Polymer Chemistry

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/polymers

Journal Name

RSCPublishing

COMMUNICATION

Facile and material-independent fabrication of poly(luteolin) coatings and their unimpaired antibacterial activity against *Staphylococcus aureus* after steam sterilization treatments

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Jingya Cheng,[a,†] Miao Guan,[a,†] Jialiang Zhu,[b] Changtao Wang,[c] Lei Su,[a,*] and Xueji Zhang[a]

Poly(luteolin) coatings on different surfaces are fabricated via a facile one-step autoxidation of luteolin in alkaline solution. They exhibit unimpaired antibacterial activities against *Staphylococcus aureus* even after high temperature steam sterilization treatments, which provides extra advantages for them for practical applications, e.g., in the field of implant materials and devices.

Introduction

Bacterial infections are of great concern in a variety of areas, such as medical devices, healthcare products, food packaging, household sanitation, etc. Infections can progress rapidly as free-swimming (planktonic) bacteria first adhere to a surface and ultimately evolve into a biofilm.[1] Therefore, there is a keen interest in developing antibacterial coatings or surfaces. Recently, antibacterial polymer coatings that effectively kill microbes on contact without releasing a biocide have attracted increasing research interest. They can potentially eliminate the health and environmental concern about the agents currently leached from antibacterial products,[2] representing a modern method for producing permanently sterile surfaces.[3] Polymeric biocides and biocidal polymers, such as quarternized polyethylenimine and quarternized chitosan, have been attached to various materials, including glass, stainless steel, cellulose, and plastics, to produce antibacterial coatings.[4-5] Flavonoids belong to an important group of naturally occurring phenol derivatives. Chemically they are C_6-C_3-C_6 compounds in which the two C_6 groups are substituted benzene rings, and the C_3 is an aliphatic chain which contains a pyran ring.[6] Luteolin (3',4',5,7-tetrahydroxyflavone, see Fig. 1A) belongs to the flavones subclass of natural occurring flavonoids.[7] Recent studies have revealed that luteolin possesses various biological effects, such as antioxidant, anti-inflammatory and anticancer properties as well as antibacterial activity against a number of bacteria.[7-8] In addition, unlike certain synthetic polymers, e.g., the polymers with quaternary ammonium as antibacterial side groups,[9] luteolin is more biocompatible to mammalian cells, favorable of their use in biomaterial applications. Therefore, it is highly desirable to develop luteolin-based antibacterial polymer coatings. However, previous research efforts on the flavonoids have focused on the low weight flavonoids and their derivatives, and little progress has been made in polymerized flavonoid coatings.[7-8, 10-11]

Recently, poly(dopamine) coatings formed through autoxidation of dopamine at alkaline pH are emerging as an extremely attractive approach for single-step surface functionalization of almost all kinds of materials.[12] Further investigation has shown this approach is also applicable for preparing polymeric coatings using other catechol-derivative compounds.[13-14] These polymeric coatings may hence become an interesting alternative to the established surface coatings like self-assembled monolayers and polyelectrolyte multilayered films, and currently have become a hot field of scientific research and technological innovation.[15-16] Herein we report on the facile fabrication of polymeric surface coatings via autoxidation of luteolin and their antibacterial activities against Gram-positive *S. aureus*. *S. aureus* is a major cause of potentially life-threating infections acquired in health care settings and in the community. In particular, among the common pathogens that cause implant infections, *S. aureus* is No. 1, followed by *S. epidermidis*.[17] The antibacterial activity of the PL coatings was evaluated according to the ASTM E2149-01, a quantitative antibacterial test method for determining the antibacterial activity of nonleaching antibacterial agents under dynamic contact conditions.[18] Furthermore, the effect of high temperature steam sterilization treatments on the antibacterial activities of the PL coatings was examined.

Results and discussions

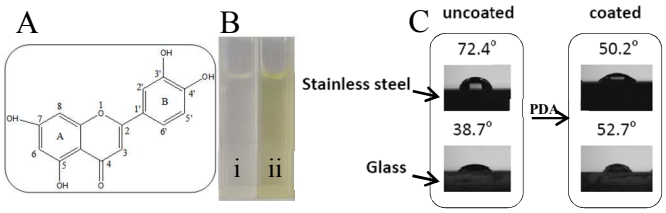

Fig. 1 (A) Molecule structure of luteolin. (B) Photographs of the fresh luteolin solution (i) and the luteolin solution after standing 18 h (ii). (C) Contact angle images of the PL-coated substrates.

