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A super-efficient and biosafe hemostatic cotton gauze with controlled balance of hydrophilicity/hydrophobicity and tissue adhesiveness

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ABSTRACT

Cotton gauze is a widely used topical hemostatic material for bleeding control in military and civil accidents and surgical operations, but its high blood absorption capacity tends to cause extra blood loss of the wounded person, which may increase the risk of shock and death. Therefore, development of rapid hemostatic cotton gauze with less blood loss is of great significance. Herein, we prepared a super-efficient hemostatic cotton gauze whose surface was slightly modified with a catechol compound which features a flexible long hydrophobic alkyl chain terminated with a catechol group. Its hemostatic performance in rat and pig injury models was far superior to standard cotton gauze and Combat GauzeTM. The latter is a well-known commercial gauze for

controlling massive hemorrhaging. Additionally, after stoppage of bleeding, the wound sites hardly re-bleed upon the gauze was peeled off. Histological analysis proved that the novel cotton gauze well kept the biosafety of cotton gauze. Interestingly, a similar impressive hemostatic performance was also achieved for chitosan nonwoven gauze modified with the same procedure. Density functional theory calculation and instrumental measurements demonstrate that their extraordinary hemostatic capability is attributable to the highly efficient formation of big and thick primary blood clot made of massive aggregated erythrocytes, due to gauze's effective controlling of blood movement through its blocking effect from tissue adhesion by catechol, platelet activation by cotton fiber, blood absorption by cotton, and hydrophobic effect from long alkyl chain. The methodology and hemostatic mechanisms presented in this work may open a new avenue for developing highly efficient hemostatic gauzes.

INTRODUCTION

Massive bleeding from nonfatal traumatic wounds is one of the leading causes of death and disability in battlefields and civilian accidents, because significant blood loss causes symptoms such as hypothermia, coagulopathy, acidosis, sepsis, and organ failure.^{1, 2} More than 50% of these mortalities is preventable if emergent and efficient hemostatic measures are applied. This inspires the development of advanced hemostatic products and technologies for prehospital bleeding control of the wounded people, to increase survival rate and reduce medical costs.³⁻⁶

Cotton gauze has a long history as an effective topical hemostatic fabric for compressible and noncompressible wounds, mainly due to its safety, non-allergy, low cost, adaptability, breathability, stability, blood absorbency, and easy applicability^{7, 8}.

It is still the most widely used hemostat for traumatic bleeding control, although lots of

1 efficient hemostatic agents have been manufactured and clinically applied in recent two
2 decades.⁹⁻¹² The hemostatic mechanism of cotton gauze counts on the activation of
3 platelets upon contacting with cotton fiber, and its quick wicking of blood fluid, leading
4 to resting of blood cells and platelets to form blood clots. However, it's often seen that
5 excessive large volume of blood is absorbed by cotton gauze before bleeding stops, due
6 to its highly hydrophilic nature, porous structure, and capillary action among the
7 weaved fibers. Those extra blood losses may be the last straw that causes morbidity or
8 mortality because the blood volume in the circulating system is critical.

9 Many endeavors to enhance the hemostatic efficacy (reduction in blood loss and
10 bleeding time) of cotton gauze have been made in the academic and industrial circles.
11 Z-Medica in USA has commercialized a gauze brand-named QuikClot Combat Gauze®
12 (QCG), which is made by binding inorganic mineral kaolin particles onto
13 rayon/polyester nonwoven. Kaolin can activate clotting factor XII to accelerate blood
14 coagulation reactions, leading to fast thrombus formation. This topical hemostat has
15 been clinically adopted in military, emergency care, and hospital for compressible
16 severe hemorrhage. However, its rapid hemostasis efficacy is subsided because of
17 possible loss of kaolin, and the detached kaolin particles may cause risks of unexpected
18 distal thrombus. To increase binding stability of inorganic particles to cotton fiber,
19 mesoporous chabazite zeolite particles were chemically anchored onto fiber surface by
20 an on-site growth route.⁸ Such a composite cotton gauze has a better topical hemostatic
21 efficiency than QCG. In the rabbit lethal femoral artery injury model, blood loss of
22 chabazite zeolite-cotton gauze was only about 40% less than that of QCG. For these
23 two kinds of hybrid cotton gauzes, it's obvious that they still absorb large precious

1 volume of blood during bleeding control. Tuning the wettability of a hemostatic fabric
2 was proposed to address this concern.¹³ A Janus gauze consisting of a top hydrophobic
3 fabric layer and a bottom hydrophilic cotton fabric layer was developed. It was thought
4 that the hydrophilic layer absorbed blood to expedite clotting, while the hydrophobic
5 layer gives a pressure to inhibit blood diffusion through gauze in lengthways. However,
6 blood permeation in warp and weft directions of the bottom cotton fabric layer is un-
7 avoidable, resulting in losses of valuable blood yet. Furthermore, in most cases, blood
8 seepage from the seam between the hemostatic fabric/wound surface, which leads to
9 massive blood loss as well. A composite gauze with a superhydrophobic
10 Poly(vinylidene fluoride)/carbon nanofiber (PVDF/CNF) bottom coating layer was
11 reported to have fast hemostatic capability and no re-bleeding potential, because of
12 synergetic effects from CNF's acceleration of fibrin fiber formation and PVDF's
13 repellency of blood.¹⁴

14 Therefore, controlling of movement of blood fluid at the gauze/tissue contact
15 surface and in gauze is the key in designing a highly efficient hemostatic gauze. Inspired
16 by mussel foot protein's good adhesion to wet substrates,¹⁵⁻¹⁷ we speculate that cotton
17 gauze with catechol group on its surface may help it adhere to blood-wetted tissue, in
18 order to hinder blood seepage from the seam of gauze/tissue contact surface; meanwhile,
19 the strong interaction among cotton fibers through catechol linkage may slow down
20 blood diffusion in gauze. Thus, in view of the great importance of fiber structure,
21 wettability, and wet biological tissue adherence to hemostatic materials, herein, we
22 design and prepare a novel hemostatic cotton fabric, which is made by slightly grafting
23 a catechol compound (Fig. 1a) with a C15 alkyl side chain onto the fabric surface. This

hemostatic fabric integrates wet tissue adhesiveness from catechol group, hydrophobicity from long alkyl chain, absorbency and breathability from cotton fiber, into one hemostatic device. In rat femoral artery and liver laceration, and pig femoral artery injury models, this modified cotton gauze shows very limited blood permeation through the gauze, no blood oozing out from the seam of gauze/wound contact surface, short bleeding time and reduced blood loss. Its biosafety is comparable to cotton gauze, as suggested by the cytocompatibility and histological examinations. This idea and methodology were also successfully applied to chitosan nonwoven fabric to fabricate a gauze with high hemostatic capability.

Fig. 1 (a) Reaction scheme for grafting catechols onto cotton gauze. Surface grafting reactions of (b) 4-allyl-1,2-benzenediol (ABO) and 1,2-benzenediol-3-(9,11,13-pentadecatrienyl) (USO), and (c) hexadecyltrimethoxysilane (HTMS) onto cotton cellulose macromolecular chain.

