Incorporating catechol into electroactive polypyrrole nanowires on titanium to promote hydroxyapatite formation

Zhengao Wang a, b, Jinquan Zeng c, Guoxin Tan d, Jingwen Liao e, Lei Zhou a, b, Junqi Chen a, b, Peng Yu a, b, **, Qiyou Wang f, ***, Chengyun Ning a, b, ***

a School of Materials Science and Engineering, South China University of Technology, Guangzhou 510641, China
b Guangdong Key Laboratory of Biomedical Sciences and Engineering, South China University of Technology, Guangzhou 510006, China
c Guangzhou Tieyi Middle School, Guangzhou 510660, China
d Institute of Chemical Engineering and Light Industry, Guangdong University of Technology, Guangzhou 510006, China
e Guangzhou Institute of Advanced Technology, Chinese Academy of Sciences, Guangzhou 510006, China
f Department of Spine Surgery, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou 510630, China

A R T I C L E   I N F O

Article history:
Received 18 April 2017
Received in revised form 11 May 2017
Accepted 15 May 2017
Available online 26 May 2017

Keywords:
Titanium
Hydroxyapatite
Nanowires
Catechol
Polypyrrole

A B S T R A C T

To improve the osteointegration property of biomedical titanium, nano-architectured electroactive coating was synthesized through the electrochemical polymerization of dopamine and pyrrole. The highly binding affinity of Ca²⁺ to the catechol moiety of doped dopamine enabled efficient interaction between polypyrrole/polydopamine nanowires and mineral ions. The results indicate that the PPy/PDA nanowires preserved its efficient electro-activity and accelerated the hydroxyapatite deposition in a simulated body fluid. The PPy/PDA nanowires coating could be applied to promote the osteointegration of titanium implant.

© 2018 The Authors. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

The clinical success of implants, such as biomedical titanium, relies on effective osteointegration at the interface between bone tissues and implant materials [1]. Improvement in implant electroactivity and surface chemistry has been applied to obtain the desirable osteointegration outcome, without any fibrous tissue intervention [2]. The universal existence of electrical signals in all functions of living organisms [3], has given researchers inspiration to design new-generation biomaterials to manipulate cellular bioelectrical signals [4]. Conductive polymer (CP) is an ideal candidate for modifying implant, because they possess electroactivity, biocompatibility, biomolecule affinity and environmental stability [5,6]. CP such as polyaniline (PANI) and polypyrrole (PPy) could promote growth and differentiation of skeletal muscle cells, cardiomyoblasts, and neurons [5]. PPy bone-like nanoarchitecture (i.e., nano-fibers) has recently received a great deal of attention due to their biocompatibility, electroactivity and biomolecule affinity [7]. PPy has been used to fabricate nanoarchitecture on the implant surface through incorporating different dopants, such as taurocholic acid [6], cetyltrimethylammonium bromide [8], taurine [9]. However, it remains a challenge to promote carbonated hydroxyapatite deposition on PPy coated implant, which is related to the osteointegration [10].

Incorporating catechol moiety into electroactive PPy is a smart strategy to facilitate the formation of hydroxyapatite. Polydopamine (PDA) is a mimic of the specialized adhesive foot protein, Mefp-5 (Mytilus edulis foot protein-5) [11]. Catechol moiety of PDA strongly binds to various metal ions, and could accelerate hydroxyapatite formation by co-precipitation of calcium and phosphate ions [12]. PDA could be synthesized through the electrochemical polymerization of dopamine [13]. Therefore, PDA can be incorporated into PPy through simultaneous
electrochemical polymerization of dopamine and pyrrole. As a green chemical synthesis method, electrochemical polymerization provides an efficient way to modulate the polymerization reaction, which potentially results in a smoother and more homogeneous coverage compared to its solution counterpart [14]. We electrochemically incorporated mussel-inspired PDA into electroactive PPy nanowires. And the biomineralization in vitro shows that the catechol moiety on the surface could improve hydroxyapatite crystallization.

2. Materials and methods

Nano-architected PPy (NAPPy) doped with PDA was synthesized on biomedical titanium via template-free electrochemical polymerization. First, under the control of an electrochemical workstation, the prenucleation film (PNF) was formed on titanium at 0.8 V (vs SCE) for 30 s at room temperature. The electrolyte contained 0.2 M KCl (Aladdin Chem Co., China) and 0.1 M Py (Aladdin Chem Co., China). Second, NAPPy was galvanostatically synthesized on PNF at a different current for 40 min. The electrolyte was a phosphate buffer solution (PBS), which contained 0.2 M Py and 0.1 M DA (Aladdin Chem Co., China). PH value was 6.8 and the concentration of phosphate was 0.5 M. And the PPy/PDA nanowires and PPy/PDA nanograins coating were synthesized at 1.5 mA cm\(^{-2}\) and 0.3 mA cm\(^{-2}\), respectively. As a control, β-naphthalene sulfonic acid (NSA) (Aladdin Chem Co., China) replaced dopamine, and PPy/NSA nanowires were synthesized according to Jingwen Liao’s research [15].

