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1 **Carbohydrate Polymers, Original full-length research papers**

2

3 **Wood-mimetic skins prepared using horseradish peroxidase catalysis to**
4 **induce surface wrinkling of chitosan film upon drying**

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20

21

22 **Abstract**

23 We previously developed bio-based wrinkled surfaces induced by wood-mimetic skins upon
24 drying in which microscopic wrinkles were fabricated on a chitosan (CS) film by immersing it
25 in a phenolic acid solution, followed by horseradish peroxidase (HRP)-catalyzed surface
26 reaction and drying. However, the detailed structure of the resulting wood-mimetic skins,
27 including crosslinking mode and thickness, has not been clarified due to the difficulty of the
28 analysis. Here, we prepare wrinkled films using ferulic acid (FE), vanillic acid (VA), and
29 homovanillic acid (HO) and characterize their structures to clarify the unknown
30 characteristics of wood-mimetic skin. Chemical and structural analyses of wood-mimetic
31 skins prepared using VA and HO indicate that the crosslinking structure in the skin is
32 composed of ionic bonds between CS and an oligophenolic residue generated by the HRP-
33 catalyzed reaction on the CS surface. Moreover, the quantity of these ionic bonds is related to
34 the skin hardness and wrinkle size. Finally, SEM and TOF-SIMS analyses indicate that the
35 skin thickness is on the submicron order (<200 nm).

36
37 **Key Words:** Chitosan, Surface wrinkling, Skin layer, Horseradish peroxidase, Phenolic acid,
38 Biomimetic materials

39 40 **1. Introduction**

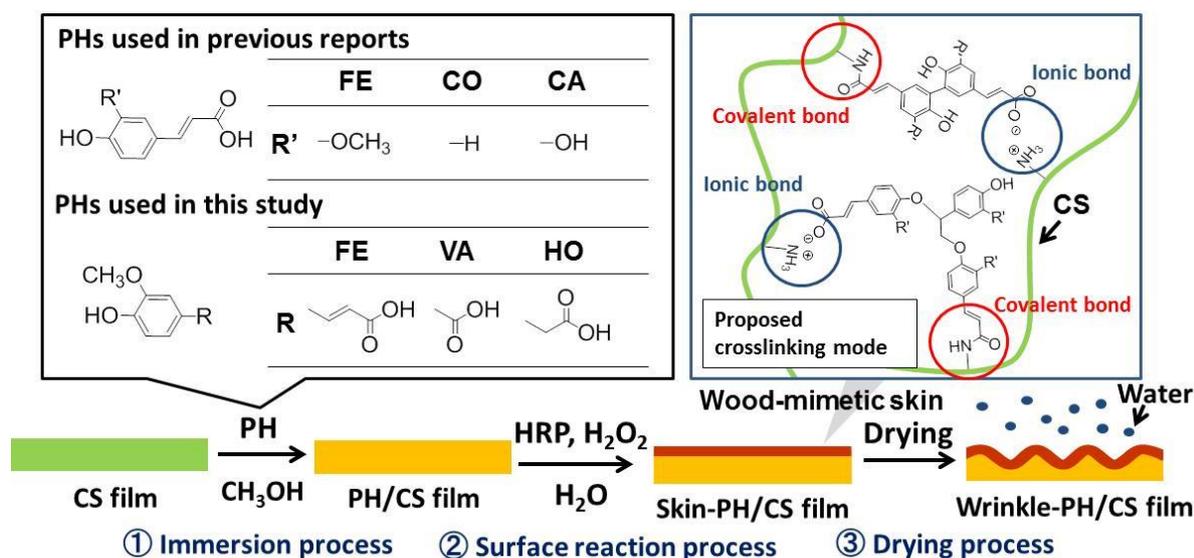
41 Biomimetic systems that imitate the design principles of nature are key technologies in the
42 progress toward environmentally benign and high-performance materials (Bhushan, 2009;
43 Bhushan & Jung, 2011). Many functional materials inspired by nature have been developed
44 (Aizenberg & Fratzl, 2013; Kawamura, Kohri, Morimoto, Nannichi, Taniguchi & Kishikawa,
45 2016; Kawamura, Kohri, Yoshioka, Taniguchi & Kishikawa, 2017; Otsuka, Fujikawa,
46 Yamane & Kobayashi, 2017; Pandian & Sugiyama, 2016; Sedo, Saiz-Poseu, Busque & Ruiz-
47 Molina, 2013).

48 Surface-wrinkling is a ubiquitous physical process that creates macro/microscopic
49 wrinkles in nature (Genzer & Groenewold, 2006; Ionov, 2012). This spontaneous process is
50 the result of inhomogeneous changes triggered by internal stresses and swelling/shrinking of
51 tissue layers possessing different elastic moduli (Ionov, 2012). The formation of fine wrinkles
52 in human skin is closely associated with decreases in the water content of the stratum
53 corneum by aging; i.e., fine wrinkles are formed by drying, and as a result of inhomogeneous
54 shrinkage (Barel, Paye & Maibach, 2009; Imokawa & Takema, 1993; Tsukahara, Hotta,
55 Fujimura, Haketa & Kitahara, 2007).

