Food waste durian rind-derived cellulose organohydrogels: towards anti-freezing and antimicrobial wound dressing

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ABSTRACT

Hydrogels synthesized from naturally derived raw materials are attracting increasing attention as compared to synthetic hydrogels. In this study the use of food waste and side-stream products which were generated from the food industry, commonly associated with environmental concerns, were instead treated as a precious resource for hydrogel fabrication. Cellulose with a high purity was extracted from the food by-product durian rind, and used as a natural raw material to prepare water-based cellulose

hydrogels. Glycerol was introduced into the water-based hydrogels to fabricate organohydrogels by a simple one step water-glycerol replacement. Our results showed the organohydrogels possessed anti-freezing and non-drying properties, and the mechanical property was enhanced by the use of glycerol. Next, natural yeast phenolics were added into the organohydrogels. This endowed the organohydrogels with antimicrobial activity. The prepared organohydrogels showed no cytotoxicity, and when applied as a wound dressing on pig skin as a proof of concept, showed strong antibacterial activity. Therefore, this suggested that durian rind-based cellulose organohydrogels have the potential to be applied as antimicrobial wound dressing in medical supplies, even at extreme temperature environments such as -30 °C.

KEYWORDS: food byproduct, durian rind, cellulose organohydrogels, anti-freezing, non-drying, antimicrobial activity

INTRODUCTION

Hydrogels are widely applied in cosmetics[1, 2], drug delivery[3, 4], tissue engineering[5, 6], and wound dressing[7, 8] due to their excellent water absorption ability and soft mechanical properties. However, due to the character of the high-water content,

hydrogels usually freeze, and become friable and inflexible, thus losing some of their original properties when the temperature drops to below zero degree. Furthermore, as water evaporates quickly and easily in most of the water-based hydrogels, it leads to undesired changes in the initial properties of the hydrogels during storage. Hence, some research efforts have been made in an attempt to solve these problems. For example, Zhang and co-workers reported anti-freezing cellulose hydrogels synthesized in $ZnCl_2/CaCl_2$ solvent.[9] This salts solvent based cellulose hydrogels could keep their conductivity and thermal reversibility temperatures at up to -70 °C. In another study, Zhao used a spinning method to prepare conductive hydrogel fibers with an anti-freezing property which were maintained at -35 °C.[10] Rong used a H_2O/ethylene glycol binary solvent as the medium to prepare hydrogels which can keep the property at a temperature of -55 °C.[11] These reported hydrogels with anti-freezing property were achieved using a synthesis process which followed strict conditions. Zhou and co-workers developed a solvent exchange method to endow their hydrogels with anti-freezing and non-drying properties, which can retain their original properties at low temperatures of up to -70 °C.[12] This method is easier and more convenient as it can be

applied at a later step after the hydrogels were prepared and the glycerol used in this one-pot solvent exchange method has good biocompatibility,[13, 14] which is preferable to be applied in biomaterials.

Most of the research on hydrogels with anti-freezing and non-drying properties focused mainly on synthetic origin hydrogels. In comparison with synthetic hydrogels, hydrogels synthesized from natural materials have attracted increasing attention due to their inherent properties of nontoxicity, biocompatibility, and biodegradability, and sustainability.[15, 16] Cellulose, which is the most abundant biopolymer resource on the earth, could be used as a raw material to prepare natural origin hydrogels. In our previous work, the soybean waste (okara) was used as raw material to prepare natural cellulose-based hydrogels, and the hydrogels were applied as wearable sensors to detect the movement of the human body.[17] Durian is a fruit which is famous all over the world, especially in Southeast Asia as its name is “King of the fruits”. Durian is popular for its special flavor and high-quality nutrients which could afford health benefits for the human body. However, less than half part of the entire durian is edible, while the other

parts (rind and seeds) are treated as food waste in the durian industry.[18] Durian residues (rind and seeds) are usually used in landfills or burned, which poses a serious problem to the environment, and in the meanwhile is also a waste of a natural resource. Durian rind is comprised of 31.6% cellulose, 15.5% hemicellulose and 10.9% lignin in dried durian rind.[19] The content of cellulose in durian rind is larger than that in okara[20], which makes durian rind a sustainable cellulose resource for hydrogel fabrication, and this can also help reduce the environmental pollution.

Hydrogels are utilized in wound dressing, implants coating, and infection treatment with physiological conditions which are favorable for bacteria growth due to the moist environment. Bacterial infection is a great health challenge for hydrogels application, and this has attracted some research focus. Copper was used to add into the alginate hydrogel as the active ingredient to fabricate an antimicrobial hydrogel.[21] Silver nanoparticles were also used to cover the surface of hydrogels and to endow the hydrogels with antimicrobial property.[22] However, copper[23] and silver[24, 25] were reported to be toxic and not safe to be used in biomaterials. Compared with

antimicrobial materials with low biocompatibility such as copper and silver, materials with good biocompatibility and antimicrobial activity are more preferable in hydrogels preparation. Leucine was added into phenylalanine to form antimicrobial hydrogels, which had antimicrobial effect against Gram-positive bacteria.[26] Quaternized chitosan was used to fabricate hydrogels to endow them with antimicrobial activity as its quaternary amine salt group has a high charge density to kill bacteria.[27, 28] Reduced graphene oxide was used for synthesizing antimicrobial hydrogels not only due to its damage on cell membrane from both sharp contact and charge transfer between reduced graphene oxide sharp nanowall and bacteria, but also its photothermal property for bacteria killing.[27, 29] Dopamine was also introduced in the system of antimicrobial hydrogel attribute to its photothermal antimicrobial ability.[29, 30] Interestingly, phenolic metabolites, which were produced by a flavonoid-producing yeast in our lab,[31] had previously demonstrated strong antimicrobial activity and the potential to be a natural preservatives from sustainable source that can be added into food. Hence, the phenolic metabolites could be a natural and sustainable antimicrobial agent that could potentially be added in a natural cellulose-based hydrogel to endow it with an antimicrobial activity.

