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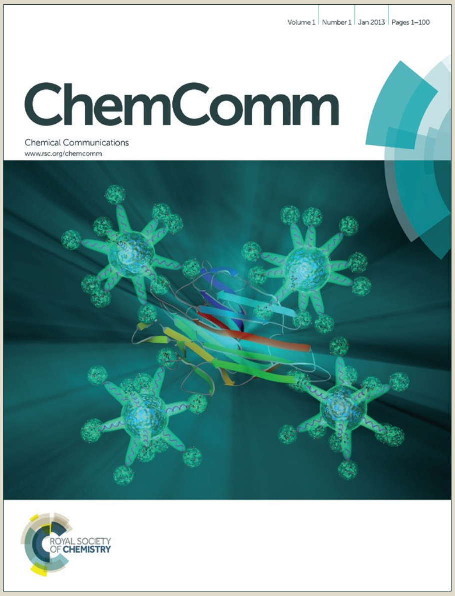

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Hydrophobized Plant Polyphenol: Self-Assembly and Promising Antibacterial, Adhesive, and Anticorrosion Coatings

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Hydrophobized plant polyphenol can be easily prepared by rational and controlled etherification of highly abundant aromatic hydroxyls with linear alkyl chains. The resultant organo-soluble polyphenols spontaneously formed fibrous structures and unravelled to be potential adhesive, anticorrosion, and antibacterial coatings.

Natural materials have long been studied and considered as a source of inspiration for wide range of scientific knowledge and innovations. Therefore, scientists from various disciplines had given a tremendous effort to exploit these natural feedstocks for wide-range practical uses.[1] Among them, water-soluble biopolymers, such as DNA, protein, polysaccharide, and others offer versatile building blocks for various chemical/physical manipulations along with their unique indigenous characteristics. For example, by introducing hydrophobic moieties, water-soluble biopolymers could be coaxed to exhibit exotic phenomena, such as formation of micelle, hydrogel and liquid crystal, leading to novel exploitation of biocompatible medical, biological, and optical applications.[2] On the other hand, ubiquitous plant polyphenols are regarded as another class of important natural raw materials.[3] However, to best of our knowledge, hydrophobized polyphenols have not been reported often, and the detailed properties, such as chemical, biological, and mechanical, remain majorly unexplored. In this context, we specifically focused on, tannic acid (TA), a naturally occurring water-soluble polyphenol dendroid (Fig. 1a). Tannic acid is present in variety of plants and fruits, so that, it is considered as a “generally recognized as safe” (GRAS) food additives. Numerous studies have reported that tannic acid exhibits natural antioxidant, antimicrobial, and antiviral activity.[4] Furthermore, more recently, it has been reported that tannic acid is capable of versatile surface binding to metal, glass, or synthetic polymer substrates and could be further tuned to fascinating structural/functional materials.[5] This is more likely that dihydroxyphenyl (catechol) and trihydroxyphenyl (pyrogallol) moieties have binding ability through chemical and physical interactions, which is similar to the adhesion mechanism of DOPA-enriched adhesive foot protein of blue mussel.[6] Therefore, tannic acid and related polyphenols are being considered as an efficient and universal surface treatment precursors. However, due to its strong hydrophilic nature, most of these studies were limited in aqueous media. For example, the majorly adopted approaches include, (1) layer-by-layer (LBL) assembly/deposition via intramolecular non-covalent interactions with polymers,[7] (2) rapid assembly of metal-phenolic coordination networks on solid surfaces,[5a] and (3) most recently by simple dip-coating under buffered saline condition.[5c] It is deduced that rich contents of H-bonding donating/accepting groups and π-π interactions among aromatic rings of galloyl subunits enable such polyphenols to associate with wide-range of (bio)macromolecules.[8] However, such weak inter-/intramolecular interactions often lead to random aggregation to non-uniform particle structures and consequently produce unstable thin film on solid surfaces (Fig. S8-10).

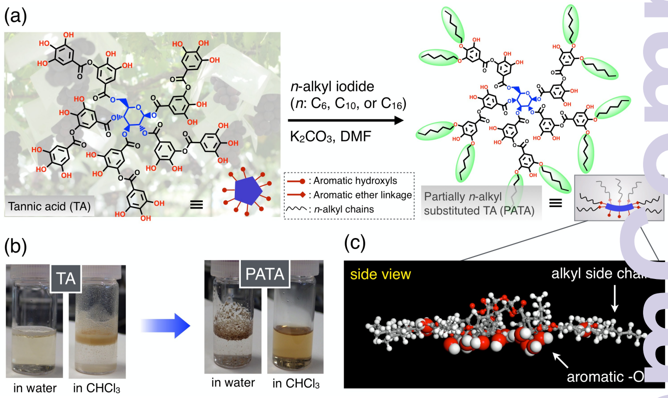

Fig. 1 (a) Chemical structure of tannic acid (TA) with image of a natural source, a bunch of red grapes in the background. Chemical modification method of TA to partially alkyl substituted TA (PATA) with different alkyl side chains. (b) Comparison of solubility between TA and PATA in aqueous/organic media. For more details see fig. S3. (c) Lowest energy structure of *n*-hexyl deca-substituted TA molecule estimated by DFT calculation.