56 Nano/microscopic wrinkled surfaces inspired by nature-mimetic surface designs have
57 been developed for optical (Ohzono, Suzuki, Yamaguchi & Fukuda, 2013) and electronic
58 devices (Lee et al., 2013), the realization of tunable wettability (Li, Dai, John & Carter, 2013)
59 and adhesion (Davis, Martina, Creton, Lindner & Crosby, 2012), and the synthesis of cell
60 culture scaffolds (Zhao, Gu, Zhao, Guan, Zhu & Zhang, 2014). Basically, a skin layer is
61 fabricated on a soft substrate via dry processing methods, including chemical vapor deposition
62 (Bowden, Brittain, Evans, Hutchinson & Whitesides, 1998), photo-crosslinking (Chen, Reed
63 & Yang, 2013), and UV/O₃ oxidation (Efimenko, Rackaitis, Manias, Vaziri, Mahadevan &
64 Genzer, 2005). The wrinkling event can be caused/controlled by mechanical stress (Efimenko,
65 Rackaitis, Manias, Vaziri, Mahadevan & Genzer, 2005), thermal expansion (Bowden, Brittain,
66 Evans, Hutchinson & Whitesides, 1998), and/or swelling-shrinking (Huraux, Narita, Bresson,
67 Fretigny & Lequeux, 2012; Rizzieri, Mahadevan, Vaziri & Donald, 2006; Zhao, Gu, Zhao,
68 Guan, Zhu & Zhang, 2014).

69 We previously developed a surface-wrinkling system inspired by the fine wrinkles and
70 design principles of wood-cell walls (Fig. 1) (Izawa, Okuda, Ifuku, Morimoto, Saimoto &
71 Rojas, 2015; Izawa et al., 2016). In this method, wood-mimetic skins are fabricated by
72 immersing chitosan (CS) film in a phenolic acid (PH)-methanol solution, then treated with
73 horseradish peroxidase (HRP) to catalyze a surface reaction. Finally, surface wrinkling is

74 induced by water evaporation during drying. The wrinkle wavelength and amplitude can be
 75 controlled by the choice of a phenolic acid (ferulic acid, FE; *p*-coumaric acid, CO; or caffeic
 76 acid, CA) and by varying the temperature of the immersion process. Using this system, we
 77 found that the wrinkle size was predominately determined by the hardness of the wood-
 78 mimetic skins (Izawa et al., 2016). However, the structure of the wood-mimetic skin layer has
 79 not been fully elucidated.



80
 81 **Fig. 1.** Illustration of the wood-inspired surface wrinkling systems used in this study and in
 82 previous reports.

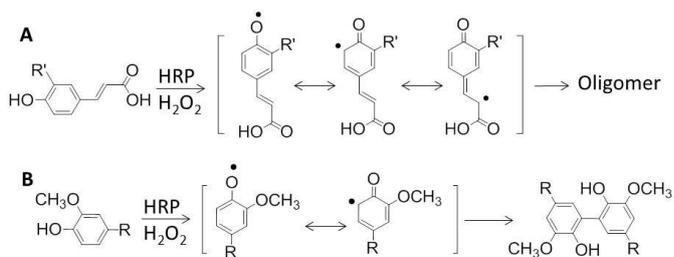
83
 84 Using this surface-wrinkling system, a dehydration-condensation reaction was observed
 85 between CS and the PHs during the immersion process (Izawa, Okuda, Ifuku, Morimoto,
 86 Saimoto & Rojas, 2015). Therefore, we hypothesized that the covalently bound PHs on the
 87 CS film acted as reaction sites for chemical and/or ionic crosslinking via the HRP-catalyzed
 88 oligomerization of the precursor molecules to yield a skin layer. However, the role of
 89 covalently bound PH in skin layer formation has not yet been proved due to the difficulty of
 90 analyzing insoluble skin layers. In addition, the detailed crosslinking mode between CS and
 91 phenolic oligomers has not been fully clarified.

92 In common wrinkled surfaces, the wavelength (λ) of the wrinkle is dependent on skin
93 thickness (d), and the mechanical properties of the film are described as follows (Chung,
94 Nolte & Stafford, 2011; Genzer & Groenewold, 2006):

$$95 \quad \lambda = 2\pi d \left(\frac{\bar{E}_s}{3\bar{E}_f} \right)^{\frac{1}{3}}, \quad (1)$$

96 where the subscripts s and f refer to the skin layer and the foundation (substrate), respectively;
97 \bar{E} is the plane-strain modulus given by $E/(1-\nu^2)$, where E is the elastic modulus, and ν is the
98 Poisson's ratio. Thus, the skin thickness is important information for understanding surface
99 wrinkling. However, the skin thickness has not been fully investigated because it is not
100 distinguishable due to its very small thickness, and also because of the similar electron density.