The adhesion strength of PPy/PDA nanowires coating was determined through ultrasonication tests. Ultrasonication (Kunshan Ultrasonic Instruments Co. Ltd., Kunshan, China, 40 kHz, 80 W) was applied to test the in vitro mechanical stabilities of coating on titanium implant for 15 min. Then overall structure microstructure of the coating was recorded by camera and field emission scanning electron microscopy (FE-SEM, ZEISS Ultra 55, Germany), respectively.

The simulated body fluid (SBF) was prepared according to Kokubo’s previous description [16]. NAPPy doped with DA and NSA were immersed in SBF for biomineralization, respectively. After being incubated in a constant temperature incubator at 37 °C for 3 days and 7 days, respectively, the specimens were washed with deionized water and dried under a vacuum.

Field emission scanning electron microscopy (FE-SEM, ZEISS Ultra 55, Germany) and atomic force microscopy (AFM, Shimadzu SPM-9600, Japan) were used to characterize PPy nanoarchitectures. Fourier transform infrared spectroscopy (FTIR, Bruker VERTEX 33, Germany) was used to characterize the chemical composition of CaP deposition. An electrochemical workstation (Zennium Zahner, Germany) was used to measure cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). CV and EIS was tested in three electrode system, including platinum as counter electrode and SCE as reference electrode and sample (pure titanium, PPy/PDA nanograins coated titanium and PPy/PDA nanowires coated titanium, respectively) as working electrode. The electrolyte was 0.1 M KCl solution. The slew rate of CV was 50 mV/s. The EIS was tested from 100000 Hz to 0.01 Hz.

3. Results and discussion

In this work, we electrochemically incorporated PDA into conducting PPy coating on titanium. The catechols moiety of PDA strongly bind to calcium. So the hydroxyapatite could form by coprecipitation of phosphate ions and calcium (Fig. 1). As shown in Fig. 2, polypyrrole nanowires and nanograins were deposited on the biomedical titanium. When the current density was 1.5 mA cm\(^{-2}\), high density of PPy/PDA nanowires about 180 nm in diameter were successfully synthesized using template-free electrochemical polymerization (Fig. 2a1, b1). And when the current density was set as 0.3 mA cm\(^{-2}\), nanograins were acquired (Fig. 2a2, b2). And FT-IR spectroscopy was used to characterize the PPy/PDA nanowires coating and PPy doped with Cl\(^{-}\) (a control group) to approve the existence of catechols in the PPy/PDA nanowires coating (Fig. S1). A broad peak at 3230 cm\(^{-1}\) was associate with the \(\nu\)(N-H) of PPy and PDA and to the \(\nu\)(O-H) stretching modes. A peak at 1265 cm\(^{-1}\) aroused from the phenolic C-O-H stretching modes of PDA. The result demonstrated that catechols exited in the PPy/PDA coating.

The adhesion strength is a crucial factor for the service lifetime

![Fig. 1. The schematic illustration of formation of calcium phosphate biominerals on the polypyrrole nanowires doped polydopamine. (a, b) the polypyrrole nanowires deposited on the biomedical titanium through the simultaneous electrical polymerization of dopamine and pyrrole. (c, d) catechol moiety on the surface of conducting polypyrrole nanowires doped polydopamine assists calcium phosphate crystal formation.](image-url)
of bioactive layer coated metallic implants within the human body. Bonding strength of PPy/PDA nanowires coating on titanium implant were measured via ultrasonication. The results showed that the PPy/PDA nanowires coating were nondestructive after strong ultrasonication for 15 min (Fig. S2).

The cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were used to characterize the electroactivity of NAPPy in the electrolyte containing 0.1 M KCl (Fig. 3). The CV of PPy nanowires showed that the oxidation peak at $0.17 \, \text{V}$ and the reduction peak at $-0.26 \, \text{V}$ appears good x axis symmetry, demonstrating good reversible redox reaction. And the redox peak intensity of PPy nanowires was much stronger than that of PPy nanograins, because the surface area of nanowires coating was much higher than that of nanograins coating and interface transfer resistance and transmission time of electrons of nanowires were much smaller than that of nanograins. What’s more, oxidation peak and reduction peak in the CV of pure titanium were not observed (Fig. S3). ZSimpWin was used to analyze the Nyquist graph, and the equivalent electro circuit was obtained (Fig. 3b). In this case, the electrolyte resistance ($R_s$) was related to the resistivity of the electrolyte, and the charge transfer resistance ($R_{\text{ct}}$) and $C_{\text{d}}$ reflect the property of nanoarchitected PPy layer doped with PDA, and

---

Fig. 2. (a1, a2) Field emission scanning electron microscopy image (FE-SEM) of nanowires and nanograins respectively and (b1, b2) atomic force microscopy (AFM) image of nanowires and nanograins respectively.

