·综述·

壳聚糖表面改性钛合金材料的研究进展

刘嘉鑫，安丽萍，贾耀飞，张广瑞，周建平，吴定，张明涛，韵向东
（兰州大学第二医院骨科 甘肃省骨关节疾病研究重点实验室，甘肃　兰州　730030）

【摘要】 钛合金材料生物性能良好，是骨科常用的内植入材料，但其骨整合性及抗菌性能较差，需进行表面改性以弥补其不足之处。壳聚糖具有良好的生物相容性及成膜性，且可作为载体将目标药物引入钛合金表面，可有效改善钛合金材料的生物学性能，增加其使用范围。本文对近几年壳聚糖表面改性钛合金材料的相关研究进行归纳总结，从壳聚糖涂层改性的方式、钛合金材料成骨性及抗菌性的改善 3 个方面展开论述，以期为钛合金材料涂层改性在临床中的应用提供指导依据。

【关键词】 壳聚糖；　钛合金；　综述
中图分类号：R683
DOI：10.12200/j.issn.1003-0034.2020.12.017　　开放科学（资源服务）标识码（OSID）：

Research advance in surface modification of titanium alloys with chitosan

LIU Jia-xin, AN li-ping, JIA Yao-fei, ZHANG Guang-rui, ZHOU Jian-ping, WU Ding, ZHANG Ming-tao, and YUN Xiang-dong. Department of Orthopaedics, the Second Hospital of Lanzhou University, Key Laboratory of Bone and Joint Diseases in Gansu Province, Lanzhou 730030, *Gansu, China*

ABSTRACT　Titanium alloy has good biological properties and is commonly used in orthopedics, but its bone integrity and antibacterial properties are poor, so surface modification is needed to make up for its shortcomings. Chitosan has good biocompatibility and film-forming ability, and can be used as a carrier to introduce the target drug to the surface of titanium alloy, which can effectively improve the biological properties of titanium alloy materials and increase its application range. In this paper, the related research of chitosan surface modified titanium alloy materials in recent years is summarized. The modification methods of chitosan coating, the improvement of osteogenesis and antibacterial properties of titanium alloy materials are discussed in order to provide guidance for the clinical application of coating modification of titanium alloy materials.

KEYWORDS　Chitosan;　Titanium alloy;　Review

钛合金多方面的优势被广泛应用于骨科内植入材料的制备中[1]。但钛合金材料存在生物惰性，骨整合性较差，并且不具备抗菌性，无法阻碍细菌在其表面粘附定植，易导致内植入材料相关感染的发生，尤其是对于糖尿病或存在感染的患者，钛合金内植入材料并不适用[1]。钛合金材料表面是骨组织长入或细菌定植引发内植入材料相关感染的关键场所，故在改善钛合金材料生物学性能方面，钛合金材料表面改性一直以来是研究的热点[2]。尽管表面改性物质的可选择范围较为广泛，但近几年有关钛合金材料表面改性的研究主要围绕壳聚糖涂层改性展开，主要原因在于壳聚糖具备优良的生物学性能及强大的载体特性。本文主要从壳聚糖涂层改性方法的选择、壳聚糖表面改性钛合金材料的骨整合性及抗菌性进行论述。

1　壳聚糖表面改性钛合金方式的选择

壳聚糖涂层改性钛合金的方式很多，主要有以下 3 类：(1)通过化学键将壳聚糖直接定植于钛合金表面。钛合金表面覆盖有二氧化钛保护膜，生物惰性较强，故在对其表面进行涂层改性时需首先对钛合金表面进行活化处理。Martin 等[3]先用 3-氨丙基三乙氧基硅烷（APTES）对钛合金表面进行处理，使其表面留有硅烷基团，随后通过戊二醛两侧的醛基分别同硅烷基团及壳聚糖的羟基相连实现钛合金表面壳聚糖涂层的制备。(2)壳聚糖表面带有正电荷，与其他带负电荷的高聚分子（如海藻酸钠、透明质酸[4]及明胶等）之间存在静电吸附作用，可通过层层自组装法或电泳沉积法在钛合金表面制备复合涂层。Liu 等[5]研究发现壳聚糖复合涂层较单一的壳聚糖涂层在抗菌性及骨整合性方面更有优势。(3)将壳聚糖融入到钛合金材料表面的其他涂层中以弥补相应涂层的局限性。羟基磷灰石涂层虽然是钛合金材料改善

