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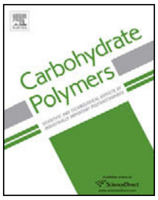

Research Paper

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

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ABSTRACT

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

1. Introduction

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

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

Nano/microscopic wrinkled surfaces inspired by nature-mimetic surface designs have been developed for optical (Ohzono, Suzuki, Yamaguchi, & Fukuda, 2013) and electronic devices (Lee et al., 2013), the realization of tunable wettability (Li, Dai, John, & Carter, 2013) and adhesion (Davis, Martina, Creton, Lindner, & Crosby, 2012), and the synthesis of cell culture scaffolds (Zhao et al., 2014). Basically, a skin layer is fabricated on a soft substrate via dry processing methods, including chemical vapor deposition (Bowden, Brittain, Evans, Hutchinson, & Whitesides, 1998), photo-crosslinking (Chen, Reed, & Yang, 2013), and UV/O_3 oxidation (Efimenko et al., 2005). The wrinkling event can be caused/controlled by mechanical stress (Efimenko et al., 2005), thermal expansion (Bowden et al., 1998), and/or swelling-shrinking (Huraux, Narita, Bresson, Fretigny, & Lequeux, 2012; Rizzieri, Mahadevan, Vaziri, & Donald, 2006; Zhao et al., 2014).

We previously developed a surface-wrinkling system inspired by the fine wrinkles and design principles of wood-cell walls (Fig. 1) (Izawa, Okuda et al., 2016; Izawa, Miyazaki, Ifuku, Morimoto, &

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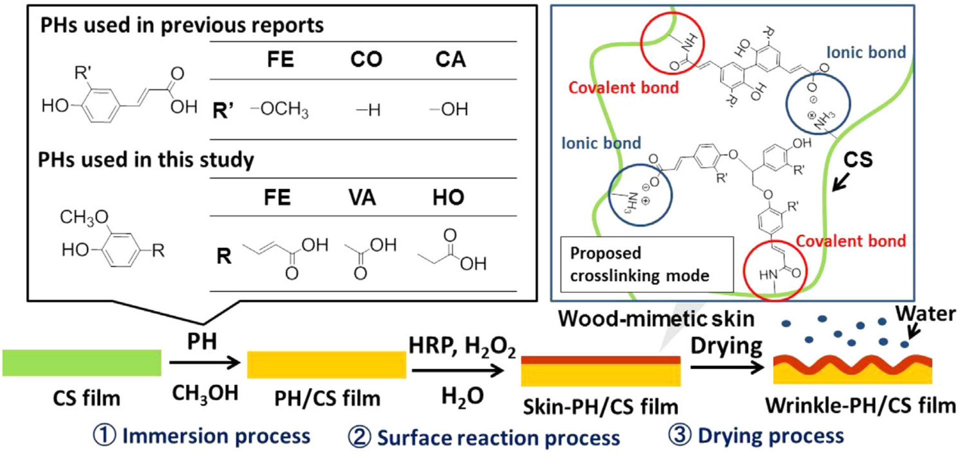

Fig. 1. Illustration of the wood-inspired surface wrinkling systems used in this study and in previous reports.

Saimoto, 2016). In this method, wood-mimetic skins are fabricated by immersing chitosan (CS) film in a phenolic acid (pH)-methanol solution, then treated with horseradish peroxidase (HRP) to catalyze a surface reaction. Finally, surface wrinkling is induced by water evaporation during drying. The wrinkle wavelength and amplitude can be controlled by the choice of a phenolic acid (ferulic acid, FE; *p*-coumaric acid, CO; or caffeic acid, CA) and by varying the temperature of the immersion process. Using this system, we found that the wrinkle size was predominately determined by the hardness of the wood-mimetic skins (Izawa, Miyazaki et al., 2016; Izawa, Okuda et al., 2016). However, the structure of the wood-mimetic skin layer has not been fully elucidated.

Using this surface-wrinkling system, a dehydration-condensation reaction was observed between CS and the pHs during the immersion process (Izawa et al., 2015). Therefore, we hypothesized that the covalently bound pHs on the CS film acted as reaction sites for chemical and/or ionic crosslinking via the HRP-catalyzed oligomerization of the precursor molecules to yield a skin layer. However, the role of covalently bound pH in skin layer formation has not yet been proved due to the difficulty of analyzing insoluble skin layers. In addition, the detailed crosslinking mode between CS and phenolic oligomers has not been fully clarified.

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

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

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

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

2. Experiments

2.1. Materials

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

2.2. Instrumentation

SEM images of film surfaces were recorded by a TM303Plus (Hitachi, Japan) without coating. SEM cross-sectional images were recorded with a JSM-6700F (JEOL, Japan). The sample was coated with an approximately 5 nm layer of Pt with an ion sputter coater. The wrinkle amplitudes of the wrinkled films were obtained with a NanoCute-NanoNavi IIs (Seiko Instruments, Japan). Elemental analysis data were recorded on a Perkin Elmer 2400 II CHNS/O (Perkin Elmer, US). Infrared (IR) spectra of the samples were recorded by a Spectrum 65 (Perkin-Elmer Japan Co., Ltd., Japan) equipped with an ATR attachment. TOF-SIMS measurement was performed with a pH I TRIFT V nanoTOF (ULVAC-pH I, Japan). The pulsed primary ion source was Bi_3^{2+}, and the ion beam was operated at 30 kV (50 fA AC) with a 50 μm × 50 μm rastering area at an incident angle of 45°. The sputtering was done with an Ar^+ ion beam operated at 300 V and 150 nA with a 0.1 mm × 0.1 mm rastering area at an incident angle of 45°.

2.3. Preparation of the CS film

CS (2.0 g) was dissolved in 100 mL of an acidic aqueous solution containing 0.5 mL acetic acid. Then, 10 mL of the CS solution