

Material Independent Surface Chemistry for Gold Shell Formation Inspired by Plant Flavonoids

Hyukjin Lee¹, Jihyun Yum², Sun Ki Hong², Haeshin Lee², Daniel G. Anderson¹, and Robert Langer^{1*}.

¹David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139.

²Department of Chemistry, KAIST, KOREA.

Statement of Purpose: Herein, we report a material-independent surface functionalization strategy using the plant flavonoids composed of non-ketone polyhydroxy polyphenol compounds. Surface property of bulk material plays pivotal roles in determining the performance of advanced functional materials in modern chemical, biological science, and material engineering.¹⁻² Although common surface modification methods, such as self-assembled monolayer (SAM) formation, organosilane chemistry, and Langmuir-Blodgett deposition, have been widely implemented for the preparation of functionally active surfaces, there are several limitations requiring a specific interaction between interfacial modifiers and bare surfaces, use of volatile organic solvents, and repeated complex instrumentation procedures.^{3,4} Inspired by the intrinsic adhesive properties of plant flavonoids, we have screened various polyphenol derivatives to find a versatile interfacial modifier for material-independent surface modification in aqueous environment. This interfacial modifier can provide a ultra-thin coating on any type of substrates and allows a secondary modification such as the growth of gold layer on the surface. We believe that this study can provide a valuable platform for in vivo imaging, photothermal therapy, and drug delivery.

Methods:

Plant polyphenol coatings: Various polyphenols (2 mg/ml) were dissolved in 10 mM Tris-HCL (pH 8.5) for surface coating. Hydrophobic polyphenols such as coumaric acid (CA) and quercetin (QN) were dissolved instead in 10 mM Tris-HCL (pH 8.5) with 10 % acetonitrile (ACN). Solid substrates were cleaned in acetone or ethanol with sonication prior to use. Polyphenol coating was performed by immersing substrates in the buffer solution at room temperature. After 12 h of incubation, the coated surfaces were rinsed extensively with ultrapure water and dried under a stream of nitrogen for further experiments.

Template independent gold seed growth/gold shell formation on PA coated micro/nano-particles: Poly(lactic-co-glycolic acid) (PLGA) microparticles were prepared as described previously.⁵ Poly(styrene) (PS) nanoparticles were purchased from Polysciences (Polysciences Inc., United States). Silica nanoparticles were synthesized using the conventional Stöber method.⁶ For polyphenol coating, all micro/nano-particles were incubated with 2 mg/ml of PA buffer solution (Tris-HCL, pH 8.5) for 4 h. After PA coating, particles were collected by centrifugation and washed three times with ultrapure water. For growth of Au seeds, PA coated nano/micro-particles were re-dispersed in water and particle solution was added to gold salt precursor solution (hydrogen tetrachloroaurate

trihydrate, 0.5 mM) at pH 4.1 for 2h. For gold shell formation, PA coated particles with Au seed on the surface were further reduced using 1.25 mM of gold salt solution with 20 ul of formaldehyde for 5 min.

Characterizations: Static water contact angle was measured using a Phoenix 300 goniometer (Surface Electro Optics Co., Ltd., Korea). Spectroscopic ellipsometry (Gaertner Scientific Co., IL) was used to determine the film thickness with a HE-Ne laser (632.8 nm) at a 70° angle of incidence. XPS spectra were obtained to measure surface atomic composition using an Omicron ESCALAB (Omicron, Taunusstein, Germany) with a monochromated Al K α (1486.8 eV) 300 W X-ray source with an ultrahigh vacuum ($< 10^{-9}$ Torr). The takeoff angle was fixed at 45° except as otherwise mentioned, and all spectra were calibrated using the hydrocarbon C1s peak (284.5 eV). AFM analysis was performed in a non-contact mode on Nanoscope IIIa multimode scanning probe microscope (Veeco, United States) with a tapping mode etched silicon probe (TESP). SEM images were obtained using FE-SEM for all micro-particles (S-4800, Hitachi, Japan). TEM images of nanoparticles were taken using a Zeiss Omega 912 transmission electron microscope (Carl Zeiss, Germany). UV-VIS absorbance of nano-particles was measure using a UV-VIS spectrophotometer (Agilent HP8453, United States).