101 Here, we investigate the unknown characteristics of wood-mimetic skin. To clarify the
102 detailed structure of the skin layers, we use surface wrinkling induced by ferulic acid (FE),
103 vanillic acid (VA), and homovanillic acid (HO), which are PHs having different substituents
104 (R), although always including a carboxyl group (Fig. 1). The previously used HRP-catalyzed
105 reaction of FE generates a phenoxide radical that can resonate with the 5'-position or the β -
106 position (Fig. 2A) (Oudgenoeg et al., 2002). The coupling reaction of these radicals provides
107 oligomers (Izawa, Miyazaki, Ifuku, Morimoto & Saimoto, 2016). However, the HRP-
108 catalyzed reactions of VA and HO provide only dimers (Fig. 2B) (Ci & Wang, 1991; Foppoli,
109 Coccia, Blarzino & Rosei, 2000; Tai, Sawano & Ito, 2012). In addition, the carboxyl groups
110 in FE and VA are conjugated to styryl and phenyl groups, respectively, while that in HO is
111 not conjugated. These differences in the HRP-catalyzed reactions and reactivity of the
112 carboxyl groups provide important information regarding wood-mimetic skin. In addition, we
113 conducted scanning electron microscopic (SEM) and Time-of-Flight secondary ion mass
114 spectrometry (TOF-SIMS) analysis of the wrinkled surface to estimate the skin thickness and
115 structure.



116

117 **Fig. 2.** Previously reported HRP-catalyzed reactions of FE (A) and VA or HO (B).

118

119 2. Experiments

120 2.1. Materials

121 CS (M_n : 5.6×10^4 ; M_w/M_n : 2.36; GPC analysis with Pullulan standards) was supplied
 122 by the Koyo Chemical Co., Ltd. (Tottori, Japan), with an undeacetylated 23.5% fraction of CS
 123 (elemental analysis). FE, HO, and VA were purchased from the Tokyo Chemical Industry Co.,
 124 Ltd. (Tokyo, Japan). HRP (274 U/mg) was purchased from Toyobo Co., Ltd. (Osaka, Japan).
 125 Other reagents were commercial grade and used without further purification.

126

127 2.2. Instrumentation

128 SEM images of film surfaces were recorded by a TM303Plus (Hitachi, Japan) without
 129 coating. SEM cross-sectional images were recorded with a JSM-6700F (JEOL, Japan). The
 130 sample was coated with an approximately 5 nm layer of Pt with an ion sputter coater. The
 131 wrinkle amplitudes of the wrinkled films were obtained with a NanoCute-NanoNavi IIs
 132 (Seiko Instruments, Japan). Elemental analysis data were recorded on a Perkin Elmer 2400 II
 133 CHNS/O (Perkin Elmer, US). Infrared (IR) spectra of the samples were recorded by a
 134 Spectrum 65 (Perkin-Elmer Japan Co., Ltd., Japan) equipped with an ATR attachment. TOF-
 135 SIMS measurement was performed with a PHI TRIFT V nanoTOF (ULVAC-PHI, Japan).
 136 The pulsed primary ion source was Bi_3^{2+} , and the ion beam was operated at 30 kV (50 fA AC)
 137 with a $50 \mu\text{m} \times 50 \mu\text{m}$ rastering area at an incident angle of 45° . The sputtering was done with

138 an Ar⁺ ion beam operated at 300 V and 150 nA with a 0.1 mm x 0.1 mm rastering area at an
139 incident angle of 45°.

140

141 **2.3. Preparation of the CS film**

142 CS (2.0 g) was dissolved in 100 mL of an acidic aqueous solution containing 0.5 mL
143 acetic acid. Then, 10 mL of the CS solution was added to a Teflon Petri dish ($\phi=50$ mm) and
144 degassed under reduced pressure. The CS solution was heated at 50°C for 24 h to yield a CS
145 film after evaporation. The film was then heated at 50°C under reduced pressure for 12 h. The
146 inhomogeneous edge of the film was cut down with scissors. The weight and thickness of the
147 CS film were ca 0.15 g and 111 ± 12 μm , respectively.

148

149 **2.4. Surface wrinkling of films**

150 In a typical experiment, a CS film was immersed in 20 mL methanol containing 0.05
151 g/mL FE at 30°C for 24 h. The resulting film (hereafter, FE/CS film) was removed and soaked
152 in 10 mL water, followed by the prompt addition of the HRP (1 mL, 137 U) and H₂O₂ (200
153 μL , 30% concentration). The system was kept at 30°C for 12 h, after which the film was
154 removed and dried at 40°C under for 12 h.