Results

Chemical composition and surface structure

Free radicals can be readily generated on cotton gauze by plasma treatment. Through radical initiated reaction with the double bonds in USO, which can be grafted onto cotton gauze surface. Thus, a long alky side chain with a catechol end group was introduced onto cotton fiber (Fig. 1a, b). Its chemical structure is confirmed by its solid state ^{13}C NMR spectrum (Fig. 2a). The peaks at region of 60-70 ppm are attributed to C6 of cellulose, while the signals at region of 70-80 ppm is assigned to C2, C3, and C5 of cellulose. The peaks at region of 80-95 and 100-110 ppm correspond to C4 and C1, respectively.¹⁸ Compared with cotton gauze, several new peaks appear for USO-g-gauze, i.e., broad peaks at 120-150 ppm corresponding to unsaturated carbons on benzene ring; peaks at region of 10-40 ppm assigning to methyl and methylene groups of the alkyl chain of USO. Therefore, ^{13}C -NMR spectra indicates that USO is successfully grafted onto cotton gauze surface.

Fig. 2 (a) Solid-state ^{13}C NMR spectra of gauzes. SEM images of (b-d) cotton gauze and (e-g) USO-g-gauze at different magnifications.

FTIR and XPS also prove that ABO, HTMS and USO were successfully grafted onto the surface of cotton gauze (Figs. S2 and S3). For cotton gauze, characteristic

absorption bands at 3200-3600 cm^{-1} ($\nu_{\text{O-H}}$), 1610 cm^{-1} ($\nu_{\text{C-C}}$), 1435 cm^{-1} ($\delta_{\text{C-O-C}}$), 1125 cm^{-1} ($\nu_{\text{C-O-C}}$) are present. In the spectra of ABO-g-gauze, HTMS-g-gauze, and USO-g-gauze, a new band at 2846 cm^{-1} appears for $\nu_{\text{C-H}}$ of $-\text{CH}_2-$ groups of ABO, HTMS, and USO grafted on the gauze. C1s XPS spectra shows the relatively strongest peak of USO-g-gauze is C-C bond, while that of cotton gauze is C-O-/C-OH bond (Fig. S3b). This is in accordance with the abundance of C-C bond from the long aliphatic chain grafted on USO-g-gauze.

Surface morphology of gauzes

Cotton gauze consists of interwoven cotton yarns (Fig. 2b-g). Pores including macropores (among yarns), capillaries (among fibers), meso- and micro-pores (inner fiber) are abundant in the gauze (Fig. 2b). After plasma treatment and radical initiated graft reaction with USO, the interwoven fiber network structure is well maintained, while the surface roughness of cotton fiber slightly increases (Fig. 2g). On the one hand, the improved surface roughness is due to the deepening effect of plasma etching.¹⁹ On the other hand, the USO thin layer grafted on the fiber surface may also enhance roughness.²⁰ In fact, the well maintenance of fiber/yarn/fabric morphology is also found for ABO-g-gauze and HTMS-g-gauze (Fig. S4).

Wettability of gauzes

The wettability of a gauze has a large effect on blood fluid absorption, protein adsorption and blood cell adhesion. For instance, it is reported that a material with a water contact angle (WCA) of 40-70° is suitable for adhesion of various cells.²¹ The wettability of gauze by simulated body fluid (SBF) and fresh rat blood was evaluated by applying 200 μL of these liquids onto a gauze. As shown in Fig. 3a, a water droplet

immediately spreads over and diffuses into gauze upon dripping onto gauze surface, due to cotton gauze has a robust intrinsic hydrophilicity and capillary structure. ABO-g-gauze exhibits similar wetting behavior, but the blood spreading area on ABO-g-gauze surface is smaller than that on cotton gauze within a same time period (Fig. 3b). HTMS-g-gauze shows a high hydrophobicity with a contact angle of 132.6° . Water or blood droplet neither spreads radially nor diffuses downwards, but stands on HTMS-g-gauze surface. This is due to this gauze is covered by 16-carbon hydrophobic alkyl chains, whose hydrophobicity prevents blood from diffusing into interior. The instant static WCA of USO-g-gauze is ca. 68.2° . Water or blood exhibits a unique and interesting wetting behavior on USO-g-gauze, namely the droplet gradually and vertically diffuses into gauze in 60 s, while the wetted area on surface is almost identical to the size of the droplet. This is totally different from the easy all-directional liquid pervasion on cotton gauze and ABO-g-gauze.

Fig. 3 Wettability of gauzes by (a) SBF and (b) blood. (c) Water vapor permeation rate, and (d) water absorption ratio of gauzes.

Water vapor permeation rate and water absorption/movement in gauze

Hemostatic gauze with proper water vapor transmission and liquid absorption can prevent dehydration and excessive accumulation of exudates. Therefore, they can control water/blood loss and create an ideal moist environment for wound healing.²² The water vapor permeation rate of cotton gauze, ABO-g-gauze, HTMS-g-gauze, and USO-g-gauze was 1028, 1023, 1021, and 1015 g·m⁻²·day⁻¹ at 37 °C, respectively (Fig. 3c), suggesting that water vapor permeability of the three surface modified gauzes is as good as that of standard cotton gauze, due to their breathable knitted fabric structure.

Cotton gauze and ABO-g-gauze are rapidly wetted by water and sink to the bottom when they are put in water. The hydrophobic HTMS-g-gauze always floats on water surface. However, USO-g-gauze shows a very different behavior, i.e., it initially floats on water surface, but sinks after 30 min (Suppl. Video 1). USO-g-gauze has a thin USO layer on its surface, weakening its hygroscopicity. This lowers the water absorption rate of USO-g-gauze, which is proven by its water absorption dynamic (Fig. 3d). Therefore, it takes a relatively long period for fully swollen to sink. The water absorption ratio of cotton gauze, ABO-g-gauze, HTMS-g-gauze, and USO-g-gauze is approximately 337.7%, 313.9%, 7.6%, and 260.5% at the saturated state (Fig. 3d), respectively. Compared to cotton gauze, the water absorption ratio of USO-g-gauze significantly lose by 77.2%, attributing to the great effect from the very thin hydrophobic USO layer on surface.

The movement of water in gauze was also quantitatively measured by moisture management test (MMT). The variation of wetting time of gauzes (Table 1) is in good correlation to their wettability as shown in Fig. 3a and b. The wetting time (top side) is

as short as 0.19 s for cotton and ABO-g-cotton gauzes, and substantially increases to 1.59 s for USO-g-cotton, and to 15.53 s for hydrophobic HTMS-g-gauze. As indicated in Table 1, the shorter wetting time is, the faster the water absorption rate and spreading speed is. The water absorption rate of gauzes follows a decreasing order of Cotton gauze > ABO-g-gauze > USO-g-gauze > HTMS-g-gauze, while the water spreading speed is in the order of Cotton gauze $\approx$ ABO-g-gauze $\gg$ USO-g-gauze $\gg$ HTMS-g-gauze. The changing patterns of these indices suggest that the surface chemical structure of gauze effectively guides moisture movement (wetting, spreading, and diffusion) in gauze, agreeing well with the results shown in Fig. 3. The designed concomitant hydrophobic/hydrophilic structure of USO-g-cotton imparts it with not only proper wetting time and spreading rate, but its ability of water diffusion from one side to the other side (indicated by the cumulative one-way transport capacity, Table 1). Such a unique property would be very helpful for controlling blood movement in gauze and at the gauze/tissue contact surface when it is practically applied as a topical hemostat, as will be shown in the following sections.

Table 1 Results of moisture management test of gauzes.