Fig. 3. (a) Cyclic voltammetry of nanowires and nanograins. (b) alternating current impedance of nanowires and nanograins. Inset: equivalent electric circuit.
the \( R_{C2} \) and \( C_{d2} \) depend on the property of prenucleation films doped with \( Cl^- \). The prenucleation films deposited on the titanium, then the nanoarchitectured PPy deposited on the prenucleation films. The process suggests that titanium and the prenucleation films and the nanoarchitectured PPy connected in series, which is corresponding to the simulation results. The \( R_{C1} \) of nanograins and nanowires are 484 and 107 \( \Omega \) cm\(^2\), respectively, which indicates that PPy nanowires could promote the electron transmission [17]. This phenomenon may result from (a) the higher specific surface area accessible to the electrolyte, and (b) smoother electron flow in oriented nanostructured matrix, which are potential applications in high sensitivity and efficiency of numerous microelectronics devices.

To prove the how the PPy/PDA nanowires promote the in vitro biomineralization, PPy/PDA nanowires nanograins coated titanium and PPy/NSA nanowires coated titanium (Fig. S4) and pure titanium were introduced as control groups. The CaP minerals deposited on the PPy-coated titanium after incubation in the SBF (Fig. 4). The CaP agglomerates formed on the surface of PPy/PDA nanograins after 3 days of incubation (Fig. 4b1) and the agglomerates covered most of the surface area of PPy/PDA nanowires in 3days (Fig. 4c1), resulted from the fact that the amounts of free catechols on PPy/PDA nanowires are much larger than that on PPy/PDA nanograins. The amounts of agglomerates on the surface of PPy/NSA nanowires was much less than that on PPy/PDA nanowires and nanograins (Fig. 4a1), which indicating that the catechols of PDA is critical for the CaP nucleation. After 7 days of incubation, the titanium substrate modified by the PPy/PDA nanowires was uniformly covered by CaP minerals (Fig. 4c2), and the titanium substrate modified by the PPy/PDA nanograins was covered by abundant spherical CaP agglomerates (Fig. 4b2). Nevertheless, a little CaP mineral deposited on the PPy/NSA nanowires (Fig. 4a2). High-magnification

![Fig. 4. FE-SEM of calcium phosphate biominerals coated on the PPy/NSA nanowires (a), PPy/PDA nanograins (b) and PPy/PDA nanowires (c) after 3 days (1) and 7 days (2).](image-url)
scanning electron microscopy images show that the agglomerates possessed a lath-like structure, which are similar to a typical form of hydroxyapatite crystals [18]. What's more, little CaP mineral deposited on the surface of polished titanium after 7 days of incubation (Fig. S5). These results demonstrated that PPy/PDA nanowires on titanium implant could promote in vitro biomineralization because of the high surface area and catechols.

The Energy Dispersive Spectrometer (EDS) analysis demonstrates that the Ca/P ratio of minerals on the PPy/PDA nanowires after deposition for 7 days was 1.71, which was closer to 1.67 in the natural bone mineral than that on the PPy/NSA nanograins (1.58) and PPy/NSA nanowires (1.42) (Fig. 5a). Our results suggest that the abundant catecholamine moieties in PDA and the high specific surface area of nanowires play a dual role in the accelerating hydroxyapatite formation. The FTIR spectra shown in Fig. 5b also support that the mineral phase is hydroxyapatite. The peaks at 980–1100 cm⁻¹, 957 cm⁻¹, 570 cm⁻¹ and 589 cm⁻¹ were assigned to PO₄³⁻ of the mineral [19].

4. Conclusion

NAPPy doped with PDA were successfully fabricated through electrochemical polymerization. The PPy/PDA nanowires possess versatile electroactivity. Because of the abundant catecholamine moieties of PDA and the high specific surface area of nanowires, PPy/PDA nanowires coated titanium play a dual role in the accelerating hydroxyapatite formation, suggests that PPy/PDA nanowires coated titanium is a suitable implant material for effective biomineralization.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (grant numbers 51372087, 51541201), the National High Technology Research and Development Program of China (863 Program) (grant number 2015AA033502), the Science and technology program of Guangzhou (grant number 201707010200), the Natural Science Foundation of Guangdong Province (grant number 2016A030308014).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bioactmat.2017.05.006.

