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Research Paper

# Compressed hybrid cotton aerogels for stopping liquid leakage

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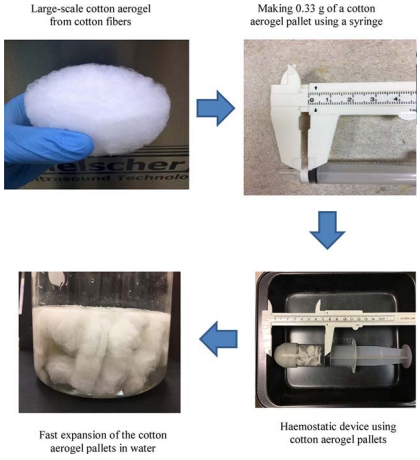
## GRAPHICAL ABSTRACT

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## ABSTRACT

Haemostatic devices can exert internal pressure to promote blood clotting and reduce blood flow, potentially reducing mortality rates of gunshot wounds or other deeply penetrating wounds. For the first time, cotton aerogels have been successfully developed from commercial cotton fibers. The developed cotton aerogels can be compressed to form aerogel pallets which can be used for haemostatic devices. The effects of various cotton fiber concentrations, their morphology and chitosan concentrations on volume expansion ratio, expansion time and hydrostatic pressure of the hybrid cotton aerogels are investigated comprehensively. The chitosan-coated cellulose-cotton aerogel pallets having 0.7 wt% of the fibers, with cellulose-cotton ratio of 1:2 and 0.5 wt% of the coated chitosan showed excellent haemostatic performance. The volume expansion ratio of 16.0, expansion time of 4.5 s and hydrostatic pressure of 11.5 mmHg of each aerogel pallet are much better than those of commercial haemostatic sponges. The developed cotton-based aerogels have several potential applications such as haemostatic devices, stopping serious liquid leakage, oil-spill cleaning, personal care and heat and sound insulation applications.

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## 1. Introduction

Haemorrhage control devices are widely used to stop bleeding in the military and civilian first responder sectors [1–6]. Haemorrhage injuries include two main types: (i) Compressible bleeding wounds such as external capillary, venous, arterial bleeding, (ii) Non-compressible wounds such as sub-dermal or internal venous and arterial bleeding [1,2]. For example, a rupture in the subclavian artery in a shoulder gunshot wound can cause penetrating trauma internal bleeding. From U.S. military data, around 67% of haemorrhagic injuries are non-compressible, and when uncontrolled, they account for 80% of combat fatalities within the first hour. Similar problems of uncontrolled haemorrhage fatalities are seen in first responder sectors [1,2].

The most commonly used method to stop bleeding is through covering the wound with a bandage, then by applying direct pressure, facilitating the formation of a blood clot. Unfortunately, these wound dressings are often too stiff and rigid to fit into the small and narrow cavity of the casualty. Also, they do not conform to the irregular tissue morphologies of the wound. These flaws have to be overcome in order to achieve quick and effective haemostasis [1,2]. Another method to facilitate the clotting of wound would be using granular and powder-based haemostatic products, as they are able to conform to irregular tissue morphologies of the wound cavity. However, the significant drawback of these products is that they can only be deployed in calm conditions [1,2].

Several works are underway to develop products that have the potentials to make surgery and interventional care faster and safer [3,4]. One approach is to develop products based on self-assembling peptide technology [1]. When applied as a liquid or spray to a wound, a hemostatic device would locally self-assemble into a nanofiber structure as a physical barrier on the tissue, mechanically sealing the wound in order to stop substances, such as blood from leaking. The hemostatic device demonstrated quick average time to hemostasis when applied to bleeding wounds in a variety of animal tissues. The technology can result in time to hemostasis of 15–30 s on animal tests. When various control substances were applied, hemostasis time is often from 80 s to 300 s.

Correct hemostasis in liver surgery is hard to achieve because of the oozing bleeding. Takacs *et al.*(2011) investigated collagen- and cellulose-based hemostatics. A standardized resection was treated by applying different hemostatics in a randomized order, and bleeding times were measured. Macroscopic evaluation of the liver and tissue sampling for histological investigations were carried out after 21 days. The bleeding times of bovine collagen (BoCo), protein-coated equine collagen (PECo), and oxidized cellulose (OxCe) were 140, 243 and 352 s, respectively. Microscopic evaluation of the PECo presented fibrosis and significant inflammation in the implantation zone, whereas BoCo and OxCe caused only fibrosis in the wound area [4].