Sample	Wetting time (s)		Maximum wetted radius (mm)		Water absorption rate (%/s)	Spreading rate (mm/s)	Cumulative one-way transport capacity
	Top side	Bottom side	Top side	Bottom side	Top side	Top side	
Cotton gauze	0.19±0.00	0.19±0.00	20.00±5.00	20.00±5.00	28.52±2.86	20.19±1.40	220.53±54.12
ABO-g-gauze	0.19±0.00	0.19±0.00	10.00±0.00	10.00±0.00	11.46±5.20	18.03±0.10	370.38±21.40
HTMS-g-gauze	15.53±0.58	48.65±5.65	5.00±0.00	3.33±0.36	3.06±0.17	0.320±0.01	843.65±56.37
USO-g-gauze	1.59±0.47	3.00±0.00	10.00±0.00	5.00±0.00	9.33±3.50	3.42±0.64	672.32±68.44

Erythrocyte and platelet adhesion on gauze

Aggregated erythrocytes and platelets are essential components of blood clot for bleeding control.²³ SEM was used to explore the interaction of erythrocyte/platelet with gauzes. As shown in Fig. 4, erythrocytes adhere on gauze fiber surface to form aggregates. The unique double concave disk structure of erythrocyte is well maintained, indicating that gauzes don't affect the normal physiological state of erythrocytes. However, the amounts of erythrocyte adhering on gauze fiber surface depend on its wettability. Due to the hydrophobicity of HTMS-g-gauze, on it significantly fewer erythrocytes are observed than on the other three gauzes (Fig. 4c). Cotton gauze accumulates erythrocytes simply by blood fluid absorption, leading to adherence of erythrocytes to its surface (Fig. 4a). However, many erythrocytes aggregate on the fiber surface of ABO-g-gauze and USO-g-gauze (Fig. 4b, d). In addition, the formation of larger platelet aggregations can be observed on USO-g-gauze than on cotton gauze (Fig. 4e, f). It is clearly seen that some platelets with pseudopods are present on USO-g-gauze surface, indicating that platelets have been successfully activated upon contact with gauze fiber.²⁴ The above phenomena suggest that USO-g-gauze can catch the main components such as erythrocytes and platelets in plasma to accelerate formation of blood clots.

Fig. 4 Erythrocytes on (a) Cotton gauze; (b) ABO-g-gauze; (c) HTMS-g-gauze; (d) USO-g-gauze. Platelets on (e) cotton gauze fiber and (f) USO-g-gauze fiber.

Hemostatic performance evaluation

Bleeding control efficiency in rat femoral artery injury model

The hemostasis efficacy of gauzes on in vitro trauma was evaluated by using the rat femoral artery injury model. The hemostatic performance of the five gauzes is significantly different from each other (Fig. 5a, Suppl. Video 2). Due to high hydrophilicity, blood quickly permeates into the outmost layer of the stacked four-layer cotton gauze within 1 s. The blood pervasive area on the gauze swiftly expands with increasing time, and the gauze surface is almost fully blood-stained in 60 s. Blood is once again welling out from the wound when gauze is removed after bleeding stops in 5 min. On the unfolded gauze, the four gauze layers are completely wetted by blood

(Fig. 5-unfolded gauze). These phenomena imply that it absorbs a large volume of blood before bleeding is controlled, and the thrombus is easily destroyed when gauze is removed from the wound (Fig. 5a). The hemostatic performance of ABO-g-gauze is similar to that of cotton gauze, i.e., blood also quickly permeates and wets the whole four-layer ABO-g-gauze. However, the area wetted by blood on ABO-g-gauze is slightly smaller than that on cotton gauze in 60 s, and the red color of the bloody ABO-g-gauze is lighter than that of cotton gauze (Fig. 5a). On the unfolded ABO-g-gauze, the piles of the four gauze layers are fully permeated by blood, which is similar to the case of cotton gauze (Fig. 5-unfolded gauze). After ABO-g-gauze is removed post-hemostasis, re-bleeding occurs. However, the blood-stained area around wound is obviously less than that in the cotton gauze group (Fig. 5a), suggesting that the hemostatic performance of ABO-g-gauze is improved to some extent.

When the hydrophobic HTMS-g-gauze comes into contact with trauma, blood does not diffuse vertically into the top gauze layer, but it steadily seeps out of the seam of gauze/tissue surface. On the unfolded gauze, only a small area of the first gauze layer in contact with the wound is stained by blood in 60 s (Fig. 5-unfolded gauze). The gauze is taken away in 10 min when bleeding ceases, it is seen that blood stains the vicinity of the wound and even wet the gauze underneath the thigh, and severe re-bleeding occurs (Fig. 5a), indicating its hemostatic ability is far worse than cotton gauze. For comparison, when HTMS-g-gauze is compressed onto the bleeding wound, blood spills and still seeps out even after 2-minute-compressing (Suppl. Video 3). This is due to the hydrophobic HTMS-g-gauze has a poor moisture management ability which is not

1 helpful for hemostat. When applied to a bleeding trauma, the hydrophobic HTMS-g-
2 gauze inhibits blood wetting, absorption, wicking and diffusion into the upper gauze
3 layer (Fig. 3 and Table 1), resulting in poor hemostatic performance. As for QCG gauze,
4 an acclaimed gauze for effective controlling of severe bleeding, the outflowing blood
5 diffuses into the four-layer gauze in 1 s, but the blood-stained area on the outmost layer
6 gauze is smaller than that on cotton gauze and ABO-g-gauze. The unfolded gauze,
7 which is removed from the post-hemostatic wound, has blood-stains on all four gauze
8 layers (Fig. 5-unfolded gauze). And the wound re-bleeds rather than maintains
9 hemostatic state (Suppl. Video 2). Very surprisingly, when USO-g-gauze is in contact
10 with the bleeding wound, blood neither diffuses through to the top gauze layer nor seeps
11 out of the seam of gauze/wound surface no matter the gauze is applied with (Suppl.
12 Video 3) or without pressure (Suppl. Video 2). Compared with the other four types of
13 gauze, there is only one small blood-stain on the first two gauze layers of four-layers in
14 total (Fig. 5-unfolded gauze). In the post-hemostasis, it is amazingly found that the
15 wound and its vicinity is dry and clean without obvious blood stain, and no re-bleeding
16 occurs. These exceptional phenomena strongly indicate that the USO-g-gauze has
17 excellent hemostatic efficiency on the rat femoral artery injury. In an extended similar
18 work (Section 5 of SI), we found that chitosan nonwoven whose surface was slightly
19 grafted with USO also showed significantly enhanced hemostatic performance similar
20 to USO-g-gauze, namely controlled blood spreading and diffusion in gauze, no re-
21 bleeding upon gauze removal, and small blood loss, compared to mean performance of
22 pristine chitosan nonwoven gauze (Fig. S5, and Suppl. Video 4).

On the rat femoral artery injury model, HTMS-g-gauze has the longest hemostasis time of ca. 356 ± 15 s; The hemostasis time of ABO-g-gauze is ca. 174 ± 13 s, down from ca. 180 ± 20 s of cotton gauze; that of USO-g-gauze astonishingly falls to ca. 34 s from 147 s of QCG gauze (Fig. 5b). Compared with QCG gauze, the hemostatic time of USO-g-gauze drops by 77%. Accordingly, the blood loss follows a decreasing order of HTMS-g-gauze (1.04 ± 0.09 g) > cotton gauze (0.52 ± 0.03 g) $\approx$ ABO-g-gauze (0.49 ± 0.08 g) > QCG gauze (0.42 ± 0.03 g) > > USO-g-gauze (0.12 ± 0.03 g) (Fig. 5c). Thus, the blood loss of USO-g-gauze is impressively reduced by about 71%, as compared to QCG gauze.

Fig. 5 (a) From left to right: gauze was put on the bleeding rat femoral artery injury, gauze was removed from the wound after hemostatic state was reached; The stacked

four gauze layers were unfolded. “1” was the layer directly contacted with the wound, “4” was the outmost layer. (b) Hemostatic time and (c) total blood loss in the rat femoral artery model. (d) Enlarged photo of rat femoral artery injury after hemostasis. This photo was taken right after the gauze was removed.