References

[1] L. Le Guennec, A. Soueidan, P. Layrolle, Y. Amouriq, Surface treatments of titanium dental implants for rapid osseointegration, Dent. Mater. 23 (2007) 844–854.
[2] X. Zhang, C. Zhang, Y. Lin, P. Hu, Y. Shen, K. Wang, S. Meng, Y. Chai, X. Dai, X. Liu, Y. Liu, X. Mo, C. Cao, S. Li, X. Deng, L. Chen, Nanocomposite membranes enhance bone regeneration through restoring physiological electric microenvironment, ACS Nano 10 (2016) 7279–7286.
[3] A.J. Barabasi, Z.N. Olivi, Network biology: understanding the cell’s functional organization, Nat. Rev. Genet. 5 (2004) 101–115.
[4] C. Ning, L. Zhou, G. Tan, Fourth-generation biomedical materials, Mater. Today 19 (2016) 2–3.
[5] M.K. Gaimard, N. Gomez, C.E. Schmidt, Conducting polymers in biomedical engineering, Prog. Polym. Sci. 32 (2007) 876–921.
[6] J. Liao, Y. Zhu, Z. Zhou, J. Chen, C. Mao, Reversibly controlling preferential protein adsorption on bone implants by using an applied weak potential as a switch, Angew. Chem. Int. Ed. 53 (2014) 13068–13072.
[7] R.A. Green, N.H. Lovell, G.W. Wallace, L.A. Poole-Warren, Conducting polymers for neural interfaces: challenges in developing an effective long-term implant, Biomaterials 29 (2008) 3393–3399.
[8] P. Liu, P. Du, J. Guo, X. Wu, J. Qiu, Facile mass production of semi-conductive polypyrrole/polystyrene composite with enhanced mechanical strength via in-situ bulk polymerization, Mater. Lett. 139 (2015) 191–193.
[9] J. Liao, H. Fan, C. Ning, G. Tan, Z. Zhou, J. Chen, S. Huang, Taurine-induced fabrication of nano-architectured conducting polypyrrole on biomedical titania, Macromol. Rapid Commun. 35 (2014) 574–578.
[10] J.G. de Castro, B.V.M. Rodrigues, R. Ricci, M.M. Costa, A.F.C. Ribeiro, F.R. Marciano, A.O. Lobo, Designing a novel nanocomposite for bone tissue engineering using electrospun conductive PPy/polypyrrole as a scaffold to direct nanohydroxyapatite electrodeposition, RSC Adv. 6 (2016) 32615–32623.
[11] J.L. Liu, K.L. Ai, L.H. Lu, Polydopamine and its derivative materials: synthesis and promising applications in energy, environmental, and biomedical fields, Chem. Rev. 114 (2014) 5057–5115.
[12] W. Zhang, Z. Pan, F.K. Yang, B. Zhao, A facile in situ approach to polypyrrole functionalization through bioinspired catechols, Adv. Funct. Mater. 25 (2015) 1588–1597.
[13] B. Lin, M. Zhong, C. Zheng, L. Cao, D. Wang, L. Wang, J. Liang, B. Cao, Preparation and characterization of dopamine-induced biomimetic hydroxyapatite coatings on the AZ31 magnesium alloy, Surf. Coat. Technol. 281 (2015) 82–88.
[14] M.N. Arechederra, C. Jenkins, R.A. Rincon, K. Artsushkova, P. Atanasov, S.D. Minteer, Chemical polymerization and electrochemical characterization of thiazines for NADH electrocatalysis applications, Electrochim. Acta 55 (2010) 6659–6664.
[15] J. Liao, S. Wu, Z. Yin, S. Huang, C. Ning, G. Tan, P.K. Chu, Surface-dependent self-assembly of conducting polypyrrole nanotube arrays in template-free electrochemical polymerization, ACS Appl. Mater. Interfaces 6 (2014) 10946–10951.

[16] A. Oyane, H.M. Kim, T. Furuya, T. Kokubo, T. Miyazaki, T. Nakamura, Preparation and assessment of revised simulated body fluids, J. Biomed. Mater. Res. Part A 65 (2003) 188–195.

[17] Gareth P. Keeley, Arlene O’Neill, Niall McEvoy, Nikos Peltekis, Jonathan N. Coleman, Georg S. Duesberg, Electrochemical ascorbic acid sensor based on DMF–exfoliated graphene, J. Mater. Chem. 20 (2010) 7864–7869.

[18] S. Koutsopoulos, J. Biomed, Synthesis and characterization of hydroxyapatite crystals: a review study on the analytical methods, J. Biomed. Mater. Res. 62 (2002) 600–612.

[19] M. Lee, S.H. Ku, J. Ryu, C.B. Park, Mussel-inspired functionalization of carbon nanotubes for hydroxyapatite mineralization, J. Mater. Chem. 20 (2010) 8848–8853.