Results: Various polyphenol derivatives were dissolved in either buffer solution (10 mM tris, pH 8.5) to test their ability for generating a thin polymer film onto inorganic and organic substrates such as noble metals (Au), metal oxides (SiO₂, TiO₂, Al₂O₃, and indium-tine oxide (ITO), ceramics (glass), and synthetic polymers (polycarbonate (PC), polyurethane (PU), cyclic olefin copolymer (COC), and poly(tetrafluoroethylene) (PTFE)). Simple immersion of substrates in a diluted solution of plant polyphenol derivatives resulted in spontaneous deposition of polyphenol compounds onto the surfaces. Analysis by static water contact angles of polyphenol coated surfaces indicated that pyrogallol amine (PA) exhibited a material-independent surface modification, resulting contact angles of 40° for most of substrates even with vastly different initial wetting behaviors (Fig. 1a). Unlike other plant polyphenols such as coumaric acid (CA), quercetin (QN), and tannic acid (TA) that produced moderate surface coatings on selective metal oxide and ceramic substrates, PA provided spontaneous deposition of a thin adherent polymer film on any bulk material surfaces. Ellipsometry analysis on metal oxide substrates confirmed that the thickness of polymer film was a function of immersion time and the maximum thickness up to 25 nm after 24 h

(Fig. 1b). XPS data also confirmed that PA allows versatile coatings on the tested substrates (Fig. 1c).

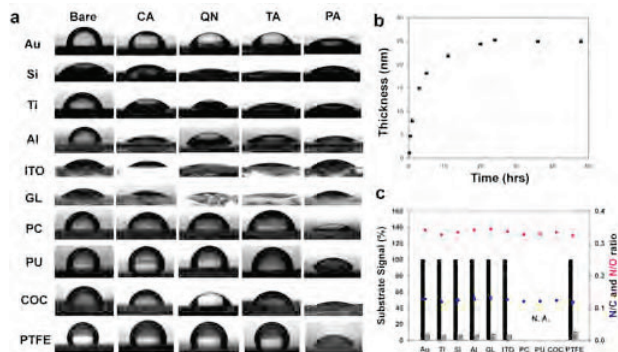


Figure 1. (a) Pictures of water droplets on several bare substrates and plant polyphenol coated substrates. Substrates investigated include noble metal (Au), metal oxides (SiO_2 , TiO_2 , Al_2O_3 , indium-tin oxide(ITO)), ceramics (glass (GL)), and synthetic polymers (polycarbonate (PC), polyurethane (PU), cyclic olefin copolymer (COC), and poly(tetrafluoroethylene) (PTFE)). Polyphenols in this study include coumaric acid (CA), quercetin (QN), tannic acid (TA), and pyrogallolamine (PA). (b) Time dependent thickness deposition of pyrogallolamine (PA) coating on SiO_2 as measured by ellipsometry analysis. (c) XPS characterization of 10 different pyrogallolamine (PA) coated surfaces. Polymer substrates with no characteristic peak are marked by 'N.A.'. The blue and red circles represent the N/C and N/O ratio after pyrogallolamine (PA) coating

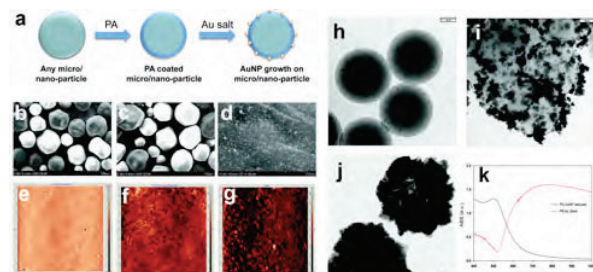


Figure 2. (a) Schematic illustration of gold nanoparticle (AuNP) growth onto any micro/nano-particles coated with pyrogallolamine (PA). SEM images of (b) bare poly(lactic-co-glycolic acid) (PLGA) microparticles, (c) PA coated PLGA microparticles, and (d) PA coated PLGA microparticles with AuNP growth on the surface. AFM images of three different microparticle surfaces, (e) bare PLGA, (f) PA coated PLGA, and (g) PA coated PLGA with AuNP growth. TEM images of polystyrene (PS) nanoparticles, (h) PA coated PS with AuNP growth, (i) reduction of gold seeds for shell formation, and (j) gold nanoshell prepared from PA coated PS nanoparticle. (k) UV-VIS spectra of PS nanoparticles with AuNP growth and PS core/gold shell nanoparticles.