Hemostasis in rat liver injury model

The gauze’s hemostatic performance in a non-compressible wound is evaluated by using the rat liver laceration model. Their behaviors of blood diffusion, flow at the gauze/tissue contact surface, and re-bleeding are similar to the hemostasis on the rat femoral artery injury model (Fig. 6a and Fig. 6-unfolded gauze, Suppl. Video 5). Obviously, the hemostatic efficacy of USO-g-gauze is significantly better than the other four gauzes. When USO-g-gauze is removed from the wound in post-hemostasis, no fresh blood wells out from the liver wound. The hemostatic time of cotton gauze, ABO-g-gauze, HTMS-g-gauze, QCG gauze, and USO-g-gauze is 172 ± 20 s, 153 ± 15 s, 344 ± 19 s, 96 ± 12 s, and 32 ± 4 s, respectively (Fig. 6b). In addition, the blood loss of cotton gauze, ABO-g-gauze, HTMS-g-gauze, QCG, and USO-g-gauze is 0.39 ± 0.08 g, 0.37 ± 0.03 g, 0.98 ± 0.09 g, 0.13 ± 0.07 g, and 0.03 ± 0.01 g, respectively (Fig. 6c). Compared with QCG gauze, the hemostatic time and blood loss of USO-g-gauze is reduced by 67% and 77%, respectively. Therefore, the high hemostatic ability of USO-g-gauze on the non-compressible liver injury is also confirmed.

In both rat femoral artery injury and liver laceration models, the survival rate within 120 min varies from one gauze to another. It is 100% for rats treated with USO-g-gauze, compared to 20% for QCG, and to no survival for cotton, ABO-g-gauze and HTMS-g-gauze (Figs. S6a and b). The survival rate is in accordance with the hemostatic

efficacy, i.e., blood loss and hemostasis time of the gauzes as demonstrated in Figs. 5 and 6.

Fig. 6 (a) From left to right: gauze was put on the bleeding rat liver injury, then it was removed from the wound after hemostatic state was reached; The stacked four gauze layers were unfolded. “1” was the layer directly contacted with the wound, “4” was the outmost layer. (b) Hemostatic time and (c) total blood loss of gauzes in the liver injury models.

Hemostatic performance in pig femoral artery injury model

In order to further evaluate the hemostatic performance of gauzes on massive bleeding wounds, the pig femoral artery injury model is used. The cotton gauze is wrapped around the wound for 3 min, then opened up to check the wound. As shown

in Fig. 7a and Suppl. Video 6, the outmost cotton gauze layer is immediately wetted by blood upon contact with the trauma. The blood diffusion area steadily increases as time goes by. Fresh blood continues flowing out from the wound when the cotton gauze is removed after 3 min (Fig. 7c). In contrast, USO-g-gauze shows far better hemostatic performance (Suppl. Video 7). Only a small blood-stain is observed on the outmost gauze layer in 60 s (Fig. 7b). Three min later, re-bleeding does not happen upon the gauze is removed from the wound (Fig. 7d). Because this is a severe bleeding wound, a relatively big area is blood-wetted in the first layer, but the 4th layer is stained by a small blood domain only. This suggests blood diffusion in the vertical and radial directions is largely restricted in USO-g-gauze. It should be pointed out that the accurate hemostatic time of every gauze is not measured on this injury model, because it is hard to judge when the wound stops bleeding due to the wound is wrapped by gauze and re-bleeding often occurs upon uncovering wound. Therefore, at the time interval of 3 min, the blood mass absorbed by gauze is measured to roughly reflect the blood loss. This is 0.80 ± 0.12 g for USO-g-gauze, while that of cotton gauze, ABO-g-gauze, QCG, and HTMS-g-gauze is 5.12 ± 0.34 g, 4.16 ± 0.40 g, 3.93 ± 0.30 g, and 8.20 ± 0.34 g, respectively (Fig. 7e). Therefore, the blood loss from the pig femoral artery wound treated with USO-g-gauze is only 15.6% and 20.4% of that with cotton gauze and QCG, respectively. In fact, the hemostasis time of USO-g-gauze on this wound model is less than 3 min, as suggested by the fact that no occurrence of re-bleeding upon uncovering the wound (Fig. 7d).

Hemostasis on the pig skin laceration model is also examined since its structure is very similar to human skin. A regular cut with a length of 2 cm and a depth of 1 cm was made with a scalpel, then a four-layer gauze was applied onto the wound. The dynamic hemostatic process of the four gauzes demonstrates that blood diffusion and absorption,

and blood flowing underneath gauze, are very similar to the hemostasis on the rat femoral artery injury and liver laceration models. Blood even rarely stains the hydrophobic HTMS-g-gauze, **but oozes out from the seam of gauze/skin surface** (Fig. S7). The blood loss of the cuts treated with cotton gauze, ABO-g-gauze, **QCG**, HTMS-g-gauze, and USO-g-gauze is 0.55 ± 0.04 g, 0.32 ± 0.03 g, **0.22 ± 0.02 g**, 0.71 ± 0.02 g, and 0.032 ± 0.01 g (Fig. 7f), respectively. Compared with cotton gauze and QCG, the blood loss for USO-g-gauze reduces by ca. 94% **and 85.5%, respectively**. The results of the pig femoral artery injury and skin laceration models further justify that USO-g-gauze has excellent hemostatic efficacy for severe bleeding wounds.

Fig. 7 Hemostasis in the pig femoral artery injury models. Hemostasis process of (a) cotton gauze, and (b) USO-g-gauze. The status of wound after (c) cotton gauze and (d) USO-g-gauze were removed **after it was treated for 3 min**. Blood loss of gauzes in the pig (e) femoral artery and (f) skin injury models.

Hemostatic mechanism of the highly efficient USO-g-gauze

In each injury model, the bleeding wounds treated with ABO-g-gauze is cleaner, and the blood diffusion area on gauze and blood loss are smaller than those treated with standard cotton gauze. ABO-g-gauze not only concentrates blood components due to its quick blood absorption ability (Fig. 3d), but also catches blood cells by its tissue adhesive catechol groups (Fig. 4b), so the hemostatic efficiency of ABO-g-gauze is slightly improved. The hemostatic performance of HTMS-g-gauze is significantly inferior to that of cotton gauze, because of strong repulsion of blood fluid by the highly hydrophobic HTMS alkyl chain (Figs. 3 and 4). However, the USO-g-gauze containing a hydrophobic long alkyl chain with a tissue adhesive catechol end group exhibits impressively excellent hemostatic efficacy.

Why has USO-g-gauze the most exceptional hemostatic capability among those gauzes? To better understand the nature behind this feature of this new hemostatic cotton gauze, we initially carried out the detailed density functional theory (DFT) calculations to investigate the adsorption interaction of sixteen different kinds of amino acid molecules (they are essential components of tissue keratin protein²⁵) with USO-g-gauze, as illustrated in Fig. S8. More details have been provided in Section 8 of SI. The adsorption energies (ΔE_{ads}) of these amino acids to USO-g-gauze are calculated by considering the main non-covalent interaction modes including π - π stacking and hydrogen bond interactions.