155

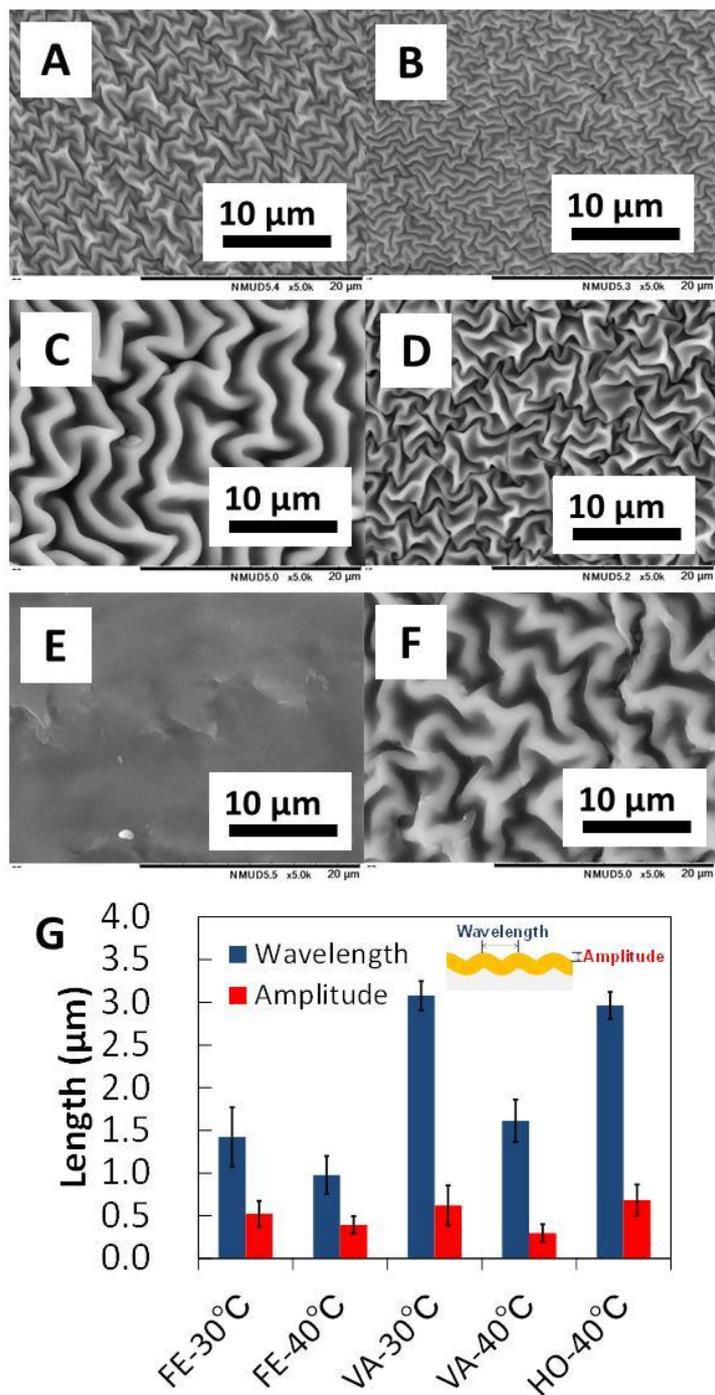
156 **3. Results and Discussion**

157 **3.1 Morphology of the obtained films**

158 Figures 3A-F show plane-view SEM images of the surface of the wrinkled films. A
159 detailed characterization of the wrinkles formed is provided in Fig. 3G. In the case of FE, the
160 results were almost the same as that in our previous report (Izawa, Okuda, Ifuku, Morimoto,
161 Saimoto & Rojas, 2015), in which the mean wrinkle wavelengths and amplitudes under
162 immersion treatments at 30°C and 40°C were 1.42 ± 0.34 and 0.97 ± 0.22 μm , respectively, and
163 0.52 ± 0.15 and 0.39 ± 0.10 μm , respectively. The mean wrinkle wavelengths and amplitudes at