In addition, the metal binding and reducing ability of pyrogallols present in the PA coating was exploited. This reduction potential was demonstrated through formation of gold seeds onto the PA coated surface via dip-coating of PLGA microparticles into gold salt solutions (Fig. 2a). Gold seed growth was confirmed by scanning electron microscopy (SEM) with energy-dispersive x-ray spectroscopy (EDX) and AFM analysis, which demonstrated successful gold seeds growth on material and size independent particles (PS, silica, and aluminum oxide) (Fig. 2b). Gold seeds uniformly distributed on any material surfaces, especially nanoparticles, suggested a

unique opportunity for gold shell formation on nanoparticle made out of any materials. In particular, photothermal effects of gold nanostructures activated by near-infrared (NIR) via the surface plasmon resonance (SPR) effect can be applied in many of biomedical applications including cancer treatment.⁷⁻⁹ Gold shell formation was achieved using PA coated PS nanoparticles functionalized with 3 nm size gold seeds (Fig. 5h-j). Upon the reduction of gold salt, seed growth and spreading was observed throughout the nanoparticles resulting a color change of solution from dark red to blue. The plasmon-based absorption band was distinctly observed after gold metal layer completely covered the PS nanoparticles as shown in figure 5 k, leading us to conclude that the PS core/Au shell nanoparticles can be implemented for photothermal therapy.

Conclusions: In conclusion, we have proposed a new facile approach for material independent surface modification in which inspired by the plant flavonoid; pyrogallolamine (PA). PA produced an adherent self-polymerized coating on a wide variety of materials. The versatility of the PA coating has been demonstrated as a secondary platform for surface mediated reactions, alteration of wetting behavior of PDMS, and leading ultimately to metal adsorption and reducing capability that can be applied to grow various metal seeds and shells on virtually any material substrates. Finally, our method of surface modification offers a facile and versatile platform for chemical, biological science, and material engineering.

References:

1. Ratner, B. D. & Haffman A. S. *Biomaterials Science: An introduction to Materials in Medicine* (Elsevier Academic, San Diego, CA, ed. 2, 2004).
2. Langer, R. Perspectives: Drug delivery – drugs on target. *Science* 293, 58-59 (2001).
3. Love, J. C., Estroff, L. A., Kriebel, J. K., Nuzzo, R. G. & Whitesides, G. M. Self-assembled monolayers of thiolates on metals as a form of nanotechnology. *Chem. Rev.* 105, 1103-1170 (2005).
4. Whaley, S. R., English D. S., Evelyn, L. H., Paul F. B. & Angela M. B. Selection of peptide with semiconductor binding specificity for directed nanocrystal assembly. *Nature* 405, 665-668 (2000).
5. Chung, H. J., Kim, I. K., Kim, T. G. & Park, T. G. Highly open porous biodegradable microcarriers: in vitro cultivation of chondrocytes for injectable delivery. *Tissue engineering Part A* 14, 607-615 (2008).
6. Stöber, W., Fink, A. & Bohn, E. Controlled growth of monodisperse silica spheres in the micron size range *J. Colloid Interface Sci.* 26, 62-69 (1968).

7. 23. Peer, D., Karp, J. M., Hong, S., Farokhzad, O. C., Margalit, R. & Langer, R. Nanocarriers as an emerging platform for cancer therapy. *Nature Nanotechnology* **2**, 751-760 (2007).

8. Yang, J., Lee, J., Kang, J., Oh, S. J., Ko, H., Son, J., Lee, K., Suh, J., Huh, Y. & Haam, S. Smart drug-loaded polymer gold nanoshells for systemic and localized therapy of human epithelial cancer. *Adv. Mater.* **21**, 4339-4342 (2009).

9. Loo, C., Lowery, A., Halas, N., West, J. & Drezek, R. Immunotargeted nanoshells for integrated cancer imaging and therapy. *Nano Lett.* **5**, 709-711 (2005).