Battlefield conditions can be harsh and subject to strong winds and dusty conditions, thus powder-based haemostatic products may not be effective. They can also be difficult to remove when the casualty reaches medical facilities as they tend have high electrostatic charges, which can cause the powder to stick to medical instruments, gloves and human tissues. All these conditions would cause it to be ineffective for deep penetration into an irregular wound cavity [1–4]. With the unsuitability of the different bleeding control methods, there is an urgent need to produce an effective and portable haemorrhage control device, which is able to quickly stop the bleeding for military usage.

There are few existing haemorrhage control products in the current market such as QuickClot Combat Gauze and XSTAT 30 from RevMedx [5–8]. The XSTAT 30 devices can deliver the compressive forces within the wound cavity while common haemorrhage control techniques can only apply the compression externally [5–8]. The XSTAT devices comprise of a syringe filled with little capsules of sponges coated with chitosan, which is an agent that promotes blood clotting. The syringe is inserted into the bullet wound of a casualty. The cellulose-based sponges are then injected into the wound where they expand quickly and absorb the blood. With the fast expansion of the capsules, a haemostatic pressure created causes to stop blood flow within the wound [6–8]. However, the XSTAT devices still have some limitations. For example, their expansion rate of the cellulose-based sponges is slow and they only achieve full length after 15 s of expansion. The cellulosebased sponges made from wood pulp may not absorb the blood very fast [6–8].

The purpose of this paper is to develop fast expanding, highly hydrophilic and mechanically robust hybrid cotton aerogels that can be safely administered for human medical treatment. The intention is to provide an effective hemorrhage treatment for first responder's situation. Cotton fibers contain approximately 90% cellulose [9] and have a very similar polysaccharide structure as cellulose, making it a hydrophilic material. Its hydrophilic properties can aid in blood absorption in the haemorrhage treatment [10]. Cellulose is also biodegradable and biocompatible. Biocompatibility property of a material does not elicit local or systemic responses when in contact with a living system or tissue, meaning that the cotton fibers are safe to use as a haemostatic agent without triggering further complications in the patient [11,12]. The cotton fibers arranged in an compressed aerogel structure may therefore provide improvements in performance compared to the *XSTAT* cellulose-based sponges in the treatment of deep haemorrhagic wounds. The large pore volume of the aerogels allows them to store copious amounts of water, while compressible nature allows them to expand and exert pressure on the wound.

However, disintegration of the hybrid aerogel occurs when the aerogel is too brittle, releasing small pieces of the aerogel when compressed and expanded in the fluid medium. This is undesirable because the pieces of aerogel may further complicate subsequent medical treatments. Cross-linking between cotton fibers is formed by Kymene 557H wet strength resin. Kymene is made of quaternary ammonium groups which form ester bonds between cotton fibers as the aerogel is dried and cured . The ester bonding can enhance the strength of the fibers and increases structural integrity of the aerogel [13].

Chitosan is also selected for its many beneficial medical properties that can improve the effectiveness of the hybrid aerogel as a haemostatic agent. Chitosan is comprised of D-glucosamine and *N*-acetyl-D-glucosamine randomly distributed along its polymer chains. It also contains a large number of hydroxyl groups [14]. Biodegradable and biocompatible chitosan can be derived from the chitin shells of shrimps and other crustaceans with the aid of alkaline substance like sodium hydroxide [14]. It can accelerate blood clotting, which reduces blood loss and chance of shock or death [15]. It is hypoallergenic and has natural anti-bacterial properties [16,17]. It can also reduce pain by blocking the nerve endings of the patient [18,19].

Based on the current technology with cellulose-based sponges as a haemostatic agent, the chitosan-coated cotton aerogels using cotton fibers, cellulose fibers and chitosan are developed. Although there are several works on cellulose aerogels reported [6,20–25], their cellulose aerogels *cannot be compressed* for the haemostatic treatment. In this paper, the highly absorptive cotton aerogel was developed to be compressed and injected into the bleeding wound caused by physical trauma such as a gunshot or incision. The primary effect of the comprssed hybrid aerogel introduction is to counter the systolic blood pressure within the wound cavity, thereby stopping further loss of blood. The secondary effect is to promote blood clotting and tissue healing through the use of additives, and to remove excess blood accumulated in the wound cavity. Three performance parameters of voulme expansion time, expansion ratio and hydrostatic pressure, relevant to applications as a haemostatic agent are determined. The effects of different compositions of cotton fibers, cellulose fibers and chitosan on the performance of the hybrid aerogels are compared with commercial *XSTAT* devices.