The PL-coated substrates were prepared by immersing the substrates of interest in a freshly prepared aqueous luteolin solution for ~18 h.

Meanwhile the color of luteolin solution changed from the initial colorless to the light yellow (Fig. 1A), indicative of autoxidation of luteolin. Due to the high chemical complexity of the polymerization reactions of polyphenolic compounds,[19-20] it is a challenge to obtain precisely the chemical composition of the PL coatings at present. Nevertheless, the formation of the PL coatings as well as the functional groups present in the PL coatings can be detected, as demonstrated below.

Fig. 1B shows the static contact angle images of substrate materials before and after PL coating. The changes of contact angles after PL coating (e.g., 72.4°→50.2° for stainless steel; 38.7°→52.7° for glass) provide evidence of formation of the PL coatings on substrate materials. Interestingly, the measured contact angle values of the PL coatings are close to those of poly(dopamine) coatings reported previously.[21] Furthermore, as the atomic force microscopy (AFM) result shown in Fig. S1A, the surface morphology of the PL coatings is homogeneous, but with apparent grains, indicating the formation of small polymeric particles. In addition, scanning electron microscopy (SEM) was used as a complemental characterization tool to image the PL coatings with a conductive indium-tin oxide (ITO) glass as the substrate. As shown in inset of Fig. S1B, bare ITO glass exhibited a polycrystalline surface morphology consisting of islands or large domains with distinct edge defects and nonhomogenous grains (inset), consistent to previous report,[22] however, after PL coating, only the large domains with edge defects could be discerned.

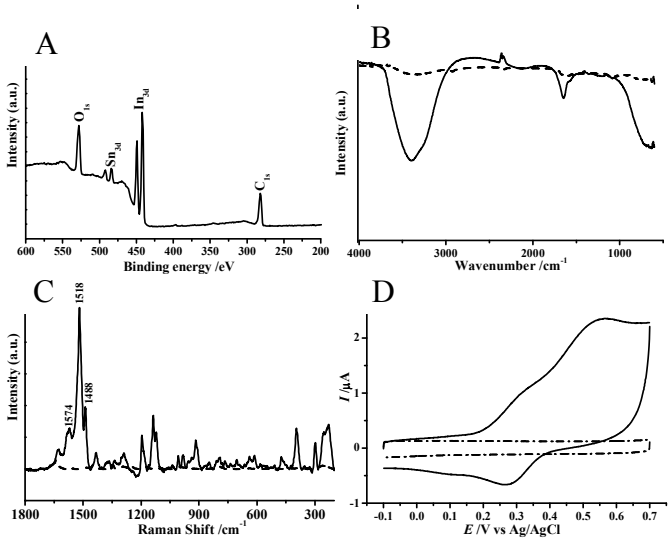

Fig. 2 (A) XPS spectrum of the PL coatings at the ITO surface. (B) FT-IR spectra of the PL coatings at the Au surface (solid line) and bare Au surface (dashed line). (C) Raman spectra of the PL coatings at the Au surface (solid line) and bare Au surface (dashed line). (D) Cyclic voltammetric responses of the ITO electrode before (dash-dotted line) and after PL coating (solid line) in a blank solution (0.1 M phosphate buffer solution, pH 7.0) at a scan rate of 50 mV s^{-1}.