Our computed results reveal that the amino acids containing π -conjugated benzene ring, such as phenylalanine (F) and tyrosine (Y), can be effectively adsorbed on the catechol of USO-g-gauze through synergistic actions of π - π stacking and hydrogen bonding (Figs. 8C and S8), where the computed ΔE_{ads} values are as large as 0.621 and 0.729 eV, respectively. Comparatively, all the remaining fourteen amino acids without

1 π -conjugated ring can effectively interact with the catechol of USO-g-gauze by adopting
2 double hydrogen bonds, as presented in Fig. 8C and S8. All these hydrogen bonding
3 distances are in the range of 1.69 ~ 1.80 Å (Table S2), and the calculated ΔE_{ads} values
4 are as big as 0.570~0.639 eV (Fig. S8), indicating strong interaction force between them.

5 Furthermore, we also examine the effect of relative position between two OH
6 groups on the benzene ring on ΔE_{ads} of a model amino acid glycine (G) on USO-g-gauze
7 (Fig. S9). The computed results reveal that when the relative position between the two
8 OH groups is changed from the original ortho- to meta- to para- arrangements, ΔE_{ads}
9 values for G is reduced from 0.617 to 0.502/0.419 and then to 0.397/0.384 eV. Further,
10 when the two OH groups are even separated by three H atoms, small ΔE_{ads} (*ca.*
11 0.383/0.355eV) is attained. Clearly, with increasing the spacing distance between the
12 two OH groups, ΔE_{ads} for the model amino acid decreases significantly, in view of the
13 fact that double hydrogen bonds cannot be effectively formed or only a single hydrogen
14 bond can be formed (Fig. S9). Therefore, the relative position between two OH groups
15 on the benzene ring has an important influence on ΔE_{ads} , where the ortho position can
16 bring the largest ΔE_{ads} due to formation of two hydrogen bonds. Further, when removing
17 either of the two OH groups in catechol of USO-g-gauze (Fig. S10), the computed ΔE_{ads}
18 values (0.248 and 0.355 eV) is about half of that of the corresponding structure with two
19 hydrogen bonds, which means that both hydrogen bonds can be effectively formed
20 simultaneously between the relevant amino acids and catechol in USO-g-gauze.
21 Obviously, all of these can reflect the superior structural match to form double H-bonds
22 between the amino acids and the catechol of USO.

23 Overall, USO-g-gauze with a long alkyl chain terminated with a catechol group
24 can effectively interact with all these amino acids via double hydrogen bonds or the
25 synergistic action of π - π stacking and hydrogen bonding. These non-covalent

interactions contribute to USO-g-gauze's strong tissue adhesiveness, where the catechol at the end of alkyl chain can play a crucial role.

Apart from the above molecular level analyses of adhesion interaction, the adhesion force (or peeling force) of those gauzes on fresh wet rat femoral tissue is measured and shows obvious variation from one to another. As expected, the hydrophobic HTMS-g-gauze has the lowest peeling force of 24 mN, while USO-g-gauze shows the largest force of 90 mN, which is ca. two times as much as that of cotton gauze (Fig. S11). From Suppl. Video 3, the adhesion of ABO-g-gauze and USO-g-chitosan to wound tissue is obviously perceivable when they were peeled off from the wounds, while it is less noticeable in the cases of cotton gauze etc (Suppl. Video 2). This vividly confirms the existence of adhesive interaction between catechol and tissue, but such non-covalent adhesion can be broken with mild peeling forces. In the case of the double -OH groups of catechol were modified such as with chelation with Fe^{3+} or oxidation into quinone, the tissue adhesion force sharply decreases to values close to that of HTMS-g-gauze (Fig. S11). This further confirms that catechol group plays an essential role in the wet tissue adhesion.²⁶⁻²⁸

In order to demonstrate the importance of tissue adhesion of catechol to high hemostatic efficiency, the catechol group on USO-g-gauze is transformed to lower its tissue adhesiveness. Hence, USOF_e-g-gauze and USOQ_u-g-gauze were fabricated. On USOF_e-g-gauze, catechol groups readily react with Fe^{3+} to form a complex, while on USOQ_u-g-gauze the catechol groups are oxidized into quinone (Fig. S12). Both catechol- Fe^{3+} and quinone groups have no or weak adhesion to wet tissue.²⁹

When USOF_e-g-gauze is applied onto the rat femoral artery injury, blood spills (1s

in Fig. 15a, pointed by yellow arrow) and seeps out of the seam of gauze/wound surface (15s in Fig. 15a, pointed by yellow arrow), it also diffuses throughout the whole four gauze layers in 15 s, but thereafter the blood-stained area on the top layer does not expand much, instead blood continuously oozes out from the gauze/tissue contact surface (Fig. S13a). The gauze is removed 5 min later when the wound fully stops bleeding, re-bleeding is observed and much red fresh blood is around the wound (Fig. S13a). Compared to USO-g-gauze, the hemostasis time and blood loss on this wound treated by USOF_e-g-gauze significantly increases to 289 ± 5 s, and 1.32 ± 0.11 g, respectively (Fig. S13b, c). The instant static WCA of USOF_e-g-gauze is 119° (higher than 68° of USO-g-gauze), but slowly reduces to 0° within 60 s with diffusion of water droplet (Fig. S12b), similar to that occurred for USO-g-gauze. Therefore, the weak adhesiveness of catechol-Fe³⁺ groups to wet skin tissue, would be responsible for the substantially longer hemostatic time and more blood loss than USO-g-gauze and cotton gauze. Similar phenomena (blood diffusion, seepage, and re-bleeding) are found for the USOQu-g-gauze on the rat femoral artery injury (Fig. S13a). This is also due to the less tissue adhesiveness of quinone groups existing on the USOQu-g-gauze surface. The hemostasis time and blood loss by USOQu-g-gauze are 193 ± 5 s and 0.87 ± 0.21 g, respectively (Fig. S13b, c).

The hemostatic performance of USOF_e-g-gauze and USOQu-g-gauze on the rat liver laceration model is similar to that on the rat femoral artery injury model (Fig. S14a). Blood flows down the liver and wets the gauze (underneath the rat liver), which does not occur when USO-g-gauze is applied. On this injury, the hemostatic time and

1 blood loss by USOF_e-g-gauze are 101 ± 4 s and 0.48 ± 0.13 g, respectively; and by
2 USOQ_u-g-gauze are 65 ± 4 s and 0.28 ± 0.10 g, respectively (Fig. S14b, c). Thus, the
3 catechol group does play a crucial role in controlling traumatic bleeding. The far
4 superior hemostatic potential of USO-g-gauze to ABO-g-gauze (ABO has a three
5 carbons alkyl chain) strongly suggests that a long hydrophobic alkyl chain is also of
6 great importance. Therefore, USO, a catechol compound with a side alkyl chain
7 having 15 carbons is a good candidate compound for surface modification of fabric
8 gauze (such as cotton gauze and chitosan nonwoven) to prepare novel highly efficient
9 hemostatic gauzes.

10 Certainly, other properties of USO-g-gauze such as its moisture management
11 ability (water absorption, wettability, diffusion, and one-way transportation) are also
12 essential to the high hemostatic efficiency. The blood fluid movement in gauze and
13 around the gauze/tissue surface governed by the unique wetting property and tissue/cell
14 adhesiveness facilitates aggregation of astonishingly massive erythrocytes, as shown in
15 Fig. 8B. There are so many congested erythrocytes that they even fill the quadrilateral
16 macro-pores among warp and weft yarns of the first two gauze layers (the layer directly
17 contacts with tissue is the first layer), but none in the 3rd layer, which are consistent
18 with the observation shown in Figs. 5 and 6-unfloded gauze. The thickness of the
19 erythrocyte layer accumulated on the 1st layer reaches as high as 220 μm . As well-
20 known, erythrocytes are the key component of the primary blood clot. Thus, more
21 erythrocytes are aggregated, bigger clot is formed, shorter bleeding time and less blood
22 loss are attained. Therefore, the thick erythrocyte layers in the first two USO-g-gauze

1 layers serve as clots for effectively controlling bleeding. However, the erythrocyte
2 accumulation ability of cotton gauze is poor as suggested by its sparse distribution on
3 cotton gauze yarns with none in the quadrilateral macro-pores of the whole four gauze
4 layers (Fig. 8A). This is because erythrocytes move along with the fast blood wicking
5 to everywhere in the cotton gauze patch, rather than group together to form a big
6 erythrocyte plug. But such a movement is retarded in USO-g-gauze by the anchoring
7 hydrophobic chain barriers at the interface of USO-g-gauze/tissue and among fibers and
8 yarns.