## 2. Experimental section

### 2.1. Materials

The recycled cellulose fibers from paper were sourced from Insul-Dek Engineering Pte Ltd (Singapore). Kymene 557H wet strength resin was sponsored by Ashland (Taiwan). All solutions were produced using deionized water (DI water). Cotton fibers were purchased off-the-shelf from local supermarkets (Singapore). Chitosan powder was sourced from Sigma-Aldrich Chemical Co.

### 2.2. Fabrication of pure cotton aerogels (C1 aerogels)

The pure cotton aerogels named as the C1 aerogels were produced from commercially available cotton fibers. Initially, the cotton fibers were mechanically blended using Tefal 400W blender and underwent ultra-sonication treatment to reduce the fiber length. The cotton fibers have a length of between 0.5–5.0 mm and a diameter from 15.45–16.53 μm. 0.5 − 1.0 g of the blended cotton fibers was then added in 100 mL deionised (DI) water at room temperature. The cross-linker Kymene was then added at 2.5 wt% of the dry weight of the blended cotton fibers. The obtained mixture was stirred for 24 h at 4000 rpm in a mixer to achieve homogeneity. Finally, the slurry was stored under refrigeration at −12 °C for 24 h for icing the supension, and then freeze-dried for 72 h to form the C1 aerogels. The C1 aerogels were cured at 120 °C for another 3 h to activate the crosslinking of the Kymene and mechanically strengthen the obtained C1 aerogels. Fig. 1a shows the pure cotton aerogels C1 having 0.5 wt% of the cotton fibers.

### 2.3. Fabrication of cellulose-cotton aerogels (C2 aerogels)

The hybrid cellulose-cotton aerogels having various cellulose-cotton ratios, called as C2 aerogels were chosen to develop further. The recycled cellulose fibers had length of 0.3–5.0 mm and diameter of 13–15 μm. The cellulose-cotton ratios were fixed at 1:2, 1:4 and 1:6, respectively. 200 mL of DI water was added to the fiber mixture; and they were blended by using Tefal 400W blender. Kymene (33.3 μL per 100 g water) was then added to the above suspension and went through a probe sonication process (Hielscher Ultrasound Technology) for 5 min at 140 W. During the mechanical blending and the sonication, the fibrous mixture achieved macro-scale homogeneity. The obtained fiber slurry was then placed in a refrigerator at −18 °C and then went through freeze-drying at −98 °C for 2-4 days to obtain the C2 aerogels. The C2 aerogels were cured at 120 °C for another 3 h to cause cross-linking of Kymene and mechanically strengthen the obtained aerogels. Fig. 1b shows the cellulose-cotton aerogel C2 having 0.7 wt% of the cellulose and cotton fibers and the cellulose-cotton ratio of 1:2.

### 2.4. Fabrication of chitosan-coated cellulose cotton aerogels (C3 aerogels)

The 0.7 wt% cotton-cellulose aerogels having the constant cellulose-cotton ratio of 1:2 were chosen to investigate further the effects of different chitosan concentrations on the performance of the chitosan-coated cotton-cellulose aerogels, called as C3 aerogels. For the fabrication of the C3 aerogel having 0.5 wt% chitosan, the chitosan solution was prepared first. Using a magnetic stirrer, 2.0 g of acetic acid was added 100 mL of DI water kept in an ice bath. 1.0 g of the chitosan powder was then slowly added into the dilute acetic acid until it became a clear yellowish solution. Next 0.467 g of cotton fibers and 0.93 g of cellulose fibers were added into 100 mL of DI water. In order to