X-ray photoelectron spectroscopy (XPS) spectrum of the PL coatings formed at the surface of ITO glass is shown in Fig. 2A. The peaks located at about 528 and 284 eV correspond to the electron states of O 1s and C 1s in the PL, respectively. The two peaks close to 445 eV correspond to the electron states of In $3d_{3/2}$ and In $3d_{5/2}$. The Sn 3d peak was relatively weak. The above results show that the PL film was very thin, so the secondary electrons of oxygen and indium atoms in the ITO substrate were excited when the PL surface was scanning. FT-IR spectrum of the PL coatings at the Au surface is shown in Fig. 2B. Compared with the spectrum of luteolin (Fig. S2), in the spectrum of the PL coatings (solid line), a broad peak centered at 3380 cm^{-1} due to the vibration of O-H linkage of phenolic and hydroxyl groups, and a peak at 1640 cm^{-1} ascribed to the carbonyl vibration[23] were observed, however, the fingerprint region between 1000 and 1500 cm^{-1} were unseen. Raman spectrum of the PL coatings at the Au surface is shown in Fig. 2C. It can be seen that in the spectrum of the PL coatings (solid line), the intense line near 1600 cm^{-1}, which attributed to the C=O and C2=C3 stretch, is considerably weakened. The line at 1518 is the strongest line in the Raman spectra, which involves the 3'OH and 4'OH in-plane bend. The three lines between 500 and 800 cm^{-1}, mainly attributable to ring C in-plane deformations, are considerably weakened.[24] Cyclic voltammetric responses of the ITO electrode before and after PL coating are shown in Fig. 2D. The bare ITO electrode exhibited a typical charging/discharging current curve without any faradic process (dash-dotted line). After PL coating, two redox peaks appeared (solid line). The first oxidation peak at 0.34 V could be attributed to the oxidation of the catechol group on the ring B of luteolin, the most redox-active group, and the second oxidation peak at 0.54 V could be attributed to the irreversible oxidation of the resorcinol group on the ring A of luteolin.[25] During the reverse wave scans, there is a single reduction peak at a potential slightly lower than the first peak potential. This reduction peak could be a result of the reduction of the oxidation product of the catechol groups on the ring B.[26] These characterizations indicate the material-independent coating formation of the PL with resorcinol groups on the A ring and catechol group on the B ring as the main chemically active groups.

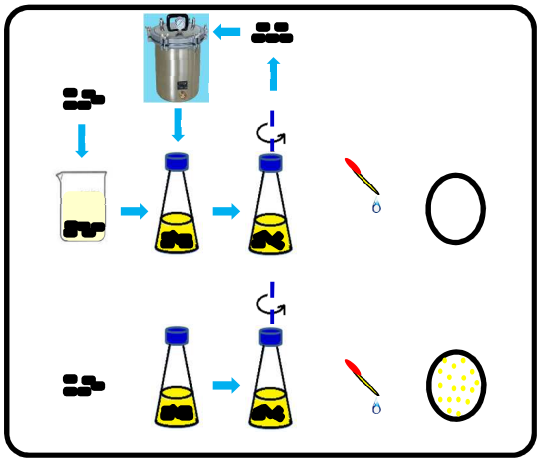

Fig. 3 An illustration of ASTM E2149-01 standard test method for determining the antibacterial activity of the PL coatings under dynamic contact condition. (a1, b1) Small pieces of stainless steel plate or glass slide modified with the PL coatings and without the PL coatings, respectively. (a2, b2) Transfer samples to 250 mL screwcap flask with 50 mL inoculum. (a3, b3) Place flask on a shaker. (a4, b4) Remove 1.0 mL solution after 10 h. (a5, b5) Plate onto agar. (c1) Remove samples from the flask after 10 h shaking. (c2) Steam sterilization of samples at 121°C for 30 min.

Antibacterial activity of the PL coatings against *S. aureus* was evaluated by the ASTM E2149-01. Fig. 3 shows a graphic representation of determination of the antibacterial activity of the PL coatings. Antibacterial activity of the PL coatings was found to be excellent. As shown in Fig. S3, no colonies formed on the plate incubated with the bacteria suspension treated with the PL coatings (Fig. S3A). The control plate, however, was covered with bacterial colonies (Fig. S3B). In other words, after incubation for 24 h with *S. aureus* cell suspension under dynamic contact conditions, the PL coating effectively killed planktonic *S. aureus* on contact and sterilized the incubation solution. Several groups reported the

structure-activity relationships of flavonoids. Mori et al observed a relationship between the structures of the flavonoids and their activity against *S. aureus*.[27] Most of the activity was related to the presence of hydroxyl groups 3', 4', 5' in ring B and at C-3. Puupponen-Pimiä et al reported that the number of hydroxyl groups in the B ring in flavones is associated with the antibacterial activity against lactic acid bacteria.[28] Accordingly, we speculated the mechanism of the antibacterial activity of the PL films could be associated with the resorcinol groups on the A ring and the catechol group on the B ring.