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To overcome these shortcomings, we developed alkyl substituted TA (PATA) by one-pot chemical modification of tannic acid. Through rational and controlled incorporation of suitable *n*-alkyl side chains, PATA exhibited “de novo” organo-solubility toward variety of organic solvents. Furthermore, PATA spontaneously formed self-assembled fibril in common organic solvents, due to its amphiphilic nature. By simple dry casting, the highly entangled fibrous mat was transformed into defect free thin film by thermal treatment. This film showed high adhesion ability toward various structural metals/alloys, leading to excellent anticorrosion against conventional corrosive hazards. Furthermore, the obtained water-insoluble PATA film has been demonstrated as a non-elution type antibacterial coating. Tannic acid extracted from plant parts like gallnuts was purchased from commercial source and converted to partially *n*-alkyl substituted TA (PATA) by etherification using alkyl iodide and potassium carbonate in dimethylformamide (DMF) (Fig. 1a & scheme S1). The as-prepared PATAs were isolated as dark-brown solids and identified by means of ^{1}H NMR and FT-IR spectroscopies (Fig. S1 & S2). Incorporation of alkyl groups to the TA was confirmed by appearance of typical alkyl $–CH_2$ and $–CH_3$ peaks around 1-2 ppm and 3000 cm^{-1} in ^{1}H NMR and FT-IR spectroscopy, respectively. Moreover, intensity of aromatic –OH stretching at 3100-3600 cm^{-1} gradually decrease with the increment of alkyl substitution. This clearly suggests hydrophobic alkyl chains were partially introduced to the aromatic hydroxyl groups of TA by ether linkages. To optimise the number and length of alkyl chain, three types of alkyl group hexyl (C_6), decyl (C_{10}) and hexadecyl (C_{16}) were incorporated to the hydroxyl groups of TA backbone with varying degree of substitution from 5 to 20, which corresponds 20-80 mol% of hydroxyl groups in TA (Table S1). Note that, generally, each TA molecule comprises of 25 reactive aromatic hydroxyl groups contributed from both catechol and pyrogallol subunits. Thermal stability and properties of these newly synthesized PATA derivatives were also examined and compared with pristine TA by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) (Fig. S4-5).

First, to estimate the most stable structure/configuration of PATA, DFT calculations were performed using Materials Studio 7.0. To simplify calculation, PATA incorporated with ten *n*-hexyl chains at the most outer galloyl aromatic rings was used as a model. As a result, optimized structure of PATA adopted disc-like structure, in which hydrophilic TA core was surrounded by hydrophobic alkyl chains. More interestingly, unreacted aromatic hydroxyl groups in the TA core located to the one side, probably reflecting chair conformation (Fig. 1c & S6). Although numerous amphiphilic (bio)molecules/polymers[9] have been demonstrated, such a disc-like amphiphile has rarely been reported; therefore, PATA can be regarded as a novel type of semi-synthesized amphiphilic macromolecule. As expected from this unique amphiphilic structure, PATA exhibited self-assembling ability in organic media, resulting in highly extended fibrous structures. The details will be discussed in the later section.

Next, solubility of PATA in various common solvents was observed by the naked eyes (Fig. 1b & S3). As a control, pristine TA dissolved in water and other polar solvents such as tetrahydrofuran (THF), ethyl acetate and DMF. On the other hand, with increase in conversion ratio of alkyl chain, hydrosolubility of PATA gradually decreased, and eventually turned to hydrophobic when ca. 40 mol% hydroxyl groups of TA were modified with alkyl units. Moreover, solubility trend among different PATAs were highly dependent on number of alkyl units incorporated to the TA backbone and alkyl chain length. Reasonably, longer alkyl units (C_{10} or C_{16}) provide better solubility in common organic solvents with wide range of polarity such as *n*-hexane, chloroform, diethyl ether and others. This hydrophobization of TA allowed us to effortlessly process (ultra)thin-film of PATA on multiple substrates by simple drop-casting. Moreover, film thickness was easily tunable in the submicron range by varying concentration of PATA (Fig. S7). Here, it is noteworthy to mention that TA could not be fabricated to stable thin-film under same casting condition. Indeed, atomic force microscopy (AFM) observation revealed that TA formed non-uniform sphere aggregation with an average diameter of 100-130 nm (Fig. 2a & S9) when a diluted THF solution (1 mg/mL) was employed, probably due to strong H-bonding interaction among hydroxyl and/or ester groups of TA in organic solvents. On the other hand, PATA produced a uniform fibre-like network assembly during drying process (Fig. 2a & S9).

