood coagulation.

**Statistical analysis.** All data points were presented as mean values ± standard deviation (SD). Statistical analysis was carried out using IBM SPSS Statistical Processor. Data were analyzed using Tukey’s test. The \(p\) values were calculated. The higher the \(p\) value, the lower the significance. In this study, \(p \leq 0.0500\) indicated that the effects were significant.

**Results and discussion**

**Fourier-transform infrared, SEM and porosity of soluble hemostatic fabric**

The FT-IR spectrum of carboxymethylated fabrics showed that the broad absorption peak between 3300-3400 cm\(^{-1}\) was the stretching vibration absorption peak of the hydroxyl
group (-OH), because carboxymethylation increased the number of hydroxyl group. Peaks at 1600 cm$^{-1}$ and 1410 cm$^{-1}$ for sodium CMC, which corresponded to asymmetric and symmetric stretch vibrations of carboxylic groups (-COOH), respectively. This suggested that the carboxylic groups in chloroacetic acid was introduced. The sodium CMC was synthesized by introducing chloroacetic acid into the C-6 hydroxyl groups of cellulose in alkaline conditions. The appearance of the peak at 1060 cm$^{-1}$ can be ascribed to the C-O-C bending vibration. The peak at 1323 cm$^{-1}$ was related to the bending vibration of -CH$_2$, which was from C-6 of cellulose and -CH$_2$ of chloroacetic acid. As reported above, this result of four new absorption peaks in Figure 3 demonstrated that the fabrics were successfully carboxymethylated.

The microstructure of the fabrics was observed by SEM (Figure 4(a) to (d)). The three fabrics underwent the same chemical treatment, resulting in the same microstructure of yarns and fibers. Taking fabric T1 as an example, Figure 4(a) and (b) showed SEM images of viscose yarns surface before and after carboxymethylation. In Figure 4(b), the irregular tooth-like groove structure and uniform protrusions were formed on the longitudinal surface, it was due to the large amount of water entering the fiber, fiber swelling caused its longitudinal contraction and cracks. The changes of yarn cross section before and after carboxymethylation were observed in Figure 4(c) and (d). Figure 4(d) showed a slight increase in diameter and cross-sectional area, the enlarged cross section formed a channel for blood transport. As can be seen from Figure 4(a) to (d), the fiber pores in the yarn were filled with sodium CMC, which will affect the porosity of the fabric.

As shown in Figure 4(e) to (f), the porosity of three original fabrics was 40.21 ± 1.72%, 46.36 ± 1.36% and 56.19 ± 1.86% respectively. After alkalization, the porosity decreased to 31.27 ± 2.11%, 38.89 ± 1.67% and 47.37 ± 2.31%. The porosity decreased obviously after carboxymethylation. This was because after carboxymethylation, viscose yarns

**Figure 3.** FTIR spectra of fabrics with three thicknesses.
absorbed sodium CMC, so that the pores of the fabric (the pores between the yarns and inter yarn pores between the fibers) have been blocked, resulting to a decrease in porosity values. Porosity was determined by the number of interlaced knitted layers inside the fabrics. A higher number of interlaced layers suggested a larger angle and porosity. Porosity is a fundamental property of hemostatic fabrics. The larger porosity is beneficial to the absorption of water, saline and blood. Meanwhile, it can be seen that the porosity were affected by carboxymethylation, such as T1 ($p = .0000$), T2 ($p = .0001$) and T3 ($p = .0002$).