In this study, durian cellulose was first extracted from durian rind and used as the raw material to prepare cellulose-based hydrogels. The hydrogels achieved an anti-freezing and non-drying property by our one-pot glycerol displacement methods. Next, natural phenolic metabolites were added into the hydrogel, to impart an antimicrobial property. Then the obtained hydrogels were applied as wound dressing in a small model as a proof of concept. The overall results showed that the prepared hydrogels had a great potential in medical treatment application and could also withstand extreme temperatures.

EXPERIMENTAL SECTION

Materials, reagents, media and strains

Durian rind was purchased from the market and stored in refrigerator at -80 °C. Sulfuric acid (H_2SO_4), 95.0-98.0%; sodium hydroxide (NaOH), BioXtra, ≥98%; pectinase from *Aspergillus niger*, powder, 1.15 U/mg; hydrochloric acid (HCl), 37%; powdered cellulose, United States Pharmacopeia (USP) reference standard, derived from cotton

linters; anthrone, analytical standard; epichlorohydrin (ECH)≥99%, 1.18 g/mL; lithium hydroxide monohydrate ($LiOH \cdot H_2O$), bioxtra; glycerol, BioXtra, ≥99% (GC); ethyl alcohol (> 99%) were all purchased from Sigma-Aldrich (St. Louis, MO, USA) without further purification. Mueller-Hinton Broth (MHB) was purchased from Fisher Scientific, USA. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and NIH3T3 cells were purchased from ATCC (USA). Phenolic metabolites from flavonoid-producing yeast was prepared in our lab according to our previous work.[31]

Extraction of durian rind cellulose and preparation of durian rind cellulose hydrogels

The method of extracting cellulose from durian rind was modified from our previous work[17] and summarized below:

The durian rind was cut into pieces and treated by a grinder to produce smaller pieces of durian rind. Then it was freeze dried, followed by ball milling. 20 g obtained durian rind powder was mixed with 80 mL deionized water. The durian rind powder suspension was adjusted to pH 3, and 0.2 g pectinase powder was added to break the

cell wall of the durian rind, then incubated at 50 °C overnight. After washing with deionized water for three times, 120 mL 5% NaOH was mixed with the solid part and the suspension was incubated at 80 °C for 3 h. 120 mL 2% surfactant was mixed with solid part and the suspension was incubated at 50 °C overnight after washing with deionized water. Finally, 10 mL 1% bleach was mixed with the solids and incubated at 75 °C for 2 h. The suspension was washed by deionized water and followed by freeze-drying to obtain durian rind cellulose powder. The purity and yield of obtained durian rind cellulose were tested and calculated according to the method in our previous study.[17]

Durian rind cellulose hydrogels were prepared according to previous research.[32] The durian rind cellulose was dissolved in LiOH·urea (weight ratio of LiOH, urea, and water is 8:15:77) solution with a concentration of 6%. An appropriate amount of ECH (molar ratio of ECH to the anhydroglucose unit is 0.7) was then added into the solution with stirring. This solution was mixed by stirring for 2 h, then poured into the molds to form hydrogels. The mold with pre-hydrogels solution was incubated at 4 °C. The

crosslinked hydrogels were obtained from the mold carefully and washed with deionized water three times a day, for 7 days to remove impurities.

Morphologies of durian rind cellulose and durian rind cellulose hydrogels

The Morphologies of the durian rind cellulose and durian rind cellulose hydrogels were analyzed by field emission scanning electron microscope (FESEM). The fracture surface of the durian rind cellulose hydrogels was obtained by immersing the hydrogels into the liquid nitrogen, followed by breaking by hands and freeze-drying. The powdered samples of durian rind and durian rind cellulose and freeze-dried hydrogels were adhered to the specimen stage to be tested by a JSM6710-FESEM.

Anti-freezing and non-drying property of durian rind cellulose organohydrogels

The original water-based durian rind cellulose hydrogels were immersed entirely in glycerol (10 times of the weight of hydrogels) for different times, ranging from 2.5 min to 6 h. The durian rind cellulose organohydrogels were obtained from the glycerol solution and the excess residual glycerol on the surface of the organohydrogels were

wiped off. The water-based hydrogels and organohydrogels with different immersion times were kept at -30 °C for 2 hours to test the anti-freezing properties. The weight of glycerol in the organohydrogels was determined by freeze drying. The drying property of the prepared organohydrogels was tested by keeping them in an electronic dry cabinet (20 °C and 50% humidity) for eight days and the weight of the organohydrogels were weighed by balance every day.

Tensile property of durian rind cellulose organohydrogels

Tensile property of durian rind hydrogels and organohydrogels was measured by an Instron 5543 Tensile Meter. Samples of 30 mm*5 mm were examined at the speed of 2 mm/min. The Young's modulus was calculated from the initial linear region of the stress-strain curves.