164 40°C immersion were smaller than those at 30°C. In this wrinkling system, higher
165 temperatures on the immersion process led to the formation of softer skins, due to the harder
166 decomposition of the CS around the film surface, inducing smaller wrinkles (Izawa et al.,
167 2016). Note that wrinkled surfaces were not observed on the FE/CS films, the control CS film
168 prepared by the adsorption of oligomeric FE, and the original CS film (Figures S1A-B, S1C,
169 and S1D, respectively). For VA, surprisingly, wrinkling occurred during both the 30°C and
170 40°C immersion treatments, even though the HRP-catalyzed reaction of VA only provided the
171 dimer as described above. The mean wrinkle wavelengths and amplitudes at 30°C and 40°C
172 were 3.07 ± 0.17 and 1.61 ± 0.25 μm , respectively, and 0.62 ± 0.24 and 0.30 ± 0.10 μm ,
173 respectively, which were larger than those of the FE/CS system. This result suggests that VA
174 produces harder skin, even though the HRP-catalyzed reaction of VA provides just the dimer.
175 When HO was used, wrinkling was observed on the wrinkle-HO/CS film at an immersion
176 temperature of 40°C. The mean wrinkle wavelength and amplitude under treatments at 40°C
177 were 2.96 ± 0.16 μm and 0.68 ± 0.18 μm , respectively, which were larger than those for the
178 VA/CS system at 40°C. No wrinkling occurred at 30°C. The same phenomenon was observed
179 in the CA/CS system. There was the suggestion that no wrinkling is due to a lack of
180 crosslinking reaction sites on the CA/CS film (Izawa, Okuda, Ifuku, Morimoto, Saimoto &
181 Rojas, 2015). The results obtained with VA and HO clearly indicated that the vinyl moiety in
182 FE is not needed to induce surface wrinkling. Note that we additionally confirmed the absence
183 of wrinkling by using 2-methoxyphenol, without a carboxyl group, for the treatment at 30°C
184 and 40°C.

185



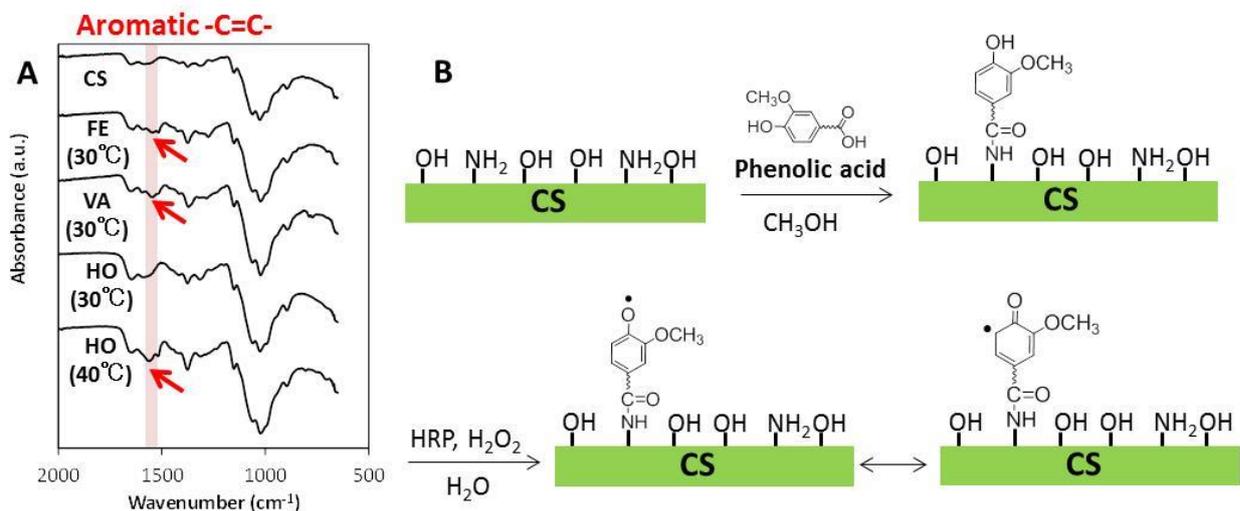
186

187 **Fig. 3.** Plane-view SEM images of the films obtained via immersion treatment at 30°C (A) or
 188 40°C (B) using FE, via immersion treatment at 30°C (C) or 40°C (D) using VA, and via
 189 immersion treatment at 30°C (E) or 40°C (F) using HO and their mean wavelength and
 190 amplitude of wrinkles (G).

191

192 **3.2 Characterization of the film surfaces**

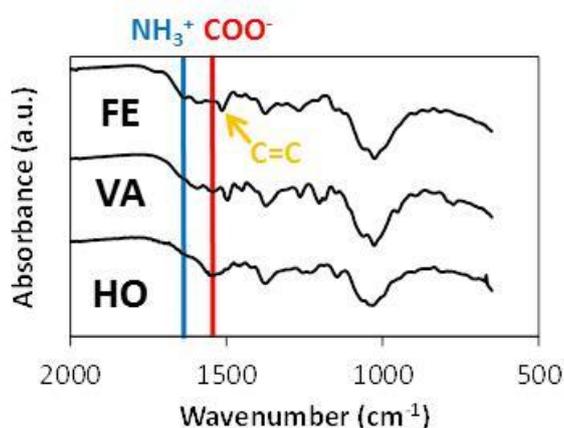
193 To confirm the presence of covalently bound PHs on the CS film, the PH/CS films were
 194 Soxhlet-extracted with methanol for 1 week in order to remove any unreacted PH, and the IR
 195 spectra of the film surfaces were measured (Fig. 4A). In the 30°C treatment, the absorption
 196 peaks attributed to aromatic -C=C- (Swislocka, Kowczyk-Sadowy, Kalinowska &
 197 Lewandowski, 2012) appeared at around 1540 cm⁻¹ for the FE/CS and VA/CS systems. The
 198 absorption peaks observed even after Soxhlet-extraction indicated the presence of the
 199 covalently bound PH via an amide bond on the CS film. In contrast, this was not seen in the
 200 HO/CS system at this temperature. We considered that the lack of a peak observed on the
 201 HO/CS system was due to the lower reactivity of unconjugated carboxyl groups than in FE
 202 and VA. On the other hand, the absorption peak due to aromatic -C=C- was observed in the
 203 HO/CS system in the 40°C treatment. These observations are consistent with the wrinkling
 204 results. Thus, this result confirmed our previous speculation that the carboxyl group in PH
 205 plays a critical role in the formation of covalently bound PH, acting as a reaction site for the
 206 HRP-catalyzed reaction to form the skin layer (Fig. 4B).