9 Therefore, the hemostatic mechanism of USO-g-gauze is proposed in Fig. 8C and
10 d. When USO-g-gauze is applied onto a bleeding wound, catechol groups of USO
11 quickly anchor to skin tissue through non-covalent bonds such as hydrogen bond and
12 π - π stacking, to form dam-like barriers around wound (Fig. 8C). This can hinder and
13 eventually prohibit blood from seepage at the gauze/tissue contact surface. The
14 repelling pressure from the massive hydrophobic interaction among the long alky
15 chains retards blood diffusion into the upper gauze layers, so only the first and second
16 layers are blood-wetted as shown in the rat and liver injury models (Figs. 5 and 6-
17 unfolded gauze). Even in the pig injury models, blood has a difficulty in diffusing
18 radially, and only little volume of blood reaches the outmost layer to result in a small
19 blood stain (Fig. 7b). This should contribute to the massive dam-like barriers formed
20 among yarns and fibers due to the interaction between USO catechol and cotton
21 cellulose, hence big blood stream moves in the pores between warp and weft yarns,
22 with small blood streams in other pathways (Fig. 8D). Finally, the blood wicking

1 capability of the moderately hydrophilic USO-g-gauze fibers facilitate platelets
2 adhesion and activation, and erythrocyte accumulation (Fig. 8D), therefore promoting
3 the formation of blood clot. Thus, the synergistic effects of tissue adhesion,
4 hydrophobic interaction, and hydrophilic fiber structure make USO-g-gauze an
5 excellent hemostatic gauze. The other impressive feature of USO-g-gauze is that no re-
6 bleeding occurs upon removing it from wound after hemostasis, while re-bleeding is
7 often experienced when cotton gauze is used. In the case of cotton gauze, with sparse
8 aggregation of erythrocytes on yarns, it is an essential part of the blood plug (Fig. 8A).
9 The plug would be easily broken since erythrocytes are removed along with peeling-
10 off cotton gauze, leading to secondary bleeding. From Fig. 8B, it's seen that the
11 erythrocytes accumulated at the injury site is so enormous and thick that removal of
12 USO-g-gauze would take away part of erythrocytes, but some erythrocytes (Fig. 5d)
13 remain on site to avoid re-bleeding. The mechanism for no re-bleeding of USO-g-gauze
14 is different from that of Bandage[®], which is a well-known no re-bleeding hemostatic
15 fabric strip for small bleeding wounds. Bandage's anti-adhesion to tissue relies on the
16 hydrophobic membrane covering on the water-absorbent fabric layer (Fig. S15). The
17 poor adhesion of hydrophobic membrane to fibrin prevents bandage from being a part
18 of blood clot. The no re-bleeding is actually very important when wounded person are
19 relocated or injured tissue/organs are moved accidentally, so new blood loss can be
20 substantially avoided. Since the hemostasis of USO-g-gauze is a physical blocking
21 effect rather than change of the body's normal physiologic clotting mechanisms, it
22 would also show hemostatic efficacy for patients with coagulopathy.

Fig. 8 Aggregation of erythrocytes on cotton gauze and USO-g-gauze after hemostasis in the rat femoral artery injury model; and hemostatic mechanism diagram of USO-g-gauze. (a) On the cotton gauze patch composed of four stacked gauze layers, erythrocytes sparsely distribute on cotton yarns of all layers. The macro-pores among yarns are vacant. (b) On the USO-g-gauze patch composed of four stacked gauze layers, erythrocytes fill the macro-pores among yarns in the first two layers, but are absent in

the pores and on the yarns of the 3rd layer. Cross-section of the 1st layer shows a thick erythrocyte layer. (c) Through adhesive bonds like π - π stacking interaction and hydrogen-bond between USO's catechol group and wound tissue's amino acid units, dam-like barriers form surrounding the wound. They retard blood seeping out of the tissue surface. The repelling pressure from the hydrophobic effect among long alkyl chains slows down blood wicking movement. (d) Dam-like barriers forming by USO between gauze layers and between fibers, retard blood diffusion in the vertical and horizontal directions, largely confine blood movement in the pores between warp and weft yarns, resulting in large accumulation of erythrocytes.

Biocompatibility of USO-g-gauze

The growth and proliferation of L929 fibroblasts on USO-g-gauze are shown in Fig. 9. On the gauze yarn, many green-dyed living fibroblasts with several red-dyed dead cells are observed. Obviously, the fibroblasts proliferate very well with incubation time increasing from 1 to 3 d. The good cell biocompatibility of USO-g-gauze is comparable to that of cotton gauze and ABO-g-gauze (Fig. S16a and b). The fibroblasts can also dwell and grow on HTMS-g-gauze, but the proliferation rate on this hydrophobic gauze is significantly smaller than that on the other three hydrophilic gauzes (Fig. S16c). Therefore, USO-g-gauze can favor cell adhesion and proliferation, due to good balance of hydrophilicity/hydrophobicity and presence of catechol groups on this gauze.

Fig. 9 Optical microscopy images of L929 fibroblast cells incubation with USO-g-gauze for 1, 2, and 3 d.

Fig. 10 shows histological changes of the subcutaneous muscle tissue treated with cotton gauze and USO-g-gauze at specific time points (3, 7, 14, 21 days) using H&E staining and toluidine blue staining. In the cotton gauze-treated tissue, plenty of neutrophils arose around the gauze on the third day after implantation (Fig.10a) and the corresponding number were counted to be about 50 ± 5 (Fig. 10c). However, very few neutrophils were observed 7 days later, which reduced to about 2 ± 1 after 21 days. Meanwhile, USO-g-gauze had a similar inflammatory response. The density of neutrophils increased in the USO-g-gauze treated tissues since implantation, but rapidly decreased to 4 ± 1 after 7 days. In addition to neutrophils, mast cell was another critical effector of inflammation. As shown in the toluidine blue staining (Fig. 10b and d), several mast cells were observed in the tissue section contacting with the cotton gauze and the USO-g-gauze on the seventh days after implantation, but sharply reduced 14 days later within the cotton and the USO-g-gauze treated tissue. The subcutaneous implantation examinations reveal that the surface

modification with USO did not compromise the biocompatibility of cotton gauze, and caused no significant inflammatory responses.

Fig.10 (a) H&E staining and (b) toluidine blue staining sections of subcutaneous tissue surrounding the gauzes at varied time intervals in the muscle implantation experiments using rats. The corresponding numbers of (c) neutrophil and (d) mast cells at different time intervals.