Anti-microbial activity of durian rind cellulose organohydrogels

The durian rind organohydrogels prepared with different immersion times were cut by a perforating machine to form 6 mm discs. Yeast phenolics stock solution (1.5 g/mL

total phenolics in ethanol) of varying concentrations (3 mg, 6 mg, 12 mg, respectively) was added into the organohydrogel discs. The antimicrobial activity of the organohydrogels was analyzed by calculating the inhibition zones of all the organohydrogels prepared with different immersion times and different amounts of yeast phenolics, against the bacteria *Escherichia coli* ATCC 25922 (Gram-negative) and *Staphylococcus aureus* ATCC 29213 (Gram-positive), respectively.[31] The two strains were streaked on fresh Mueller-Hinton agar (MHA) plates and incubated at 37 °C. After overnight incubation, individual colonies were suspended in MH broth to achieve the OD_{600} value approximated 0.5 McFarland standard, which indicated the approximate concentration of $1\text{-}3\times10^8$ CFU/ml. Cotton swabs (sterile) were applied to inoculate MHA plates with corresponding MHB. Organohydrogels discs prepared with different immersion times and different amounts of yeast phenolics were carefully put on the plates. Organohydrogels discs with ampicillin antibiotic and pure ethanol were used as positive and negative controls, respectively. All the MHA plates with organohydrogels discs were incubated at 37 °C overnight. The antimicrobial assay was examined by measuring the zone of inhibition (mm), minus the diameter of the

organohydrogel disc. All tests were repeated three times.

Cytotoxicity of durian rind cellulose organohydrogels

The prepared durian rind cellulose organohydrogels discs (prepared with the immersion time of 3 hours with addition of 6 mg yeast phenolics) were sterilized. The sterile organohydrogels (15 mg) were immersed in 3 mL of Dulbecco's Modified Eagle Medium (DMEM) at 4 °C for one month. The organohydrogels extracted DMEM was then diluted to the concentration of 25% and 50% with normal DMEM. The cultured NIH3T3 cells were seeded in 96-well plates and incubated at 37°C, 5% CO_2 for further use. After the cells were attached to the bottom of the plate, different concentrations (100%, 50%, 25%, 0%) of organohydrogels extracted DMEM were added into the plate wells and cultured with cells for 72 h. Thereafter, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays were carried out according to the protocols.[33] The optical density (OD) value of the solution in MTT was measured by a microplate reader at 570 nm. The cell viability was calculated by comparing OD value of cells cultured by organohydrogels extracted DMEM with the OD value of cells

incubated using DMEM without treatment as control.

Application of durian rind cellulose organohydrogels as wound dressing

As a proof-of-concept, the durian rind organohydrogels were applied as a wound dressing with a modified method from Yang *et al*.[34] Briefly, fresh and shaved pig skin was bought from a local market. The wound on the pig skin was created by a scalpel to expose the dermis. Samples was collected from the wound to calculate the total bacteria at the start (0 hour). For treatment group one, the wounds were treated by covering the wound with the antimicrobial organohydrogels discs (prepared with immersion time of 3 hours with addition of 6 mg yeast phenolics) as dressing, while for group two the wounds were treated by covering with a moist gauze as the control. Then the wounds from treatment group one and two were placed on the surface of MH agars (with antibiotic) and kept at room temperature. Samples were collected to quantify the total bacteria at the time points of 24 h, 48h, and 72 h, respectively.

RESULTS AND DISCUSSION

The schematic of this study was shown in Figure 1. Cellulose was derived from durian rind and crosslinked with ECH to prepare water-based hydrogels. Then the obtained water-based cellulose hydrogels were immersed into glycerol to prepared cellulose organohydrogels by a water-glycerol replacement. Next, the cellulose organohydrogels were combined with yeast phenolics and applied as a wound dressing using pig skin as a model. The purity and productivity of cellulose extracted from durian rind was 93% and 19.4%, respectively, which were higher than that from okara[17] which indicated that durian rind was a suitable natural cellulose resource.

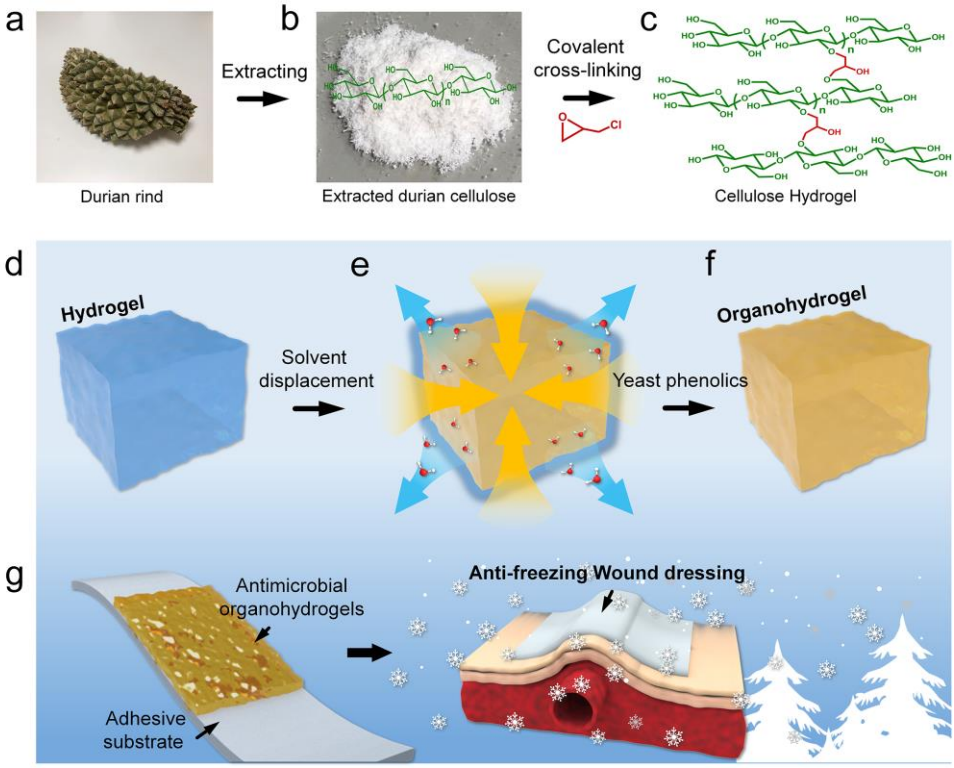

Figure 1. (a) Durian rind was used to extract (b) durian cellulose to fabricate (c) durian

cellulose hydrogel. (d) The durian cellulose hydrogel was modified by (e) water-glycerol displacement and (f) addition of yeast phenolics and applied as (g) anti-freezing and antimicrobial wound dressing.