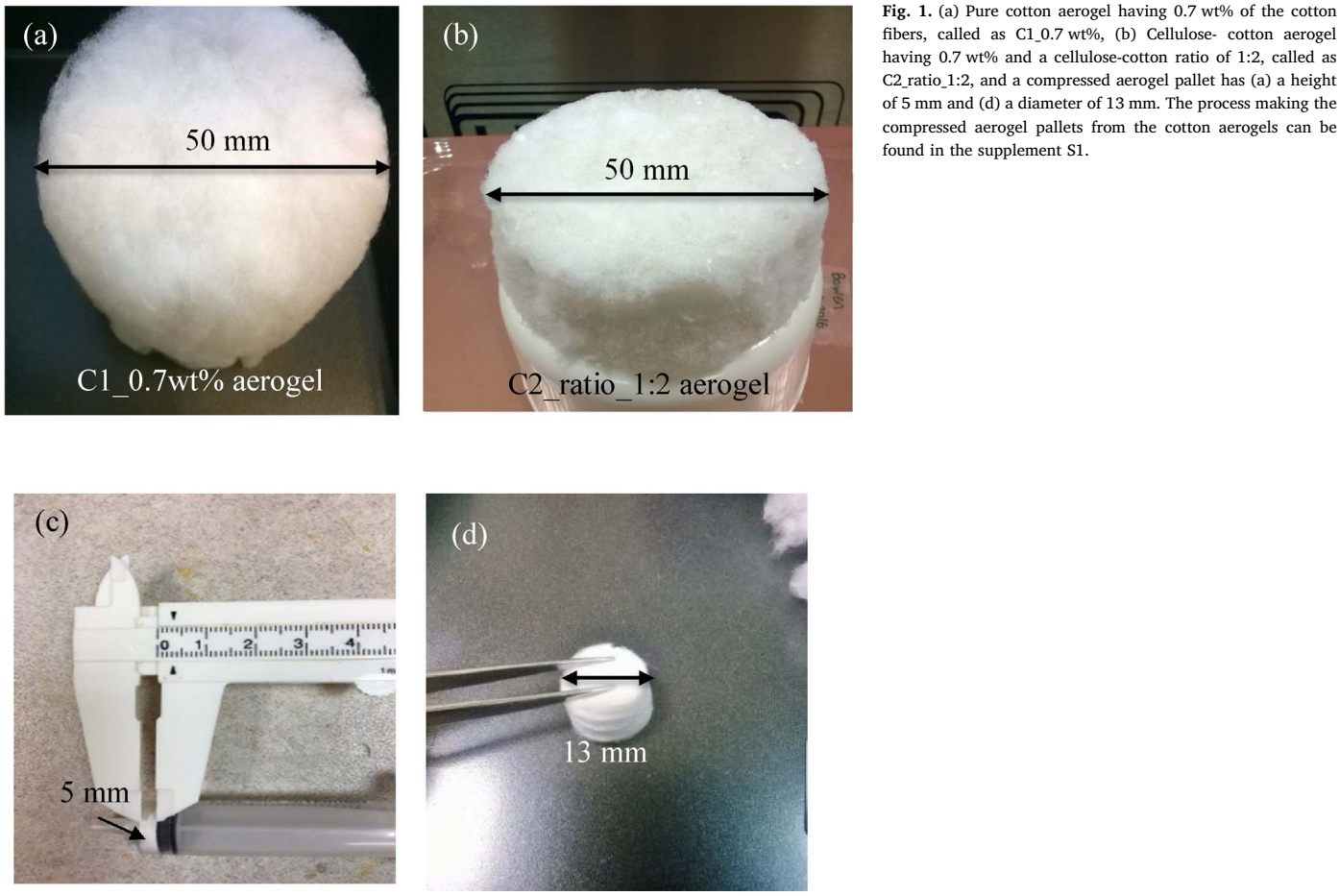


**Fig. 1.** (a) Pure cotton aerogel having 0.7 wt% of the cotton fibers, called as C1_0.7 wt%, (b) Cellulose- cotton aerogel having 0.7 wt% and a cellulose-cotton ratio of 1:2, called as C2_ratio_1:2, and a compressed aerogel pallet has (a) a height of 5 mm and (d) a diameter of 13 mm. The process making the compressed aerogel pallets from the cotton aerogels can be found in the supplement S1.