207
 208 **Fig. 4.** IR spectra of the extracted FE/CS, VA/CS, HO/CS films and CS (A), and illustration
 209 of the confirmed role of covalently bound PH (B).

210

211 In order to analyze the chemical structure of the skin, we performed IR analysis of the
212 wrinkle-FE/CS, VA/CS, and HO/CS films from the 40°C treatment (Fig. 5). In the wrinkle-
213 FE/CS film spectrum, the absorption peaks attributed to -COO^- and -NH_3^+ (Hu, Jiang, Ding,
214 Ge, Yuan & Yang, 2002) were observed at 1565 cm^{-1} and 1630 cm^{-1} , respectively. Meanwhile,
215 absorption peaks due to aromatic alkene and glycosidic ether were observed as significant
216 ones at 1511 cm^{-1} and 1024 cm^{-1} , respectively, indicating the skin layer was composed of both
217 CS and oligomeric FE. By using VA, the absorption peak due to -COO^- was slightly enhanced
218 compared to that of the wrinkle-FE/CS film. Interestingly, using HO further enhanced the
219 absorption peak attributed to -COO^- , to the point where the absorption peak attributed to the
220 aromatic alkene was completely overlapped by it. These results suggested a higher quantity of
221 the ionic bonds in the case of VA and HO than in FE. Indeed, the HRP-catalyzed reaction of
222 FE involves decarboxylation that reduces the quantity of the carboxyl group in the system
223 (Oudgenoeg et al., 2002). In addition, it was suggested that there was a higher quantity of the
224 ionic bonds in the HO/CS system than in the VA/CS system. As described above, the
225 products from the HRP-catalyzed reaction of VA and HO are dimers with a biphenyl
226 framework (Fig. 2B). The only structural difference between those dimers is whether the
227 methylene spacer is present or not. The carboxyl group in HO has higher mobility than that of
228 the VA by virtue of the methylene spacer. We consider that the higher mobility facilitates
229 ionic bonding in the HO/CS system.



230

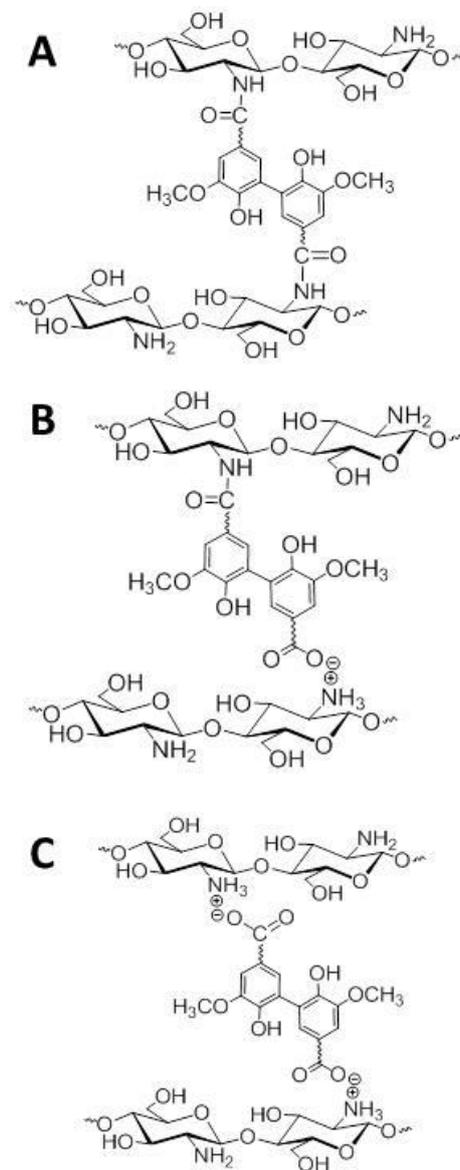
231 **Fig. 5.** IR spectra of surfaces of the wrinkle-FE/CS, VA/CS, and HO/CS films.