Morphologies of durian rind cellulose and durian rind cellulose hydrogels

The morphology of durian rind cellulose and durian rind cellulose hydrogels were characterized by FESEM as shown in Figure 2. Obvious fibers could be found in the extracted durian rind cellulose (Figure 2b) as compared to the mixed substances shown in the raw durian rind powder (Figure 2a), which indicated successful extraction of cellulose from durian rind. Porous structures could be seen on the fracture surface of the durian rind cellulose hydrogels (Figure 2c) after the crosslinking of extracted pure cellulose had taken place.

Anti-freezing property of durian rind cellulose organohydrogels

The original durian rind cellulose hydrogels were immersed in glycerol for different times to form organohydrogels with different amounts of glycerol. Certain parts of the

water present in the hydrogels were exchanged with glycerol during the immersion. The prepared organohydrogels attained the properties of anti-freezing and non-drying, which was attributed to the immersion in glycerol for the appropriate time. The anti-freezing property of organohydrogels and normal water-based hydrogels was tested by incubating in the fridge at -30 °C for 2 hours, as shown in Figure S1. All of the organohydrogels were found to shrink obviously within the first 10 min of immersion in glycerol during the solvent exchange, and then shrank much slower when the immersion time exceeded 10 min. With increasing immersion time, the volume and weight of the organohydrogels decreased. When the immersion time was more than 10 min, the volume of the organohydrogels decreased less as compared to the first 10 min and showed only a slight difference. All of the organohydrogels with an immersion time from 2.5 min to 6 h demonstrated a good transparency. It was found that organohydrogels with an immersion time of ≥10 min were not frozen after being incubated in the fridge at -30 °C for 2 hours. (Figure S1b) However, the organohydrogels with an immersion time of less than 10 min were frozen in varying levels according to the immersion time. (Figure S1a) The watershed of frozen or non-

frozen for Ca-alginate/PAAm organohydrogels[12] required an immersion time of 60 min, which indicated that the water-glycerol replacement of durian rind cellulose hydrogels was much faster than that of Ca-alginate/PAAm hydrogels. The results indicated that different immersion times in glycerol resulted in different anti-freezing property for the obtained organohydrogels. The organohydrogels with immersion time of more than 10 min were tough and able to be folded and twisted at -30 °C. (Figure 2d) The anti-freezing property of organohydrogels were kept after combining with yeast phenolics. (Figure 2e) The glycerol content of organohydrogels with different immersion times was examined by freeze drying. The photos of freeze-dried water-based hydrogel and organohydrogel were shown in Figure 3a. The weight retention (W/W_0, W was the weight of hydrogels/organohydrogels after freeze drying, W_0 was the weight of hydrogels/organohydrogels before freeze drying) increased sharply when immersion time was from 0 min to 20 min. (Figure 3b) The increasing of the weight retention slowed down when the immersion time exceeded 20 min. The results of glycerol contents at different immersion time were shown in Figure 3c. The content of glycerol increased from 0 to 52% from 0 min to 20 min, increased to 61% from 20 min to 2 h,

and only increased by 3% from 2 h to 6 h. These phenomena could be explained by the fact that during the first 20 min, the “free water” exchanged fast with the glycerol, and after that only a few of “free water” and “intermediate water” were displaced by the glycerol.[35]

Non-drying property of durian rind cellulose organohydrogels

Most water-based hydrogels face the following problems of water evaporation and stability during storage which seriously limits their applications. The water retention property of the obtained organohydrogels were examined by storing them in an electronic dry cabinet (20 °C and 50% humidity) for eight days and their weights were monitored every day. The photos of water-based hydrogel and organohydrogel after storage in the dry cabinet for 20 days were shown in Figure 3d. The ratio of the weights of the organohydrogels with different immersion times on each day (W_t) as compared to the weights of organohydrogels with different immersion times on day 0 ($W_{t=0}$) was assessed during the storage time and were shown in Figure 3e. The results indicated that the water retention ability of the organohydrogels had a positive correlation with

the length of immersion time in glycerol (weight ratio of glycerol in oragnohydrogels). The water-based original hydrogels lost 90% of the initial weight within the first 24 h. When the immersion time in glycerol was more than 5 min (included), the weight loss of the obtained organohydrogels were found to be less than 30% during the storage period. When the immersion time was longer than 2 h, the obtained organohydrogels could absorb water from the ambient environment from the first day. The weight of all the organohydrogels were found to be stabilize after being incubated in the cabinet for 48 h, which was faster than that of the stabilization time of Ca-alginate/PAAm organohydrogels.[12] These results showed that water evaporation/absorption of cellulose-based hydrogels/organohydrogels was faster than that of Ca-alginate/PAAm hydrogels/organohydrogels. This finding was in line with the speed of water-glycerol exchange. The anti-freezing and non-drying properties of durian rind cellulose organohydrogels could be attributed to the presence of glycerol, as itself possesses ice-inhibiting and water capture ability.[36] The organohydrogel (immersion time of 3 h and yeast phenolics of 6 mg/9πmm^2) lost about 9% of its weight, while the weight of organohydrogels (immersion time of 3 h without yeast phenolics) increased by about

8.9% during the first day of storage. This phenomenon may be attributed to two reasons. First, the wt% of the glycerol in organohydrogels decreased after adding yeast phenolics, which weakened the non-drying property of the organodydrogels. Second, yeast phenolics were added into the organohydrogels in the atmosphere with humidity of 70-80%, the organohydrogels may absorb water from the ambient environment during the combination with yeast phenolics before weighing weight on day 0, and the organohydrogels with yeast phenolics may lose water after being transferred from the atmosphere (humidity of 70-80%) to the dry cabinet (humidity of 50%). However, the weight of organohydrogels with yeast phenolics did not keep decreasing and became stabilized during the storage time, which indicated a favourable non-drying property of organohydrogels after the addition of yeast phenolics.