To further clarify the detailed assembly structure, TA and PATA thin films were subjected to both small and wide-angle X-ray diffraction (XRD) measurements. Thin film of pristine TA was used as a control and had no significant diffraction except a broad peak around 2θ = 25°, indicating presence of amorphous carbon. On the other hand, PATAs had two distinct diffraction regions (Fig. 2b-c). One is around 2θ = 1-3° which corresponds to a one-dimensional columnar structure with average diameter of 5-3 nm. Diffraction angle significantly increases with shorter alkyl chain length, which is consistent with decreasing average diameter of PATA molecules. Another distinct diffraction peak was observed around 2θ = 20-21°, which is typical aligned alkyl chains with an average distance of 0.42-0.44 nm (Fig. 2c).[10] Moreover, with increasing chain length from C_6 to C_{16}, width of these peaks became narrower along with elongated *d*-spacing from 0.42 nm (C_6) to 0.44 nm (C_{16}). This phenomenon clearly suggests that PATA with longer alkyl chain tends to form the developed fibrous structures, due to better crystallinity of longer alkyl chains. After thorough consideration of all these outcomes, we surmise that pristine TA molecules randomly aggregate via intra-/intermolecular H-bonding to particle

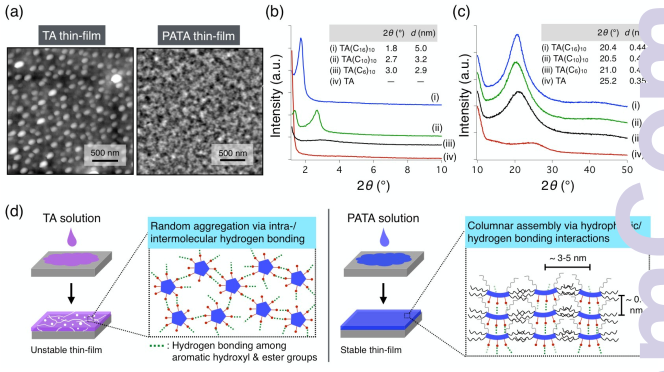

Fig. 2 (a) AFM phase image of as-coated TA and PATA samples on glass surface. (b) and (c) Comparison of XRD and WAXD diffraction pattern among as-coated TA and PATA thin-films, respectively. (d) Schematic illustration of possible molecular assembly of TA and PATA during thin-film formation on solid surfaces.

amorphous morphology but introduction of long alkyl chains promote spontaneous organization to a columnar network assembly with the help of dual intermolecular hydrophobic and H-bonding interactions (Fig. 2d).

From practical viewpoint, hydrophobization of TA tremendously contributed to expand their applications, especially as coating precursors. As we mentioned earlier, TA has been majorly processed to (ultra)thin film under aqueous condition by LBL, dipcoating, or in-situ chemical conversion methods.[5,7] Often, these methods need careful combination of multiple components, optimization of initial concentration, pH, immersion time and/or other parameters to obtain a suitable film for practical purposes. However, PATA affords stable thin film on various metal/alloy substrates of aluminum (Al), copper (Cu), iron (Fe), zinc (Zn), and magnesium (Mg), by simple solvent casting. Surface morphology was directly observed by a scanning electron microscope (SEM) and cross-sectional SEM. Consequently, it was revealed that PATA afforded entirely smooth and defect-free film, and well-adhered on the base material (Fig. S10 & S11). On the other hand, TA showed extended cracks over the film, indicating poor mechanical stability in the film state. Furthermore, several (un)coated corrosion susceptible metal/alloy plates of Al, Cu, Mg, Zn, and Fe were immersed in 3.5 wt.% NaCl solution for specified time as shown in Fig. 3a. In sharp contrast to the uncoated surface, PATA coated surface retained their surface morphology and no corrosion was detected even after long immersion (96-480 h) period.