Furthermore, the effect of steam sterilization treatments on antibacterial activities of the PL coatings against *S. aureus* was examined. The PL coatings were treated with steam sterilization at 121 ^{o}C for 30 min and their antibacterial activity was evaluated. The steam sterilization and the subsequent incubation process were repeated for three consecutive cycles. All antibacterial activity results are summarized in Table 1. It can be seen that the PL coatings exhibited unaffected antibacterial activity even after high temperature steam sterilization at 121 ^{o}C for three times. Such unimpaired antibacterial activity by steam sterilization treatments will facilitate the practical application of the PL coatings. For instance, it is well-known that all materials implanted within the body or placed in contact with corporeal fluids must be sterilized. The preferred sterilization process is high temperature steam sterilization, which is considered to be one of the safest and most practical means of sterilizing medical devices and fluids.[29] However, steam sterilization treatment may impair seriously the performances of polymeric materials and devices due to the physicochemical alterations, short-or long-term loss of functionality, and biocompatibility.[29] For example, Rao and Sharma found that chitosan film underwent degradation when autoclaved at 121 ^{o}C for 15-30 min.[30] Therefore, the unimpaired antibacterial activity of the PL coatings by steam sterilization treatments could provide extra advantages for the PL coatings for practical applications. In addition, the remained antibacterial activity of the PL coatings after incubation under shaking for four times (each for 24 h, see Experimental Section) indicates that the PL coatings are stable in aqueous environment.

Table 1. Antibacterial properties of the PL coatings.

Samples (cycle numbers of steam sterilization treatments)	R (%, S. *aureus*)
0	100
1	100
2	99.4
3	100

In addition, it is worthy of note that typically, the formation of antibacterial polymer coatings involves a two-step approach in which the substrate surface is firstly treated to provide functional groups amenable to attachment chemistry and then the antibacterial is, either, tethered by reaction involving these functional groups, or assembled onto the surfaces.[18] These approaches, however, are quite elaborate, and are usually dependent on the surface chemistry of the substrate material.[31] On the other hand, the polymerization of biocidal monomers is very sensitive to the tethering method and the nature of the monomer, and often, the polymerization of biocidal monomer does not lead to active antibacterial polymers.[32] Therefore, the fabrication process and the observed antibacterial activity of the PL coatings against *S. aureus* indicate several important advantages of the PL coatings: the fabrication method of the PL coatings is one-step, green, material-independent; the PL coatings possess antibacterial activity against *S. aureus*, which is unimpaired by steam sterilization treatments. These advantages make the PL coatings attractive in the field of developing antibacterial polymer coatings for reducing *S. aureus* infections, for instance, implant-associated infections.

Conclusions

In summary, we have demonstrated the facile fabrication of the PL coatings on different surfaces through the autoxidation of lutoelin in alkaline aqueous solution. The fabrication process is "green" as it does not involve any harmful chemicals. The fabricated PL coatings are stable in aqueous environment, and exhibit antibacterial activities against *S. aureus*. Most importantly, they maintain almost unaffected antibacterial activities even after steam sterilization treatments), which provides extra advantage for the PL coatings for practical applications, e.g., in the field of implant materials and devices.

Acknowledgements

This work was financially supported by the NSF of China (Grant No. 21175010), International Joint Key Project from Chinese Ministry of Science and Technology (2010DFA12780), the Project-sponsored by SRF for ROCS, SEM, and the grant from Beijing Municipal Science and Technology Commission (z131102002813058).

Notes and references

[a] Research Center for Bioengineering and Sensing Technology, School of Chemistry and Biological Engineering, University of Science and Technology Beijing, Beijing 100083, P.R. China

[b] Institute of Orthopedics, the First Affiliated Hospital of the General Hospital of PLA, Beijing 100048, P.R. China

[c] School of Science, Beijing Technology and Business University, Beijing 102488, P.R. China

[*]Corresponding author. E-mail: sulei@ustb.edu.cn.

† J.Y. Cheng and M. Guan contributed equally to this work.

Electronic Supplementary Information (ESI) available: Experimental section, AFM and SEM images, FT-IR spectrum of luteolin, and the photographs of LB agar plates incubated with the unmodified surfaces and the PL coatings. See DOI: 10.1039/c000000x/