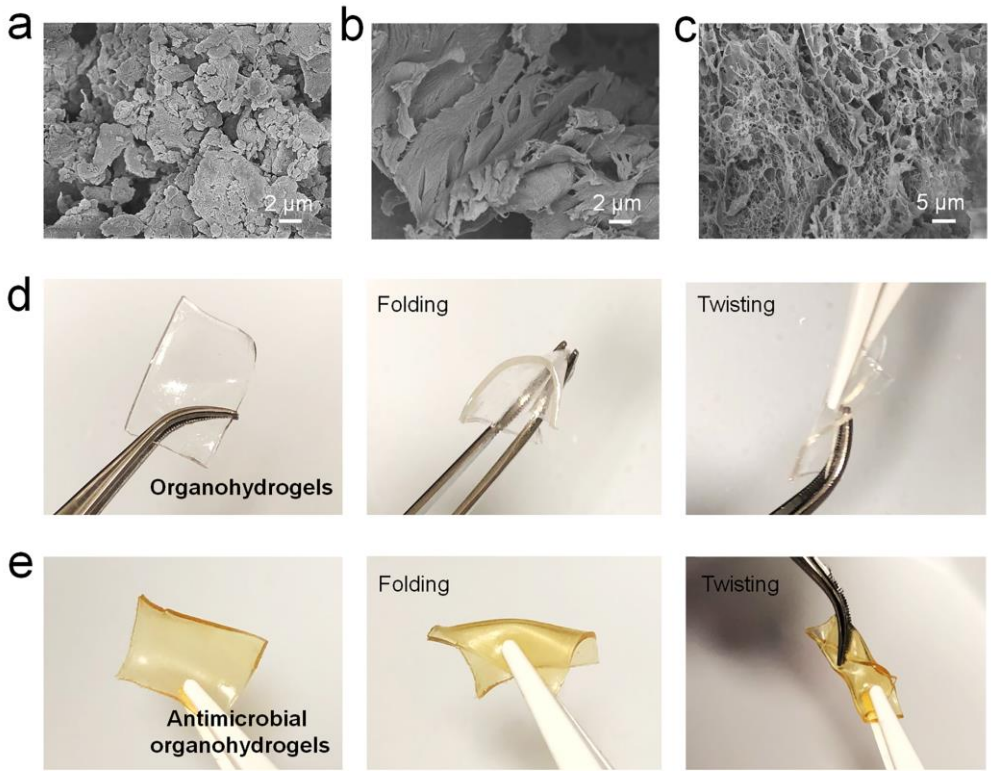

Figure 2. FESEM images of (a) durian rind powder, (b) durian rind cellulose, and (c) fracture surface of durian rind cellulose hydrogels. Mechanical deformation of (d) organohydrogels and (e) antimicrobial organohydrogels at -30 °C.

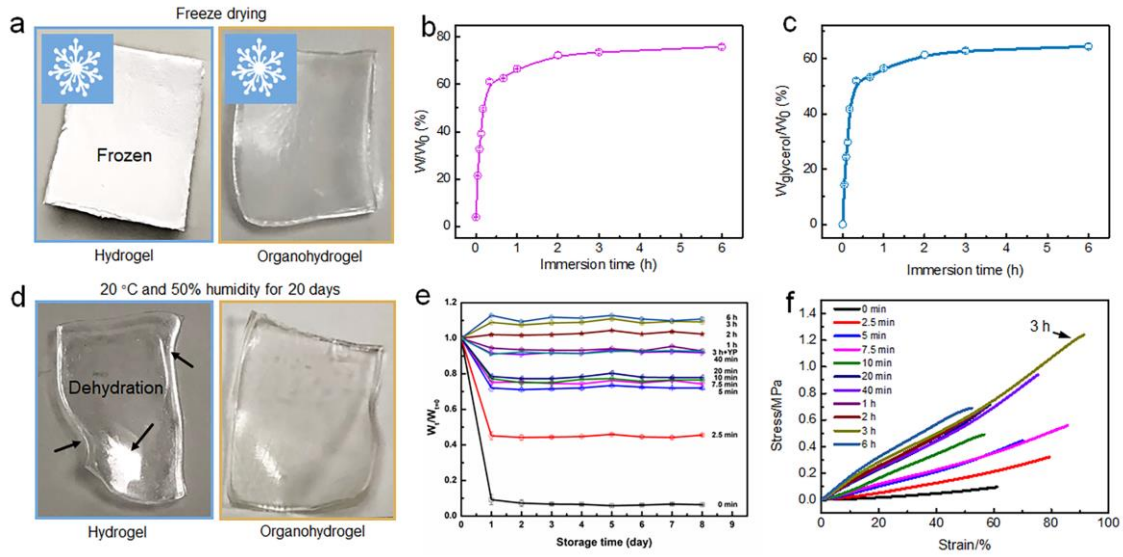

Figure 3. (a) Images of water-based hydrogel and organohydrogel after freeze-drying. (b) The weight ratio of organohydrogels with different immersion times after freeze-drying to that of before freeze-drying, where W_0 and W are the weight of organohydrogels before freeze-drying and the weight of the freeze-dried organohydrogels prepared with different immersion time, respectively. (c) The wt% of the glycerol within the respective organohydrogels with different immersion times. (d) Images of water-based hydrogels and organohydrogels after storage at 20 °C and 50 % humidity for 20 days. (e) Weight variation of hydrogels and organohydrogels upon storing at 20 °C and 50% humidity for eight days. (f) Tensile stress-strain curves of the hydrogels and organohydrogels with different immersion times under tension.