**Table 1**
Summary of the C1, C2 and C3 aerogel performance in DI water.[a]

| Aerogel Pallets | Expansion Ratio | Expansion Time (s) | Hydrostatic Pressure ($mmH_2O$) | Hydrostatic Pressure (mmHg) |
|---|---|---|---|---|
| | Pure Cotton Aerogels (C1 Aerogels) | | | |
| C1_0.5wt% | 18.6 | 15.9 | 120 | 8.8 |
| C1_0.7wt% | 15.7 | 20.8 | 109 | 8.0 |
| C1_1.0wt% | 15.5 | 21.0 | 104 | 7.7 |
| | Cellulose-Cotton Aerogels (C2 Aerogels) | | | |
| C2_ratio_1:2 | 14.8 | 7.0 | 98 | 7.2 |
| C2_ratio_1:4 | 10.8 | 8.2 | 94 | 7.0 |
| C2_ratio_1:6 | 10.0 | 11.7 | 92 | 6.8 |
| | Chitosan-Coated Cellulose-Cotton Aerogels (C3 Aerogels) | | | |
| C3_chi_0.5wt% | 16.0 | 4.5 | 155 | 11.5 |
| C3_chi_1.0wt% | 11.8 | 5.2 | 143 | 10.6 |
| C3_chi_1.5wt% | 12.2 | 30.0 | 146 | 10.8 |

[a] The max. standard deviation of the data is 1.0%.

produce a slurry, the solution was sonicated 5 times in 3 min intervals. The chitosan solution and 2.1 g of Kymene were added into the cellulose-cotton slurry solution using a pipette. The solution was sonicated 5 times in 3 min intervals and placed in the freezer for 24 h to form the wet gel. To obtain the final C3 aerogels, the wet gel was freeze-dried for 72 h, and finally cured for 120 °C for 3 h.

Finally, the obtained C1, C2 and C3 aerogels were compressed by using a 15-ml clinical syringe having a diameter of 15 mm to form the aerogel pallets as shown in Fig. 1c and d. The compression steps can be found in the Supplement Fig. S1. All the compressed C1, C2 and C3 aerogel pallets in this paper are summarised in Table 1.

### 2.5. Characterization

Sample morphology was investigated by a scanning electron microscope (SEM, JSM-6010 of Japan). Before the testing, samples were sputtered with a thin layer gold via JEOL sputter (JFC-1200) at 20 mA for 30 s to enhance their electrical conductivity.

Expansion time and volume expansion ratio were measured using the same experiment, but with different measurement methods. For the expansion tests, the aerogel was first compressed to form a pallet having the thickness of 5 mm. It was then placed into a petri-dish filled 2/3 with DI water. Expansion time was measured from the moment the aerogel contacting the water until it reached full expansion. Full expansion of the aerogel can be observed by the darkening of the aerogel across its entire length due to saturation by water. Full expansion can also be observed by the point where further expansion is insignificant. Expansion ratio was measured by taking the ratio between the fully expanded length and its original compressed length of the aerogel. Expansion ratio = (Final Length ($L_f$) − Initial Length ($L_i$))/Initial Length ($L_i$)

A U-tube manometer filled with DI water was used to measure the hydrostatic pressure provided by the compressed aerogelpallets as shown in the Supplement Fig. S2. The manometer was first filled to the brim with DI water. The hybrid aerogel was compressed into a syringe using a plunger and the syringe was then attached onto one end of the manometer. A small jet of air was pumped into the other end of the manometer to excite absorption. The final height difference between the ends of the manometer was the hydrostatic pressure provided by the aerogel in $mmH_2O$, which was then converted to mmHg.