Conclusions

A novel highly efficient hemostatic cotton gauze was developed through simply grafting a catechol compound USO (with a long hydrophobic alkenyl side chain) onto cotton gauze surface. The special pendent groups offer wet tissue adhesiveness and hydrophobicity to the gauze. On basis of findings of the density functional theory calculation and measurements, a new physical hemostatic mechanism is proposed for this gauze, namely many dam-like barriers are formed at the interface of gauze/wound

tissue surface and among adjacent fibers. These barriers are capable of retarding blood flowing and controlling the movement of bloodstream at the seam of gauze/wound contact surface and in the gauze, resulting in quick aggregation of erythrocyte to form a thick blood clot stopping bleeding. Thus, this gauze can overcome the intrinsic high blood absorption capacity of conventional cotton gauze, making it to significantly reduce additional blood loss from bleeding traumas, and therefore increasing survival rate and decreasing medical such as transfusional costs. Excellent hemostatic efficacy (including short hemostatic time, low blood loss, and no re-bleeding) of this gauze was observed on the rat femoral artery and liver laceration models, pig skin laceration and femoral artery massive bleeding wound. Its hemostatic performance is much superior to standard cotton gauze and QCG. The USO-g-gauze displays similar cell and tissue compatibility to cotton gauze. Such an idea and methodology were also successfully applied to make USO-g-chitosan gauze, whose hemostatic efficacy is much better than the chitosan control. We speculate this magic gauze may find very promising emergency-care and clinical applications for controlling traumatic massive bleeding for wounded soldiers in battlefields, civilians in accidents, patients in operation rooms, and patients with coagulopathy.

Methods

Materials

Sterile cotton gauze and chitosan nonwoven fabric were purchased from the commercial market in China. Sodium alginate (SA), acetic acid and anhydrous ethanol were bought from Sinopharm Group Chemical Reagent Co., Ltd. QuikClot Combat Gauze (QCG) was provided by Z-Medica and used as received. The catechol compound 1,2-benzenediol-3-(9,11,13-pentadecatrienyl) (USO) was extracted from the raw

lacquer provided by Xi'an Raw Paint Research Institute by a reported procedure.³⁰ Briefly, 1 kg of Chinese lacquer sap was dissolved in 2 L of ethanol under mechanically stirring for 24 h at room temperature, followed by filtration to collect filtrate. From it USO was obtained after ethanol was removed by vacuum-rotary evaporation at 60 °C. Then the C15 triene urushiol was purified by the gel permeation chromatography (GPC).³¹ Eugenol, triethylsilane (TES), tris(pentafluorophenyl)borane (TPFPB), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) and hexadecyltrimethoxysilane (HTMS) were purchased from Aladdin Reagent Co., Ltd. All chemicals were used as received without further purification. Sprague-Dawley (SD) rats with weight of ca. 200-250 g were bought from Shanghai Slack Experimental Animal Co., Ltd, China.

Preparation of USO grafted cotton gauze (USO-g-gauze)

The preparation scheme and chemical reaction of USO-g-gauze are shown in **Fig. 1**. Cotton gauze was sequentially washed with distilled water and ethanol, two times in each solvent and 30 min each. Then it was dried under nitrogen stream. To introduce free radicals onto gauze, it was treated by a low temperature plasma in N₂ at 400 Pa, 80 W for 3 min (PT-5S, Sanhe Poda Co., Ltd, China). The preparation conditions for USO-g-gauze were optimized as described in (Table S1, Section 1 of SI). In an ideal condition, USO-g-gauze was fabricated by placing the plasma-treated gauze in a mixture of 2.0 wt% USO/ethanol and refluxing at 70 °C for 2 h. Then, after washed 3 times with ethanol, it was dried in a vacuum oven at 80 °C for 2 h to obtain USO-g-gauze. About 0.1 wt% USO was grafted, as determined by the gravimetric method.

4-allyl-1,2-benzenediol (ABO) containing a catechol group was prepared according to the scheme shown in **Scheme S1**.³² Typically, eugenol (8.6 mL, 56 mmol)

and TES (19 mL, 112 mmol) were added to a 100 mL round bottom flask with a condenser and stirred at 24 °C for 5 min, then TPFPB (57 mg, 0.112 mol) was added into the mixture and reacted for another 3 h. Subsequently, the mixture was passed through a neutral alumina column with dichloromethane as the eluent to eliminate TPFPB. Then the dichloromethane and unreacted TES in the collected mixture were removed by a vacuum rotary evaporator at 60 °C. As-obtained eugenol protected by TES was present in a yellowish liquid. Finally, 5 mL of 0.1 M hydrochloric acid aq. solution was added and stirred at 80 °C for 6 h to de-protection for obtaining ABO. The chemical structure of TES protected eugenol and ABO were confirmed by ¹H NMR analysis (**Section 1 of SI**). The ABO grafted gauze (ABO-g-gauze) was made by the same way as that for USO-g-gauze, except that ABO was used to replace USO in the reaction mixture (**Fig. 1b**). HTMS with a long alkyl chain in its chemical structure was grafted onto the cotton gauze to make HTMS grafted gauze (HTMS-g-gauze) in a similar way for preparing USO-g-gauze (**Fig. 1c**).

Characterization.

The morphology of gauzes was observed by scanning electron microscopy (JEOL-7500LV, Japan). The solid-state ¹³C NMR spectra were characterized by a superconducting fourier transform nuclear magnetic resonance spectrometer (Bruker Avance III 400 WB, AVANCE III, Switzerland). X-ray photoelectron spectra was measured by scanning XPS microprobe instrument (Thermo K-Alpha+, UK). Fourier transform infrared (FTIR) spectra in KBr form were obtained on an FTIR spectrometer (PerkinElmer, 1600) in the range of 4000 - 400 cm⁻¹. The wettability was determined by DSA 100 (Krüss, Germany).

Moisture management test

The tests (wetting time, wetted radius, water absorption rate, water spreading speed, and cumulative one-way transport capacity) were performed on a moisture management tester (M290, SDL ATLAS, USA) according to AATCC 195 by measuring the electrical resistance of the top and bottom sides of the gauze.³³ The gauze size was 5 cm x 5 cm. 0.2 mL of standard test solution was dropped onto the gauze in the test. 5 replicates were run for each gauze.

Water vapor transmission rate and water absorption capacity of gauzes.

To measure water vapor permeability, a beaker containing 50 mL of distilled water was covered with a gauze. The circumferential border was tightly sealed to prevent any water vapor loss through the boundary. The water vapor transmission rate (W_{evap}) was determined by measuring the mass loss of water in the beaker after 24 h at 37 °C. The W_{evap} ($\text{g}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$) was calculated according to Equation (1):

$$W_{\text{evap}} = (m_b - m_a) / A \quad (1)$$

where A , m_b , and m_a are the area of the beaker mouth (m^2), the weight of the beaker before and after water evaporation, respectively.

For measurement of water absorption ratio, a square gauze with size of 2 cm × 2 cm was immersed in simulated body fluid (SBF), then at certain time interval it was taken out and placed on a filter paper to absorb free water, followed by weighing its mass. The gauze mass before (m_b) and after water absorption (m_a) was measured, and water absorption ratio (W_{abs}) was calculated by Equation (2):

$$W_{\text{abs}} = (m_a - m_b) / m_b \times 100\% \quad (2)$$

Hemostasis evaluation

All animal experiments were carried out in accordance with the guidelines for the protection and use of experimental animals in Fujian Normal University. The experiments were approved by the Animal Ethics Committee of Fujian Normal University. The gauze was tailored into rectangle swatch with a size of 12 cm × 2.5 cm, and four swatches were stacked together before used on a bleeding wound. The hemostatic study included four animal injury models: rat femoral artery and liver injury, pig femoral artery and skin laceration. Five rats or pigs were randomly selected as a group in each animal model and assigned to each sample. QCG is the standard military hemostatic agent recommended by the Tactical Combat Injury Care Committee for use as a control. Anesthesia was injected intraperitoneally with a 10 wt% chloral hydrate solution (0.4 mL/100 g). After complete hemostasis was reached, all animals were observed for 2 h or until death. The survived animals were euthanized with 10% chloral hydrate at the end of experiment.