Tensile property of durian rind cellulose hydrogels/organohydrogels

Tensile test was used to analyze the mechanical property of prepared sdurian rind cellulose hydrogels and organohydrogels. The modulus of hydrogels/organohydrogels increased significantly while the immersion time in glycerol increased. (Figure 3f). The stress at break (σ), break strain (ε), and Young's modulus (E) under tension of hydrogels/organohydrogels with different immersion times in glycerol were summarized in **Table 1**. The stress at break, break strain, and Young's modulus of durian rind cellulose hydrogels (water-based) were 0.095 MPa, 61.1%, 0.0012 MPa, respectively. When the water-based hydrogels were immersed in glycerol for just 2.5 min, the stress at break, break strain, and Young's modulus of the organohydrogels (2.5 min) increased to 0.321 MPa, 79.4%, and 0.0037 MPa, respectively. The Young's modulus of the organohydrogels increased as the immersion time in glycerol increased. This phenomenon may be attributed to the enhanced density (shrink of the hydrogels) as a result of water-glycerol exchange. The organohyrogels with immersion time of 3 h owned the highest tensile strength at fracture (1.24 MPa) and the largest tensile strain (91.4 %) amongst all organohydrogels with different immersion times. Hence, the

organohydrogels with 3 h immersion time were selected for further use.

Table 1. Mechanical properties of durian hydrogels and organohydrogels.

Sample	Immersion time	σ/ MPa	ε/ %	E/ MPa
1	0 min	0.095	61.1	0.0012
2	2.5 min	0.321	79.4	0.0037
3	5 min	0.442	70.01	0.0055
4	7.5 min	0.557	85.6	0.0062
5	10 min	0.49	56.7	0.009
6	20 min	0.72	58.6	0.0108
7	40 min	0.94	75.4	0.0114
8	1 h	0.68	56.6	0.0116
9	2 h	0.63	52.6	0.0118
10	3 h	1.24	91.4	0.0126
11	6 h	0.69	52.27	0.0136

σ, ε, and E are stress at break, break strain, and Young's modulus under tension.

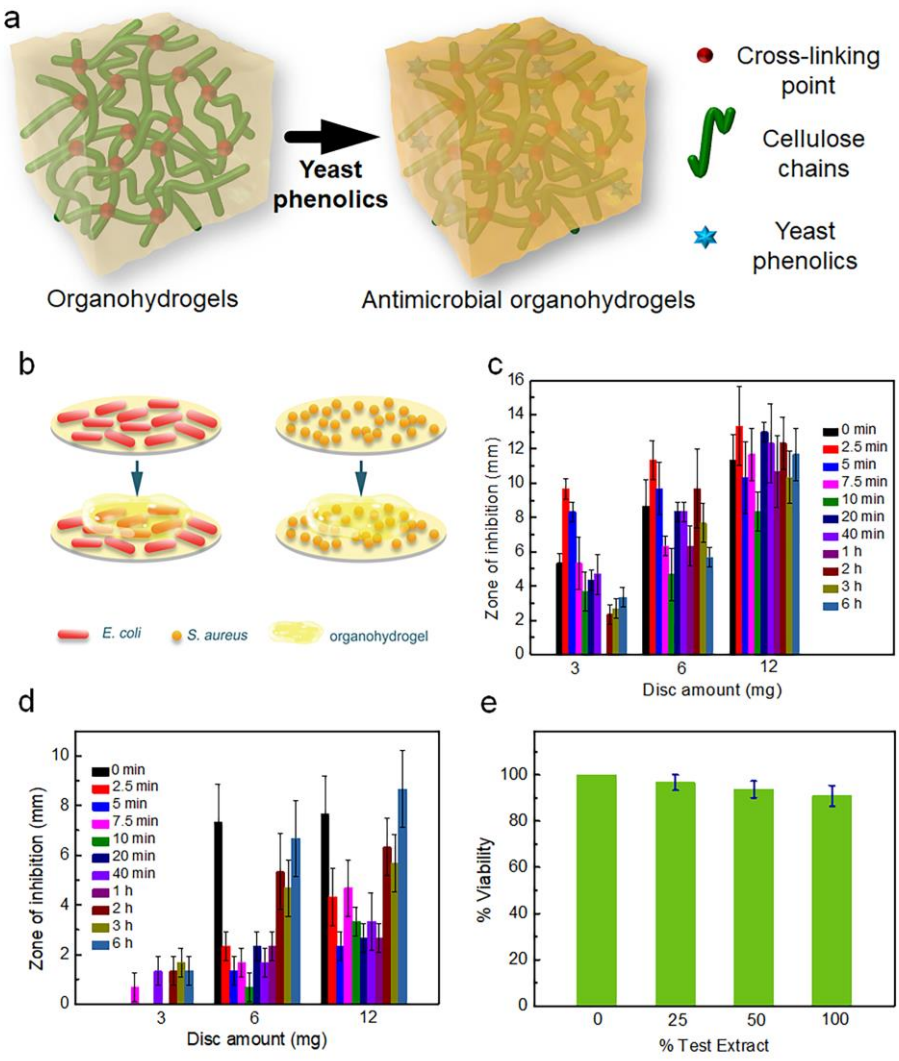

Figure 4. (a) Scheme of antimicrobial organohydrogels fabrication using durian rind cellulose organohydrogels with the addition of novel yeast phenolics. (b) Scheme of antimicrobial test of organohydrogels with yeast phenolics against *E. coli* and *S. aureus*, respectively. Antimicrobial activity of water-based hydrogels and organohydrogels

with different immersion times in glycerol and different amounts of yeast phenolics against (c) *S. aureus* and (d) *E. coli*, respectively. (e) Viability of NIH3T3 cells after culturing with antimicrobial organohydrogels extracted DMEM at different concentration.