Water vapor transmittance, liquid absorption and SR of soluble hemostatic fabric

The ideal wound dressing should have an appropriate water vapor transmittance (WVTR). In general, in order to prevent fluid accumulation and keep the environment moist, a moderately low WVTR is beneficial to the wound. The WVTR required for skin injury is 279–5318 g/m$^2$/day, besides the WVTR of normal skin was 200–500 g/m$^2$/day. However, too low WVTR can lead to prolonged moisture and discomfort on the surrounding skin. As shown in Figure 5(a), the WVTR of fabrics T1, T2 and T3 were 842.2 g/m$^2$/day, 789.6 g/m$^2$/day and 736.9 g/m$^2$/day, respectively. Under the moist environment, the
WVTR of the three fabrics were in the range of 700–900 g/m²/day, which was much higher than the WVTR of normal skin. The WVTR of fabrics was at a proper medium level and suitable for application as wound dressings.33,34 According to the above porosity test, the porosity of the three fabrics was generally low, but the hygroscopicity of the carboxymethylated fabrics was obviously improved. In the WVTR test, the effect of moisture absorption was more obvious than that of porosity. Moreover, in a humid environment, the fabric absorbed water vapor and formed gel, which blocked the pores and reduced the channels for water vapor evaporation, thus reducing the WVTR value. Besides, higher thickness gived water vapor longer distance to pass through and more yarns made water vapor pass with more difficulty. The inverse ratio of WVTR to porosity is mainly attributed to the properties of fabric that become gel after moisture absorption. However, the effect of thickness on WVTR was not significant ($p = .74$).

Fluid absorption capacity is the key to evaluate hemostasis rate and wound humidity. After the carboxymethyl modification of the fabric, the pores of the fabric mainly referred to the holes in the middle layer. The soluble fabric became gel after absorbing the liquid, leading to the reduction of the pores. So the liquid absorption was mainly related to the yarn performance. As shown in Figure 5(b), there was a tendency of improvement of liquid absorption with the increase of thickness for all liquids that were evaluated. When comparing the same thickness, water and saline medium presented higher absorption than
blood, and the absorption degree of salt water is slightly lower than that of water. On the one hand, the inorganic salts in the saline and blood affected the osmotic pressure inside and outside the fabric, so absorption of saline was slightly reduced. On the other hand, blood was more viscous than water and salt water, and a large number of platelets in the blood attach to the rough surface of the fabric and cause channel blockage. At the same time, soluble carboxymethyl gauze contained a large number of negative ions after dissolution, which can activate coagulation factor XII and promote the generation of thrombin. Blood coagulation affected the absorption of fabrics. The liquid absorption of the three fabrics was lower than that of conventional carboxymethyl dressings. For example, the water absorption of the fabric in this experiment was 1–1.2 g/g, while the water absorption of the traditional CMC woven gauze can reach 8.3 g/g.35 The hydroscopicity of CM-CKF knitted fabric treated by Zhao et al. in water system was 1.86 g/g, but the hydroscopicity of CM-CKF treated by Zhao et al. in isopropanol-water (V/V = 3/1) was 14.86 g/g.21 Therefore, the performance of CMC wound dressing depended not only on the fabric structure, but also on the solvent. The solvent of fabrics T1-T3 was water, resulting the less liquid absorption. Experiments with isopropyl alcohol-aqueous solvents will then be carried out. Moreover, the effect of thickness on water absorbency was not significant (p = .5), as well as saline (p = .089) and blood (p = .844).

In soluble dressings, the SR can represent the contact area. More detailed data was shown in Figure 5(c). From 0 to 240 min, the curve presented an upward trend. The swelling rate before 60 min increased faster, because the fabrics retained the fabric structure when starting absorbing water. After absorbing a certain amount of water, the fabric became a gel structure. After 240 min, the fabrics gradually dissolved and had no water storage capacity. A thicker fabric had more yarns overlapped in it, suggesting it can deform to a greater extent after absorbing water. The maximum of SR was 458.69% of T3 fabric. But the modified fabrics in this experiment can quickly clot blood through porous structure. Compared with two-dimensional gauze, the hemostatic knitted fabrics had swelling properties. The swelling process was shown in Figure 5(d). The effect of thickness on swelling was significant (p = .0000).