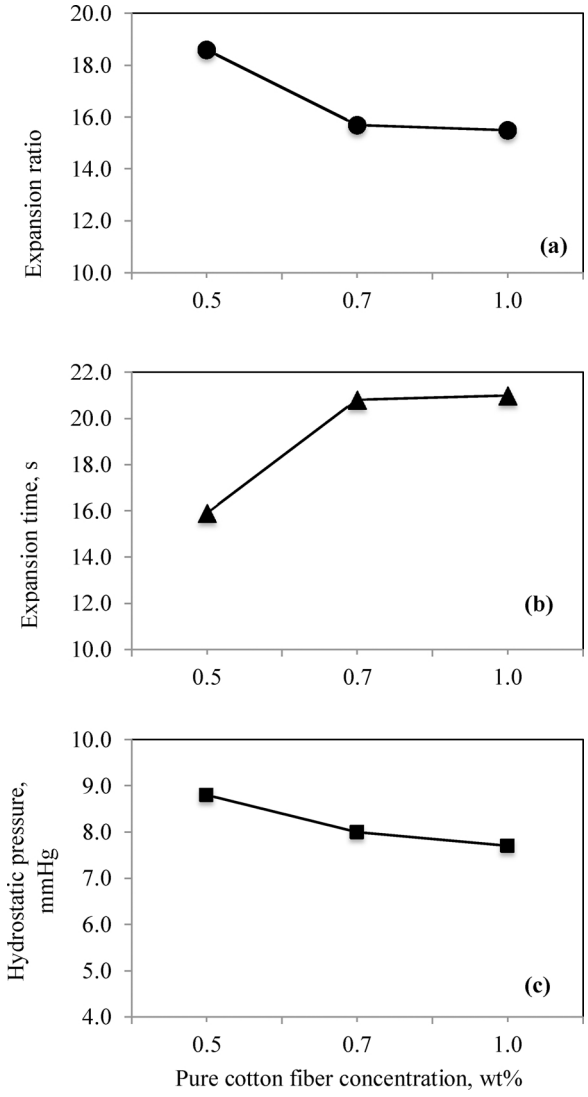


**Fig. 2.** (a) Volume expansion ratio, (b) expansion time and (c) hydrostatic pressure of the pure cotton aerogels C1 having different wt% of the cototn fibers.

## 3. Results and discussion

### 3.1. Performance of the C1 aerogels with different cotton fiber concentrations

Fig. 2 compares volume expansion ratio, expansion time and hydrostatic pressure of the C1 aerogels having the different cotton concentrations. When the cotton fiber concentration of the C1 aerogel pallets increases, their volume expansion ratio and hydrostatic pressure decease. This happens because the C1 aerogel having the larger cotton fiber concentration has a more packed structure as shown in Fig. 3a–c. The expansion time of the C1 aerogel pallets having the larger cotton fiber concentration is also larger as more compressed cotton fibers need more time to expand back. Table 1 shows the pure cotton aerogel pallet having 0.5 wt.% of the cotton fibers, called as the C1_0.5 wt% aerogel pallet exhibits the largest expansion ratio of 18.56, the fastest expansion time of 15.85 s, the largest hydrostatic pressure of 8.8 mmHg. The expansion performance of the C1_0.5 wt% and C1_0.7 wt% aerogel pallets are very competitive with the commercial XSTAT sponges. However, for compression process of the aerogel pallets, the C1_0.7 wt % aerogel pallets can be handled easier and disintegrate less than the C1_0.5 wt% ones for use as a haemostatic agent. Therefore, the C1_0.7 wt% aerogel pallets are chosen to investigate further in next

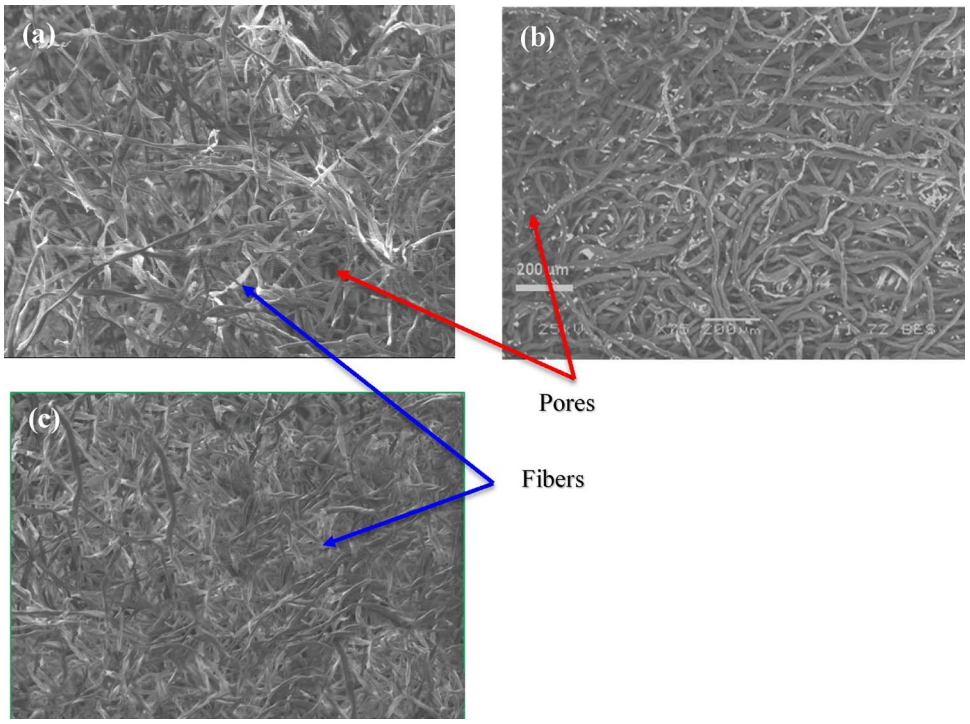


**Fig. 3.** The FESEM images of the pure cotton aerogels (C1 aerogels) having (a) 0.5 wt%, (b) 0.7 wt%, and (c) 1.0 wt% of the cotton fibers.

session 3.2.