Antimicrobial activity of durian rind cellulose organohydrogels

Yeast phenolics were added in the durian rind cellulose organohydrogels (Figure 4a) and agar disc diffusion method (Figure 4b) was applied to evaluate the antimicrobial activity of durian rind cellulose hydrogels/organohydrogels with different immersion times and different amounts of yeast phenolics. All of the hydrogels and organohydrogels with different immersion times showed no zone of inhibition (no antimicrobial activity) against either Gram-negative *E. coli* or Gram-positive *S. aureus* with pure ethanol as the negative control. In general, the zones of inhibition (which indicated antimicrobial activity) of all hydrogels and organohydrogels with different immersion times were larger for *S. aureus* (Figure 4 c) than that for *E. coli* (Figure 4 d) when the same concentration of yeast phenolics was used. These results were in line

with the results reported in our previous work[31] that the yeast phenolics had stronger antimicrobial activity to *S. aureus* than that to *E. coli*. When the amount of yeast phenolics added was 12 mg, all of the hydrogels/organohydrogels had strong antimicrobial activity against both *E. coli* and *S. aureus*. However, when the amount of yeast phenolics added decreased to 6 mg, all of the hydrogels/organohydrogels showed strong antimicrobial activity (from 4.67 to 11.33) to *S. aureus*, while only water-based hydrogels and organohydrogels with an immersion time of 2 h to 6 h showed strong antimicrobial activity (from 4.67 to 7.33) to *E. coli.* All of the hydrogels/organohydrogels with different immersion time had antimicrobial activity to *S. aureus*, while only several of them had weak antimicrobial activity (from 0.67 to 1.67) to *E. coli* when the amount of yeast phenolics added was 3 mg. Therefore, the organohydrogels with immersion time of 3 h and yeast phenolics amount of 6 mg/9πmm^2 was chosen for further use.

Cytotoxicity of durian rind cellulose organohydrogels

The cytotoxicity of durian rind cellulose organohydrogels was examined by the MTT

assay. The viability values of NIH3T3 cells after incubation with organohydrogels extracted DMEM with different dilution were shown in Figure 4 e. The viability of NIH3T3 cells were 90.9%, 93.6%, and 96.6% with the concentrations of 100%, 50% and 25% of organohydrogels extracted DMEM, respectively. The results showed that the durian rind cellulose organohydrogels with yeast phenolics had no cytotoxicity on NIH3T3 cells. This was similar to the okara cellulose hydrogels prepared in our previous work.[17] The non-cytotoxicity of the durian organohydrogels may be attributed to the natural and nontoxicity of the hydrogels' polymer (cellulose from the natural food byproduct), the metabolites from yeast served as the natural antimicrobial ingredient, and the use of non-toxic glycerol.

Application of durian rind cellulose organohydrogels as a wound dressing

The prepared durian rind cellulose organohydrogels were applied as wound dressing as a proof-of-concept. (Figure 5) Pig skin was chosen as a model instead of human skin in this test. After the skin was cut to expose the dermis, some of the samples were covered by the prepared durian rind cellulose organohydrogels, while other samples were

covered by moist gauze. All of the samples were kept at 37 °C for three days and the total viable bacteria were counted at the time of 24 h, 48h, and 72 h, respectively. The total viable bacteria were found to be $2.05*10^6$ colony forming units (CFU) when the wound was freshly created on the pig skin (day 0). After 24 hours, the total viable bacteria of sample covered with only moist gauze was $1.09*10^8$ CFU, while the sample covered with organohydrogels was $1.58*10^6$ CFU, which indicated that the prepared organohydrogels inhibited the growth of the bacteria obviously on the wound at the first day. The total bacteria count of the sample which was covered only with moist gauze had increased to $2.15*10^8$ CFU, while that of the sample covered with organohydrogels decreased to $3.36*10^5$ CFU, which indicated that the inhibition effect of the prepared organohydrogels was maintained for two days. On the third day, the total bacteria count of the sample covered with only moist gauze further increased to as much as $4.9*10^8$ CFU, while that of the sample covered with organohydrogels increased to $3.7*10^7$, which indicated that the inhibition effect of the organohydrogels became weaker on the third day. This weaker antimicrobial activity of the organohydrogels on the third day may be resolved by cleaning the wound and replacing the wound with a new

organohydrogels at day two.[34] This pig skin-based wound dressing application implied that the prepared organohydrogels have the potential to be applied in wound dressing.