232

233 There are three possible crosslinking modes in the VA/CS and HO/CS systems. The
234 first is the covalently crosslinked structure generated by the radical coupling of each of the
235 covalently bound PHs (Fig. 6A). The second is the ionically crosslinked structure between a
236 dimeric side chain, generated by radical coupling of PH and the covalently bound PH, and
237 CS (Fig. 6B). The third and final possible modes are the ionically crosslinked structure
238 composed of CS and dimeric PH (Fig. 6C). When covalently bound HO was not formed,
239 surface wrinkling did not occur, as mentioned above. Therefore, this third crosslinking mode
240 is not important for skin layer formation. Indeed, the top layers fabricated by the adsorption
241 of oligomers on the CS film could not induce surface wrinkling (Fig. S1C). Elemental
242 analysis of the VA/CS and HO/CS films and the extracted VA/CS and HO/CS films
243 provided evidence that the VA/CS and HO/CS films included ca 50-fold greater amounts of
244 VA and HO than covalently bound VA and HO, respectively. Under this condition, the
245 radical coupling of PH and the covalently bound PH proceeds more readily than that
246 between the covalently bound PHs themselves. In addition, the HRP-catalyzed reaction of
247 the extracted VA/CS and HO/CS films did not provide wrinkled surfaces upon drying. Thus,
248 the second crosslinking mode is the most likely crosslinking structure for the VA/CS and
249 HO/CS systems. This is important information which suggests that ionic crosslinking by the
250 dimeric/oligomeric side chain is capable of surface wrinkling upon drying. In addition, we
251 observed a crucial phenomenon that underscored the importance of the ionic bonding for the
252 skin formation. Namely, the wrinkles were maintained in water even after 1 week, while
253 they disappeared in 100 mM NaOH aqueous solution due to the dissociation of the ionic
254 bond (Fig. S2), indicating that the wood-mimetic skins were formed by ionic crosslinking.

255 Our theoretical calculations (see Table S1) show that the ionic bonds between
256 glucosamine and each of the PHs (FE, VA, HO) have the similar strength. Thus, the

257 production of larger wrinkles in the VA/CS and HO/CS systems could be explained by the
258 quantity of the ionic bonds. The order of the speculated quantity of the ionic bonds by IR
259 analysis was as follows: the HO/CS system > VA/CS system > FE/CS system. The wrinkle
260 wavelengths and amplitudes decreased in the following order: the HO/CS system > the
261 VA/CS > the FE/CS. This relation indicates that a higher quantity of the ionic bonds results
262 in a harder skin layer, leading to larger wrinkling (Izawa et al., 2016).



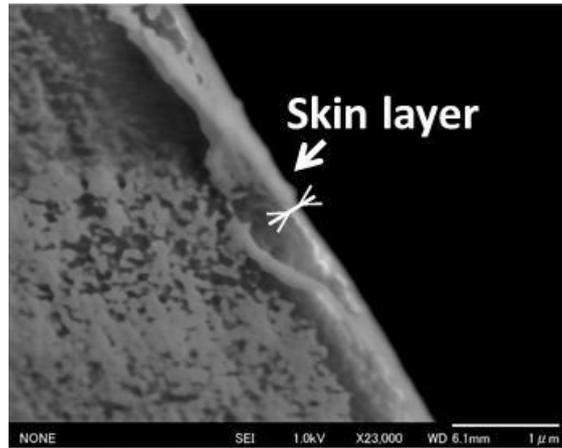
263

264 **Fig. 6.** Possible crosslinking structure in the VA/CS and HO/CS systems.