Due to the same yarns and chemical treatment, it was reasonable that three fabrics of the same weight had the same dissolution time, all fabrics dissolved in water for about 5 h. The dissolution process was shown in Figure 5(e).
The fabrics were placed in an environment of 80% relative humidity and 25°C for 10 min. In a certain humidity environment, this condition was to simulate the actual situation of the fabric on the skin surface. As shown in Figure 6(a), the tensile stress was proportional to the fabric thickness and the strain was inversely proportional to the fabric thickness. The yarn interlaced layers and interlaced points had an effect on tensile strength \( p = .0001 \) (Figure 6(b)). The more interlaced points suggested the greater friction and tensile strength. At the same time, the larger knitting angle suggested the smaller yarn strain range \( p = .009 \) (Figure 6(c)). Tensile stress of T1 fabric was obviously lower than the other two fabrics, because the interlaced points of the two layers was unstable, yarns were easy to slip when bearing the stress, and the corresponding strain was larger. Compared with commercial soluble woven gauze, the tensile stress of knitted fabrics was obviously smaller, while the strain was obviously larger, this was due to the characteristics of knitted structure. Therefore, when the wounds covering larger area, the knitted hemostatic fabrics with better elongation had an advantage over the commercial gauze. Figure S1 showed the stretching and bending processes of fabrics.

As shown in Figure 7(a), a thicker fabric had higher compressive strength \( p = .0000 \). The fabrics were compressed to 80% of its original thickness. During the compression, the curve rised gently firstly, and then the fast rising stage laterly suggested the fabric damage. T3 fabric had reached the stress failure when the displacement was small, but the yarns were still piled up, so the compression force can continue to increase. The maximum was shown in Figure 8(b). Compared with the other two fabrics, T3 fabric presented greater pressure resistance because of the greater number of interlaced yarns. Both tensile strength and compressive strength were higher than 3D porous sponge. The unit of compressive stress of sponge was mainly Kpa, and the maximum of 3D nanofiber sponge was 4 Mpa when the strain was 11\% 36,37 3D knitted hemostatic fabrics with high compressive performance are good for resisting impact and collision.
Clotting time test of soluble hemostatic fabric

The rapid hemostasis ability of wounds is the key to wound treatment and can be tested by blood coagulation test. Figure 8(a) showed clotting time of hemostatic fabrics. The results suggested that T0 pure blood group had the longest clotting time (16.55 ± 0.4 min). The clotting time decreased with the increasing thickness of fabrics. The clotting time of fabrics T1, T2, and T3 was 10.12 ± 0.11 min, 9.02 ± 0.45 min, and 8.16 ± 0.17 min, respectively. The clotting time of commercial gauze in the control group was 9.32 ± 0.09, which was longer than T3 fabric and similar to T2 fabric. Clotting time was related to fabric structure, yarn porosity and blood transport capacity ($p = .0004$). A thicker fabric had more pores, yarns and contact area, which can store more blood in the pores to reduce blood flow, so the clotting time was short. On the other hand, commercial gauze had small contact area and pores. Therefore, when blood was a lot, two-dimensional gauzes cannot store blood like the 3D fabrics, it can stop bleeding only in the contact range of blood, so clotting time was longer. In this experiment, the clotting time of T1 was longer than commercial gauze, because the porosity was smaller, the effect of pore was inferior to contact area of commercial gauze.

**Figure 8.** In vitro hemostatic efficacy of different thicknesses fabrics. (a) clotting time and images on 0#, T1, T2, T3 fabrics and gauze respectively, (b) the Abs value of fabrics and gauze after 5 min of clotting time, (c) the whole blood clotting index of gauze and different fabrics with the time changing, (d) the photographs of simulative the whole blood clotting process.

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The BCI test of soluble hemostatic fabric