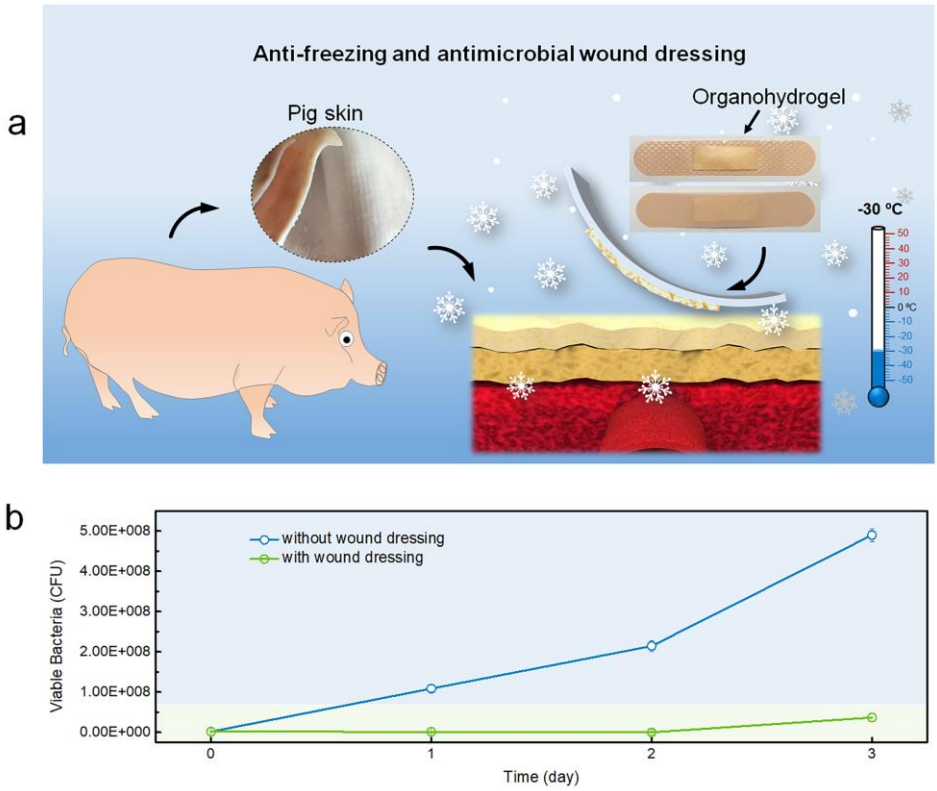

Figure 5. (a) Scheme of durian rind cellulose organohydrogels applied as anti-freezing and antimicrobial wound dressing. (b) Total viable bacteria (CFU) on the surface of pig skin with or without wound dressing.

CONCLUSIONS

In this study, natural cellulose extracted from durian rind was used to prepare water-

based hydrogels. The water-based cellulose hydrogels were modified by water-glycerol exchange to form durian rind cellulose organohydrogels. The prepared organohydrogels showed favorable anti-freezing, non-drying, and mechanical properties. Next, natural yeast phenolics were added into the organohydrogels to fabricate antimicrobial organohydrogels and the obtained antimicrobial organohydrogels showed strong antimicrobial activity against both Gram-negative *E. coli* bacteria and Gram-positive *S. aureus* bacteria according to agar disc diffusion assay results. Furthermore, the antimicrobial organohydrogels showed no cytotoxicity on NIH3T3 cells in the results from the MTT assay. As a proof of concept, the antimicrobial organohydrogels were applied as a wound dressing using pig skin as a model, and it showed good antimicrobial effect for up to 48 h. This work demonstrated a strategy to reuse side-stream products from the food processing industry, and the natural prepared organohydrogels have the potential to be used as environmentally friendly antimicrobial wound dressing which can withstand freezing temperatures over a long period of time.

ASSOCIATED CONTENT

The Supporting Information is available free of charge on the ACS Publication website at http://pubs.acs.org

Additional photos of water-based hydrogels and organohydrogels in room temperature and at -30 °C. (PDF)

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ACKNOWLEDGMENT

The authors would like to thank the Nanyang Environment and Water Research Institute (NEWRI), School of Chemical and Biomedical Engineering (SCBE), and the

Interdisciplinary Graduate School (IGS), College of Engineering, Nanyang Technological University, Singapore for the award of research scholarship to Xi Cui and the support from FoodTech@NTU grant for this research.

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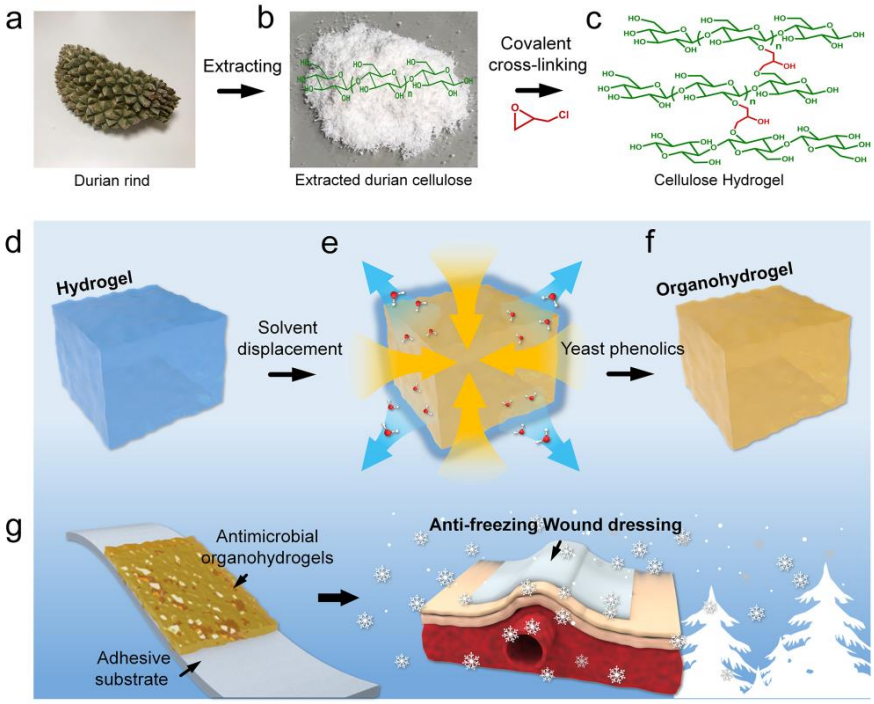
Synopsis

Hydrogels prepared by cellulose derived from food byproduct show good properties and exhibit excellent performance in wound dressing.

TOC figure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