1. J. W. Costerton, P. S. Stewart and E. P. Greenberg, *Science*, 1999, **284**, 1318-1322.
2. N. R. Panyala, E. M. Pena-Mendez and J. Havel, *J. Appl. Biomed.*, 2008, **6**, 117-129.
3. A. D. Fuchs and J. C. Tiller, *Angew. Chem. Int. Ed.*, 2006, **45**, 6759-6762.
4. J. C. Tiller, *Adv. Polym. Sci.*, 2011, **240**, 193-217.
5. X. Liu, H. Zhang, Z. Tian, A. Sen and H. R. Allcock, *Polym. Chem.*, 2012, **3**, 2082-2091.
6. T. P. T. Cushnie and A. J. Lamb, *Int. J. Antimicrob. Agents*, 2011, **38**, 99-107.
7. J. Qiu, H. Li, H. Meng, C. Hu, J. Li, M. Luo, J. Dong, X. Wang, J. Wang, Y. Deng and X. Deng, *Lett. Appl. Microbiol.*, 2011, **53**, 238-243.
8. T. Y. Lin, C. W. Lu, C. C. Chang, S. K. Huang and S. J. Wang, *J. Agric. Food Chem.*, 2011, **59**, 8458-8466.
9. B. C. Allison, B. M. Applegate and J. P. Youngblood, *Biomacromolecules*, 2007, **8**, 2995-2999.
10. S. Niu, B. Han, W. Cao and S. Zhang, *Anal. Chim. Acta*, 2009, **651**, 42-47.

11. A.J. Smith, P. Kavuru, L. Wojtas, M. J. Zaworotko, R. D. Shytle, *Mol. Pharmaceutics*, 2011, **8**, 1867-1876.
12. H. Lee, S. M. Dellatore, W. M. Miller and P. B. Messersmith, *Science* 2007, **318**, 426-430.
13. Q. Ye, F. Zhou and W. Liu, *Chem. Soc. Rev.*, 2011, **40**, 4244-4258.
14. J.-R. Jeon, J.-H. Kim and Y.-S. Chang, *J. Mater. Chem. B*, 2013, **1**, 6501-6509.
15. Y. Liu, K. Ai and L. Lu, *Chemical reviews*, 2014, DOI: 10.1021/cr400407a.
16. W. J. Yang, K.-G. Neoh, E.-T. Kang, S. Lay-Ming Teo and D. Rittschof, *Polym Chem*, 2013, **4**, 3105-3115.
17. C. R. Arciola, D. Campoccia, P. Speziale, L. Montanaro and J. W. Costerton, *Biomaterials*, 2012, **33**, 5967-5982.
18. A. Kugel, S. Stafslien and B. J. Chisholm, *Prog. Org. Coat.*, 2011, **72**, 222-252.
19. S. Sanchez-Cortes, O. Francioso, J. V. Garcia-Ramos, C. Ciavatta and C. Gessa, *Colloids Surf., A*, 2001, **176**, 177-184.
20. H. Lee, J. Rho and P. B. Messersmith, *Adv. Mater.* , 2009, **21**, 431-434.
21. S. H. Ku, J. Ryu, S. K. Hong, H. Lee and C. B. Park, *Biomaterials*, 2010, **31**, 2535-2541.
22. D. Kato, G. Xu, Y. Iwasaki, Y. Hirata, R. Kurita and O. Niwa, *Langmuir*, 2007, **23**, 8400-8405.
23. L. Mejias, M. H. Reihmann, S. Sepulveda-Boza and H. Ritter, *Macromol. Biosci.*, 2002, **2**, 24-32.
24. Z. Jurasekova, J. V. Garcia-Ramos, C. Domingo and S. Sanchez-Cortes, *J. Raman Spectrosc.*, 2006, **37**, 1239-1241.
25. M. d. Vestergaard, K. Kerman and E. Tamiya, *Anal. Chim. Acta*, 2005, **538**, 273-281.
26. P. Janeiro and A. M. Oliveira Brett, *Anal. Chim. Acta*, 2004, **518**, 109-115.
27. A. Mori, C. Nishino, N. Enoki and S. Tawata, *Phytochemistry*, 1987, **26**, 2231-2234.
28. R. Puupponen-Pimia, L. Nohynek, C. Meier, M. Kahkonen, M. Heinonen, A. Hopia and K. M. Oksman-Caldentey, *J. Appl. Microbiol.*, 2001, **90**, 494-507.
29. P. R. Marreco, P. da Luz Moreira, S. C. Genari and A. M. Moraes, *J. Biomed. Mater. Res., Part B*, 2004, **71B**, 268-277.
30. S. B. Rao and C. P. Sharma, *J. Biomater. Appl.*, 1995, **10**, 136-143.
31. N. Ayres, *Polym. Chem.*, 2010, **1**, 769-777.
32. F. Siedenbiedel and J. C. Tiller, *Polymers* 2012, **4**, 46-71.