Abs value refers to the amount of residual hemoglobin and can be used to calculate BCI. Abs values was to measure the absorbance of the resulting solution at 540 nm in this experiment. Low BCI value indicates good clotting ability. Abs values tested at 5 min were shown in Figure 8(b). Abs value of pure blood was the largest. Abs value of fabrics decreased with the increasing thickness. Blood Clotting index was related to the contact area and porosity of fabrics along the thickness direction ($p = .001$). The more pores and were more beneficial to contact and store more blood, so the BCI value was smaller. The BCI value of commercial gauze was smaller than all knitted fabrics before 3 min. Because the overlapped woven gauzes had a large surface area, so it was easier to clot blood at the beginning, but the blood was free in the gap of overlapped gauzes later, so the hemostatic effect was weakened. After 3 min, the porous knitted fabrics transported blood to the yarns and pores, the blood contacted with a large area of hemostatic fabrics, therefore it can quickly clot. As shown in Figure 8(c), the BCI of fabrics decreased at an accelerated rate, while the BCI of commercial gauze gradually flattened. The BCI value was consistent with the results of clotting time test. Pictures of dynamic coagulation process of fabrics were shown in Figure 8(d). When the blood was completely clotted on the surface of the fabrics, the solution was light in color, indicating the hemostasis effect was good. The solution turned red obviously because of the unclotted blood, indicating that the fabrics had poor hemostatic ability. By looking at the color of the water, you can tell how clotted the blood is.

Compared with the traditional dressings and soluble hemostatic materials, the BCI value of cotton yarn was 66.3 ± 5.5%, and the BCI value of carboxymethyl fabric coated with carboxymethyl chitosan was 45.7 ± 5.5%.18 Fully soluble hastatic fiber (FHF) was the product of cotton yarn after carboxymethylation, and its Abs value at 540 nm was as low as 0.04 at 5 min.19 Among the carboxymethyl chitosan - gelatin - alginate modified cotton fabric (AGCCg), AGCCG-5 dressing had the lowest BCI value (only 34.9% at 5 min).28 Among the double-bonded carboxymethyl chitosan/ cysteamine-modified Chondroitin Sulfate Composite, the relative BCI value of ME-CMC/CSS-2 in dressing was 42.29 ± 6.01.38 The BCI value of Tykesman gauze tested in this experiment was 29.8 ± 0.54% at 5 min, while the BCI value of T3 fabric was only 11.18 ± 0.74% at 5 min. Under the condition of soluble dressings, the BCI value of T3 fabric was much lower than the above soluble hemostatic gauzes. However, the BCI value of soluble carboxymethyl chitosan sponge was about 41% at 3 min, and the BCI value of T3 fabric was 39.62 ± 6.25% at 3 min.39 The BCI value of T3 fabric was very close to that of soluble sponge, and the fabric with larger thickness was more similar to soluble sponge.

In addition, insoluble CMC calcium/chitosan blend nonwovens can quickly reach a low BCI value within 30 s, and the Abs value at 540 nm was as low as 0.05,40 while the Abs value at 540 nm of T3 fabric can only reach 0.05 after 5 min. It can be seen from this that whether soluble greatly influenced blood clotting speed in the early stage, but just because of solubility, the BCI of soluble fabrics decreased at a faster rate in the later stage, and thicker fabrics had more obvious effects. Insoluble dressings can achieve a good
coagulation effect in a very short time due to the characteristics of non-disintegration of the structure after absorption.

**Conclusion**

In conclusion, this work used sodium hydroxide and chloroacetic acid to carboxymethyl modify three-dimensional porous knitted fabrics, developing a new kind of soluble three-dimensional hemostatic material. This hemostatic material combined the characteristics of good elongation and porous structure of knitted fabrics. The experimental results suggested that the thicker fabric had smaller tensile strain, higher tensile stress and compressive strength. At the same time, the thicker fabrics had greater porosity, contact area and SR, allowing for faster blood transport, storage and clotting. The three fabrics had poor fluid absorption but good blood clotting effect. T3 fabric had the best coagulation effect, and its BCI value was as low as 11.18 ± 0.74% at 5 min, far lower than soluble hemostatic gauzes and slightly lower than soluble sponges. Due to its low fluid absorption, three-dimensional knitted hemostatic material is not suitable for heavy bleeding, but suitable for hemostatic healing of small bleeding skin wounds, On the one hand, 3D knitted fabrics have similar characteristics of thickness and structural porosity to sponge hemostatic materials, which is convenient for blood to fill the pores and better clotting. On the other hand, 3D knitted fabric retains the strength and softness of the fabric. This study is the first to introduce three-dimensional knitted fabrics into the field of hemostasis, the 3D knitted fabric has a potential application prospect as a medical hemostatic material.

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**Supplemental Material**

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