Furthermore, to quantitatively assess adhesion property of PATA onto the metal surfaces, polarization test was carried out, which usually used to electrochemically evaluates corrosion of metal or alloy. PATAs with different alkyl substitution were coated on magensium alloy (AZ31) substrate, and subjected to the polarization test in NaCl solution after monitoring open circuit potential for 10 min. At first, we investigated effect of degree of alkyl substitution (5, 10, 15, or 20) and chain length (C_6, C_{10}, or C_{16}) on anticorrosion efficiency. One should take into account that, coating acts like a barrier between metal substrate and corrosive ions. Therefore, smooth and lower current density change of any coated substrate than that of uncoated one with voltage gradient implicates better corrosion resistance.[11] As shown in Fig. 4a and 4b, corrosion current density (i_{corr}) of PATA with different decyl (C_{10}) unit substitutions were in the range of 10^{-5}-10^{-9} µA cm^{-2} which is much lower than uncoated or TA coated (control) substrate, ca. [illegible] µA cm^{-2}, both in the cathodic and anodic sweep. Interestingly, deca-substituted compound was most efficient among others, displayed lowest and uniform i_{corr} over a wide range of potential sweep (Fig. 4a-b, blue solid line). This was further confirmed by the visual inspection after electrochemical test (Fig. S15), and imply that deca-substitution gives unique balance between anchoring catechol/pyrogallol and hydrophobic alkyl moieties to inhibit water/ions penetration to the metal surface. Next, we studied effect of alkyl chain length with fixed substitution (deca) and we found that hexadecyl (C_{16}) is best among other groups employed in the present study (Fig. 4c-d, green line). In addition, we examined effect of (non)annealing and longer immersion time before final polarization measurement. Notably, annealing has a significant impact for better efficiency and this coating was stable for long immersion (40 h) as revealed by this method (Fig. S16). (De)attachment of scotch tape (peeling test) also elucidates necessity of annealing and as shown in Fig. S13, as coated film was damaged while thermally treated film was completely unaffected by repeated (de)attachment indicating better film stability.

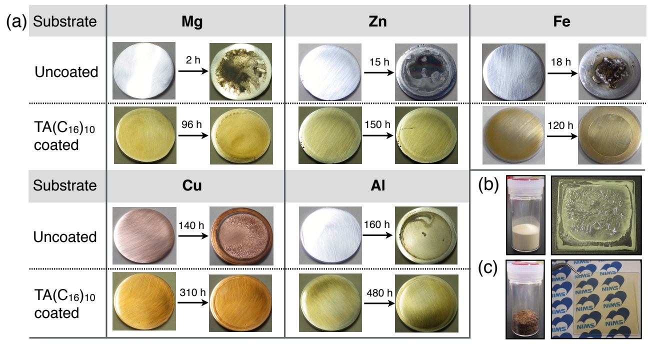

Fig. 3 (a) Photographs of various (un)coated metal/alloy substrates before and after immersion in salt water for specified time. (b) and (c) Photographs of glass plates (5 x 5 cm^2) coated with TA and PATA, respectively. Physical appearance of solid powders of respective samples has also been shown.

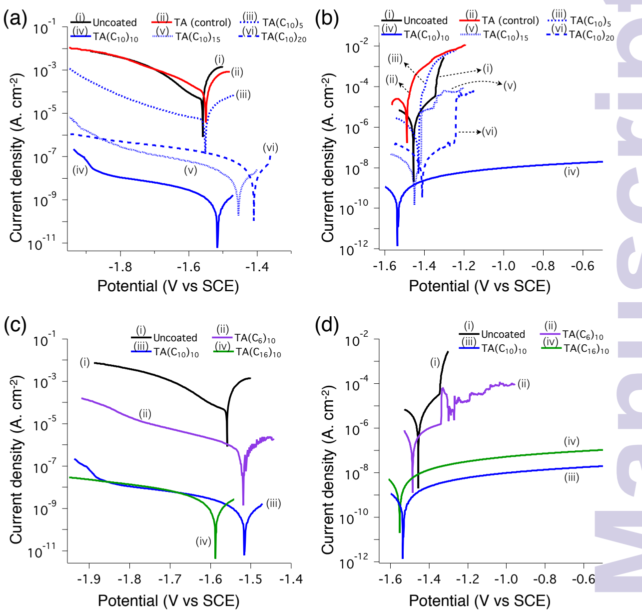

Fig. 4 (a) Cathodic and (b) anodic current comparison among uncoated, TA (control), and PATA with variation of alkyl unit mol% substrates under electrochemical polarization test. (c) Cathodic and (d) anodic current comparison among uncoated and PATA with variation of alkyl chain length under same test. Scan rate was 1 mV/s for all cases.