265

266 **3.3 SEM and TOF-SIMS analysis for the skin layer**

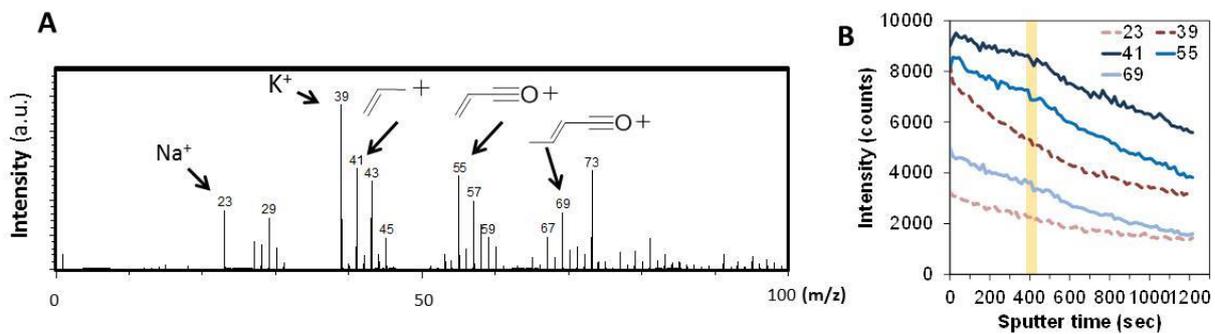
267 Figure 7 shows an SEM image of the cross-section of the wrinkle-FE/CS film. The
268 topmost layer, which is considered to be the skin layer, is shown. This topmost layer was
269 approximately 120 nm thickness. Fig. 8A shows TOF-SIMS spectra of positively charged
270 secondary ions produced from the wrinkled surface. Characteristic fragment ions for the α,β -
271 unsaturated carboxylic acid groups of oligomeric FE moieties in the skin layer were observed
272 at m/z 41 ($C_3H_5^+$), 55 ($C_3H_3O^+$), and 69 ($C_4H_5O^+$) (Lawrence, Tripathi & Jeyakumar, 2009;
273 Pati, Crupi, Benucci, Antonacci, Di Luccia & Esti, 2014). The depth profiles of the
274 characteristic fragment ions are illustrated in Fig. 8B. The intensities of these peaks were
275 linearly decreased with the increase in the sputter time. The negative slope increased after 420
276 s. The depth profile for impurities (Na^+ and K^+) were completely different from those for the
277 characteristic peaks, which were exponential decay curves. These results imply that the
278 component of the topmost layer changes after 420 s. The depth at 420 s under the sputter
279 condition for SiO_2 was ca 40 nm. The sputter rate for the organic materials was 2-4 times
280 higher than for the SiO_2 (Fearn, 2015). This observation shows good agreement with the
281 observed thickness of the topmost layer. Thus, this result indicates the topmost layer observed
282 in the SEM analysis is the wood-mimetic skin, and the thickness of the skin layer is on the
283 submicron order (<200 nm). Note that the thickness of wood-mimetic skins produced by the
284 HRP-catalyzed reaction does not depend on the choice of PH or the conditions of the
285 immersion process, because the previously reported correlation between the wrinkle sizes and
286 mechanical properties indicated that there was no large difference in the skin thicknesses of
287 the wrinkled films prepared under different immersion conditions (Izawa et al., 2016).



288

289 **Fig. 7.** SEM cross-section image of the wrinkle-FE/CS film. Scale bar is 1 μm .

290



291

292 **Fig. 8.** TOF-SIMS spectrum (A) of the wrinkle-FE/CS film and depth profile of the
 293 characteristic fragment ions (B).

294

295 **4. Conclusion**

296 We have analyzed the chemical and structural characteristics of wood-mimetic skins that
 297 were produced by a horseradish peroxidase (HRP)-catalyzed reaction of ferulic acid (FE),
 298 vanillic acid (VA), and homovanillic acid (HO) on a chitosan (CS) film and that exhibited
 299 surface wrinkling upon drying. When HO was used, covalently bound HO was not observed
 300 on immersion treatment at 30°C. This means that no wrinkling occurred at this temperature. In
 301 contrast, wrinkled surfaces were observed when covalently bound FE, VA, and HO were
 302 formed. Therefore, we determined that the carboxyl group in PH plays a critical role in that
 303 the formation of covalently bound PH acts as a reaction site for the HRP-catalyzed reaction to

304 form the skin layer. In addition, the observation of surface wrinkling using VA and HO
305 revealed that an ionic crosslinking structure composed of CS and dimeric phenolic acid
306 residues on CS enables skin layer formation, and induces surface wrinkling upon drying.
307 Furthermore, SEM and TOF-SIMS analyses indicated that the thickness of the skin layer was
308 on the order of submicrons (<200 nm). This study underscores the importance of ionic
309 crosslinking for skin layer formation. This perspective should result in novel polysaccharide-
310 based wrinkled materials being created for application to various fields.

311

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317

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416 **Figure Captions**

417 **Fig. 1.** Illustration of the wood-inspired surface wrinkling systems used in this study and in
418 previous reports.

419 **Fig. 2.** Previously reported HRP-catalyzed reactions of FE (A) and VA or HO (B).

420 **Fig. 3.** Plane-view SEM images of the films obtained via immersion treatment at 30°C (A) or
421 40°C (B) using FE, via immersion treatment at 30°C (C) or 40°C (D) using VA, and via
422 immersion treatment at 30°C (E) or 40°C (F) using HO and their mean wavelength and
423 amplitude of wrinkles (G).

424 **Fig. 4.** IR spectra of the extracted FE/CS, VA/CS, HO/CS films and CS, and illustration of
425 the confirmed role of covalently bound PH (B).

426 **Fig. 5.** IR spectra of surfaces of the wrinkle-FE/CS, VA/CS, and HO/CS films.

427 **Fig. 6.** Possible crosslinking structure in the VA/CS and HO/CS systems.

428 **Fig. 7.** SEM cross-section image of the wrinkle-FE/CS film. Scale bar is 1 μm .

429 **Fig. 8.** TOF-SIMS spectrum (A) of the wrinkle-FE/CS film and depth profile of the
430 characteristic fragment ions (B).

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