### 3.2. Performance of the C2 aerogels with different cellulose-cotton ratios

Although the expansion time of the C1-0.7 wt% aerogel pallets is less than 16 s, this value can be improved with the addition of cellulose fibers. While the weight percentage of the fibers in the C2 aerogel pallets is kept constant at 0.7 wt%, the cellulose-cotton ratios of 1:2, 1:4 and 1:6 are varied. Table 1 shows the cellulose fibers from paper added in the C2 aerogel pallets can reduce significantly the expansion time. For example, the cellulose-cotton aerogel pallets having the ratio of 1:2, called as the C2_ratio_1:2 aerogel pallets can reduce the expansion time from 20.8 s to 7.0 s. This happens due to two possible reasons. The first reason is the cellulose fiber length and diameter may be smaller than the cotton fiber length in the C2 aerogel pallets and therefore the cellulose fibers need less time to expand. The second reason can be the cellulose fibers from paper have an amorphous structure which may absorb the water faster than the semi-crystalline structure of the cotton fibers [9].

Comparing with the C1-0.7 wt% aerogel pallet with no cellulose fiber addition, the C2_ratio_1:2 aerogel pallet in Fig. 4 exhibites a greatly improved expansion time up to three times, but its expansion ratio and hydrostatic pressure decrease slightly. Comparing with the 15-s expansion time of the XSTAT cellulose-based sponges, all the C2 aerogel pallets expand much faster from 7.0–11.7 s. They also exhibit good structural integrity without disintegration. As the C2-ratio_1:2 aerogel pallets show the best performance among three C2 aerogel pallets investigated in Table 1, they are chosen to further investigate the effects of different chitosan concentrations on their volume expansion ratio, expansion time and hydrostatic pressure in next session 3.3.

### 3.3. Performance of the C3 aerogels with different chisosan concentrations

Fig. 5 shows the 0.5 wt% chitosan added in the C2-ratio_1:2 aerogel pallets can reduce further the expansion time from 7.0 s down to 4.5 s, increase the expansion ratio up to 16.0 and the hydrostatic pressure up to 11.5 mmHg. This can be explained by chitosan having a large number of hydroxyl groups in its structure which can improve the hydrophilic properties of the C3 aerogel pallets [14,17]. Comparing to these parameters of the XSTAT cellulose-based sponges, the C3_chi_0.5 wt% aerogel pallets can reduce three times of the expansion time with the similar expansion ratio. This means the C3_chi_0.5 wt% aerogel pallets in this work can stop the serious liquid leakage like gun

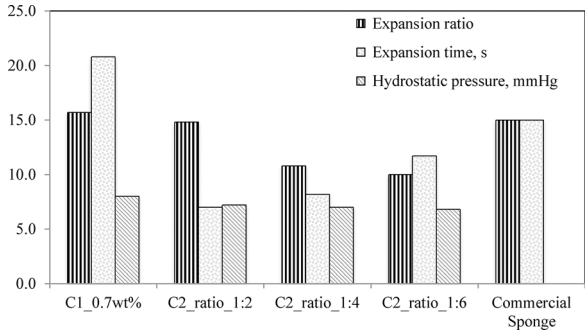


**Fig. 4.** Effects of different cellulose-cotton ratios on the haemostatic performance of the C2 aerogel pallets. Commercial sponge data is from the references 4–6 with no given hydrostatic pressure data.