Remarkable stability of PATA thin-film against water/saline condition further foreshadowed a new direction of utilizing these materials for antibacterial coating purpose. Plant-derived polyphenols are well-known to exihibit high efficiency as antibacterial materials in solution phase.[12] There are several hypotheses on the antibacterial mechanism of such polyphenols such as, inhibition to hydrolytic enzymes, specific interactions to inactive microbial adhesions, binding to cell wall/membrane, metal ion complexation, and also some reports suggested hydrogen peroxide generated by oxidative polyphenols.[13] Here, we hypothesized that partial modification of TA might not effect the inherent antibacterial nature due to the presence of unreacted

(a)

coatings	viable cells (10^5 cfu/mL) *E. Coli*[a]	 *MRSA*[a]	 *S. Aureus*[a]
Control[b]	140.0	1.7	1.1
$TA(C_6)_{10}$[c]	0[d]	0[d]	0[d]
$TA(C_{10})_{10}$[c]	0.003	0[d]	0[d]
$TA(C_{16})_{10}$[c]	0.002	0[d]	0[d]

[a]The initial concentration of bacteria was ~1.1 × 10^5 cfu/mL for all cases. [b]Uncoated glass plate (5 × 5 cm^2). [c]Drop-casted (1.2 mg/cm^2) on glass plates. [d]Less than 1 × 10^{-4} or no colony formation was observed. Immersion time was 24 hours for all cases reported in table (a).

(b)

Conditions Amount drop-casted (mg/cm^2)	 Time of immersion (h)	*MRSA*[a] (10^5 cfu/mL) Control[b]	 $TA(C_6)_{10}$
1.2	1	1.2	0.016
	2	1.2	0[d]
	4	1.4	0[d]
	8	1.2	0[d]
0.8	24	1.3	0[d]
0.4		1.3	0[d]
0.2		1.3	0[d]
0.1		1.3	0[d]

Table 1 (a) Comparison of dynamic contact antibacterial activity of different PATA coated substrates against three types of bacterial strains. (b) Effect of immersion time and amount coated per unit area on antibacterial efficiency against *MRSA*.

aromatic hydroxyls as well as H-bonding acceptors (ester groups) in PATAs. Therefore, we studied dynamic contact based antimicrobial activity of PATA coated glass surfaces against three types of bacterial colony *E. coli*, *MRSA*, and *S. aureus* (See ESI for details). Uncoated glass substrate was used as a control and the immersion time was maintained for 24 h unless mentioned otherwise.

Interestingly, complete killing of all types bacterial colony was observed for PATA coated surfaces, while cell viability remained unchanged or increased for control substrate (Table 1a). To further examine the effect of immersion time and deposited amount per unit area, we studied $TA(C_6)_{10}$ coated substrates in MRSA solution with varying immersion time (1-8 h) and coated amount (0.1-0.8 mg/cm^2). To our surprise, only 2 h immersion time was enough for complete killing of MRSA bacterial colony and very small amount (0.1 mg/cm^2) was sufficient for high efficiency (Table 1b). To the best of our knowledge, methods such as self-assembled monolayer or grafting poly(ethylene glycol) based polymers, zwitterionic polymers, glycomimetic (macro)molecules, *etc*[14] have been widely investigated for antibacterial coating purposes but utilization of inexpensive plant polyphenols as bulk or additive materials for coating on solid surfaces have rarely been explored. Although, a comparative study among TA and PATAs would provide more insight about bactericidal efficiency but poor thin-film stability of TA on solid surfaces under aqueous condition preclude proper evaluation by contact based bacteria killing. However, high potency of PATAs in terms of destroyed cell counts and rapid killing (< 2 h) opens a new direction to utilize these materials for wide-range applications especially in marine, medical, or military sectors.

In summary, we report a facile chemical modification approach to utilize renewable polyphenols for multipurpose surface coatings. Although, numerous properties of such natural precursors have been studied for long time in aqueous media, but method to improve organosolubility and/or properties in non-aqueous or co-solvent systems have rarely been carried out. Present study reveals that incorporation of suitable side chain to the polyphenol backbone significantly enhance solubility in organic media and enable stable thin-film by rapid self-organization on solid surfaces with minimum optimization of time, cost, or other parameters. Furthermore, thin-film of modified polyphenols on solid surfaces (metal, glass) were found to have good barrier ability against corrosive media and contact based bacteria killing. Further, we speculate that present approach could lead to utilize low-cost readily available plant polyphenols in organic solvent mediated processes, (co)assembly in (hetero)solvent systems/interfaces and produce bulk or additive functional materials for novel applications beyond the ones we described here.

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