Rat femoral artery injury model

The proceeding of femoral artery injury model was conducted as follows.³⁴ Rats were randomly selected and anesthetized. Then the fur on the rat thigh was shaved off to expose the femoral artery. Pre-weighed cotton gauze was placed beneath the thigh. Then the artery was transected. After bleeding for 2 s, pre-weighed gauze was gently applied or compressed onto the trauma (compressing for 150 s). Blood diffusion in the gauze was recorded. Bleeding time and blood loss were measured (n=6). We defined that hemostatic stage is reached when the blood-stained area on gauze doesn't expand and no blood seeps out of the seam at gauze/wound contact surface.

For observing the micro-morphology of the blood-stained gauze after hemostasis, a patch stacked with four gauze layers was dressed onto the rat femoral artery injury to reach

hemostasis. The gauze was placed in centrifuge tubes and fixed with 2.5% (v/v%) GA/PBS solution at room temperature for 4 h. Then it was rinsed twice with distilled water, followed by sequential dehydration in 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% (v/v%) ethanol/PBS solution. consequently, it was dried at 37 °C. The gauze was sputter-coated with gold before SEM observation.

Rat liver laceration model

Liver laceration model was conducted according to a standard procedure.³⁵ Rats were randomly selected and anesthetized. Subsequently, the epithelial tissue of the abdomen was cut to expose the liver, and pre-weighted cotton gauze were placed under the liver. A scalpel was used to make a linear incision trauma of about 1 cm in length on the left lobe of the liver. After bleeding for 2 s, pre-weighted gauze was applied onto the trauma. Blood permeation in the gauze, bleeding time, and blood loss were recorded (n = 6).

Pig skin laceration and femoral artery injury models

Two-month-old Bama miniature pigs (weight 1.6 - 1.8 kg) were used to simulate massive bleeding trauma.³⁶ Anesthesia was injected intraperitoneally with a 10 wt% chloral hydrate solution (0.4 mL/100 g). After the hairs on the back and leg were shaved off, the following two different traumas were created: (1) A linear incision of 2 cm (length) x 1 cm (depth) was made on the back of the pig with a scalpel; (2) A distally extending 4 cm longitudinal incision was made in the right femur region to expose the femoral artery.³⁷

Peeling force of gauze on wet rat femoral tissue

Rats were pre-treated as in the femoral artery injury model, then the peeling test was conducted before the artery was transected. Gauze (5 x 2.5 cm²) was immediately put on the exposed wet femoral tissue and kept there for 10 min. The gauze was then peeled off with a Digital Push Pull Gauge (Locosc Ningbo Precision Technology Co., Ltd., China), and the peak force was recorded (n = 5).

Effect of catechol functional groups on hemostasis

In order to elucidate the effect of catechol structure on hemostatic efficiency of USO-g-gauze, two models were designed: (1) The catechol groups were protected by chelating with Fe³⁺ ions.³⁸ Typically, USO-g-gauze was soaked in 100 mL of 0.1 M FeCl₃ aq. solution for 10 minutes at 37 °C to allow the occurrence of coordination reaction between catechol groups and Fe³⁺. After washed with distilled water, it was dried in a vacuum oven at 80 °C for 0.5 h. As-obtained gauze was coded as USOFe-g-gauze. (2) The catechol groups were oxidized to quinone. The USO-g-gauze was treated in 100 mL of 20 mM Tris-HCl buffer solution (pH 9.8) for 10 minutes at 37 °C to convert the phenolic structure to quinoine structure.³⁹ After washed with distilled water, it was dried in a vacuum oven at 80 °C for 0.5 h. As-obtained gauzed was coded as USOQu-g-gauze.

Accumulation of erythrocytes and platelets on gauzes

The platelet-rich plasma (PRP) was obtained by centrifuging rat whole blood at 3000 rpm for 20 min at 4 °C.⁴⁰ In a volume ratio of 1:10, the whole blood or PRP was

added into a phosphate buffer solution (PBS, pH 7.4) containing a piece of gauze (1 cm × 1 cm), followed by incubating for 90 min at 37 °C. Subsequently, the gauze was rinsed three times with PBS to remove physically adhered blood cells and platelets, and fixed with 2.5% (v/v%) GA/PBS for 2 h. Then it was rinsed twice with distilled water, followed by sequentially dehydrating with 25%, 50%, 75%, 85%, 90%, and 100% (v/v%) ethanol/PBS solution. Finally, it was air-dried at 37 °C. The adhesion of erythrocyte and platelet on gauze surface was observed by SEM.

Biocompatibility of gauze

The proliferation of L929 cells on gauze was measured by using a live/dead assay kit (Beyotime Biotechnology, Shanghai, China).⁴¹ Briefly, after treated with 75% medical alcohol and 0.1 mg/mL physiological saline, the gauze was transferred into a 24-well plastic culture plate. 1 mL of fibroblast with a density of 2.5×10^5 cells/mL was gently added on the surface of the gauze surface and cultured at 37 °C in a humidified atmosphere of 5% CO₂ for 1, 2, and 3 d. Then, 10 µL of the combined Live/Dead cell-staining solution (2 µM Calcein-AM and 4 µM PI) was added into 400 µL of culture medium and was incubated at 37 °C for 4 h. Finally, images of the live (green fluorescence) and dead (red fluorescence) cells were obtained using an inverted fluorescence microscope.

In vivo inflammatory assay

Sprague Dawley rats maintained under a 12 h light/12 h dark schedule with a continuous supply of food and water. After a week of adaptation, the rats were randomly

divided into 2 groups: cotton gauze group and USO-g-gauze. Each group contained 20 rats. The gauze was cut into a square (1 cm × 1 cm) and were sterilized by ultraviolet irradiation for 2 h. The rats were anesthetized by inhalant anesthetics-ether and fixed on the surgical plate. After the shave and the iodine disinfection, a longitudinal incision about 2 cm were made symmetrically on both sides of the spine. Then, the cotton gauze or USO-g-gauze were respectively implanted into the subcutaneous sac of the back. The incision was sutured by the simple intermittent suture method. Finally, the cut was sprayed with penicillin powder and then covered with aseptic dressing paste to prevent infection. The animals were returned to cages alone after surgery. At specific times (3, 7, 14, 21 days), the rats were sacrificed and the wounds along with surrounding tissues were collected. The collected tissues were fixed by 4% paraformaldehyde. After formaldehyde fixation for 1 week, the calcium was decalcified with 10% EDTA reagent for 2-4 weeks. Subsequently, H&E staining and toluidine blue staining were performed, and staining was performed using a fluorescence inverted microscope for microscopic examination and image acquisition analysis.

Statistical analysis

All data are shown as the mean ± standard deviation (SD) with five replicates for each test. One-way analysis of variance (ANOVA) was applied for statistical analysis. * $p < 0.05$ and ** $p < 0.01$ were considered significant and greatly significant difference, respectively.

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Author contributions

H. Liu and Y. Fang conceived and directed the project. H. He, W. Zhou, A. Liu, W. Zhang, and Y. Weng designed and performed the synthesis, characterization and hemostatic measurements of gauzes. F. Wang, S. Wang and Z. Wang carried out the tests of biocompatibility. J. Gao, Y. Gao and W. Chen run the density functional theory calculation and analysis. H. Liu., and W. Chen wrote the manuscript.

Competing interests

The authors declare no competing interests.

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