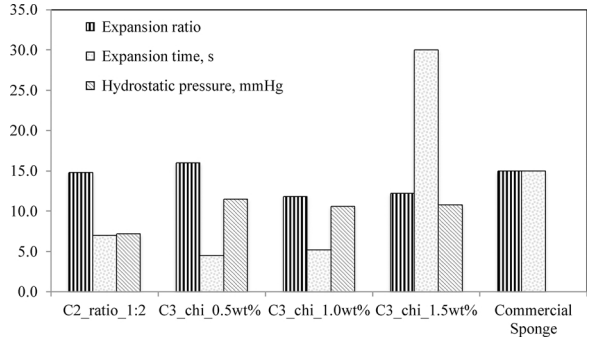


**Fig. 5.** Effects of different chitosan concentrations on the haemostatic performance of the C3 aerogel pallets. Commercial sponge data is from the references 4–6 with no given hydrostatic pressure data.

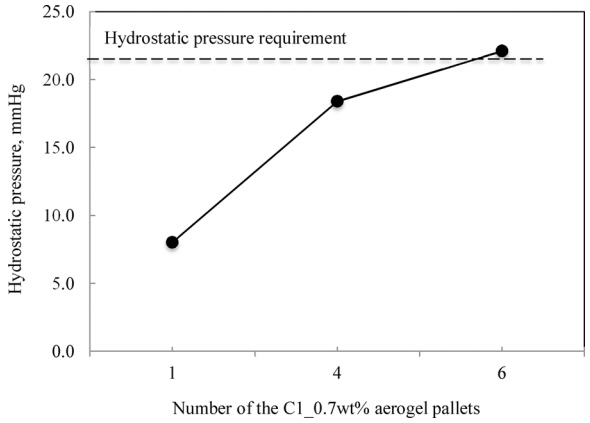


**Fig. 6.** Effects of the C1_0.7 wt% aerogel pallet number on the hydrostatic pressure. The dash line indicates the minimum hydrostatic pressure requirement of the haemostatic requirement [24].

wound three times faster than the commercial sponges. Prayson et al. [26] reported that the hydrostatic pressure ranges from 10 to 62 mm Hg in the injured leg. If the hydrostatic pressure requirement to stop the gun wound is assumed to be 21 mmHg, the potential haemostatic devices need only two C3-chi-0.5 wt% aerogel pallets. Although the chitosan coating can improve the haemostatic performance of the hybrid aerogels [19], there is also an inverse relationship at 1.5 wt% of chitosan. It can be explained that more chitosan used may cause the chitosan agglomeration in the C3 aerogel pallets. Therefore, it is essential to optimize further chitosan concentration versus the expansion and hydrostatic pressure performance of the C3 aerogel pallets in the future work.

### *3.4. Effects of the aerogel pallet number on the hydrostatic pressure*

A number of the C1_0.7 wt% aerogel pallets are placed randomly at the same time in the U-tube manometer filled with DI water to measure the hydrostatic pressure provided by themselves. Fig. 6 shows the effects of the C1_0.7 wt% aerogel pallet number on the hydrostatic pressure. As can be seen in Fig. 6, six cotton aerogel pallets C1 can prodcue 22.1 mmHg of hydrostatic pressure, which is larger than the minimum hydrostatic pressure requirement (the dash line) of the haemostatic requirement [27]. This implied that the haemostatic device using only 6 C1 aerogel pallets shown in the graphic abstract can stop the liquid leakage effectively. Compared to the previous works [1–6] such as the self-assembling peptide technology [3], the compressed aerogel pillars can stop the bleeding much faster and effectively in few seconds. The cost of the haemostatic device with the same volume using the hybrid cotton aerogel pallets in this work is much cheaper than that of the XSTAT devices. The details of the cost estimation can be found in Table S1 of the supplement document.

## 4. Conclusions

The fabrication processes of the cotton aerogels and their hybrid aerogels developed in this work can be scaled up for mass production. The cellulose-cotton (C2) aerogel pallets have better performance in terms of the volume expansion ratio and the hydrostatic pressure compared to the pure cotton (C1) aerogel pallets. Changing the ratio composition (cotton fiber: cellulose fiber content) can affect significantly the performance of the C2 aerogel pallets. However, the expansion mechanisms of the C2 aerogel pallets are still not clear and will be conducted in the future work. The C3 aerogel pallets can be used as the haemostatic agents and show much improvement over XSTAT cellulose-based sponges, especially in terms of the volume expansion time, with a reduction from 15 s to less than 5 s, even while retaining the structural integrity of the C3 aerogel pallets. A X-ray detectable marker like barium sulphate can be added in the C3 aerogel pallets so they can be visible and removed completely from the wound under the X-ray scanning.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.colsurfa.2017.10.067.

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