基金项目：国家自然科学基金地区科学基金项目（编号：81560361）
Fund program: Regional Fund Program of National Natural Science Foundation of China (No. 81560361)
通讯作者：韵向东　E-mail: xiangdongyun@126.com
Corresponding author: YUN Xiang-dong　E-mail: xiangdongyun@126.com

骨整合性常用的方式，但该涂层与成骨细胞间的生物相互作用较为缓慢。Suo 等[6]将壳聚糖融入羟基磷灰石涂层后发现，壳聚糖-羟基磷灰石涂层与单纯的羟基磷灰石涂层相比，前者在成骨细胞活性、碱性磷酸酶活性及钙盐沉积方面更具优势，更利于骨组织的长入。以上涂层方式虽都可将壳聚糖植入钛合金表面，但在选择应用何种方式进行改性时需明确：(1)不同的改性方式各具优缺点，如化学键连接方式可赋予钛合金与壳聚糖之间较高的键合力，但作用较为单一，无法携载其他物质，而静电吸附可将壳聚糖与其他高聚物相结合制备复合涂层加强涂层的稳定性，并且可携载目标药物实现药物的缓释以进一步发挥内植入物改性或治疗的作用，但静电吸附作用键合力较化学键弱，涂层易被破坏[7]。(2)壳聚糖涂层方式的选择应取决于钛合金材料表面改性的目的，例如是利用壳聚糖改善其他涂层的特性还是需要携载目标药物，亦或是为了连接其他物质进一步对钛合金表面改性。因此，只有明确改性目标并综合考虑涂层的优缺点，才能更好地发挥壳聚糖改性的优势。

2　壳聚糖表面改性钛合金材料提高其骨整合性

虽然钛合金材料的生物惰性使其具有较好的抗腐蚀性能，但不利于成骨细胞的粘附生长，降低了其骨整合性能。钛合金材料表面是成骨细胞粘附增殖的基础，如何让成骨细胞在内植入材料表面持续粘附并保持生物活性是提高钛合金材料骨整合性的关键。壳聚糖作为钛合金表面改性物质可较好地满足成骨细胞粘附增殖的需要。细胞间结缔组织的主要成分为黏多糖，而壳聚糖是 N-乙酰氨基葡萄糖与氨基葡萄糖的共聚物，其化学结构与黏多糖类似，具备黏多糖的特性，可与生长因子、黏附蛋白等发生相互作用，能更好地模拟细胞生长所需的微环境[8]。Bumgardner 等[9]在钛合金表面制备壳聚糖涂层，并将其放入实验动物体内，组织学研究表明改性后的钛合金周围组织炎症反应较少，且新生骨质量较对照组(未涂层的钛合金)更为明显。成骨细胞的粘附增殖及矿化基质的合成除需要适宜的生长微环境外，还需要适宜浓度的细胞生长因子。该类因子主要为蛋白质及核酸分子，包括 siRNA、miR-21、骨形态发生蛋白、胰岛素样生长因子、转化生长因子-β 及血小板源性的生长因子等[10-12]。将壳聚糖作为搭载载体既可减少此类物质在局部过量释放而导致骨质的过度再生，又可作为保护剂，避免此类物质在体内水解破坏。Wang 等[13]在钛合金表面制备携载有 siRNA 的壳聚糖-三磷酸盐-透明质酸复合物纳米微球，结果表明改性后的材料可通过沉默肿瘤坏死因子-α(tumor necrosis factor-α，TNF-α)的表达促进骨髓间充质干细胞向成骨细胞分化。另外，就内植入材料本身而言，提高钛合金材料的骨整合性还可通过增大成骨细胞与内植物表面的接触面积实现[14]。因此，接触面积的增大与壳聚糖表面涂层改性对于钛合金材料骨整合性的提高都具有显著的作用，将两者相结合可进一步提高钛合金材料的骨整合性。Wang 等[15]应用微弧氧化技术钛合金表面制备多孔结构，并在其表面携载 miR-21/壳聚糖-透明质酸微球，细胞实验表明该材料可促进成骨标志物的高表达，如骨桥蛋白、骨钙素及转录因子 RUNX2。但两种因素对于钛合金材料骨整合性的影响作用并无相关的对比研究，哪种因素的影响更大还需进一步研究。

3　壳聚糖表面改性钛合金材料改善其抗菌性能

骨科内植入材料相关感染是内植入材料在临床中应用所面临的主要问题。而钛合金材料由于并不具备抗菌性能，其发生感染的风险更高。内植入材料表面细菌的粘附定植是内植入材料相关感染的主要原因，尤其是当内植入材料表面形成生物膜时，感染更难控制。故改善钛合金内植入材料抗菌性的关键在于阻碍细菌在其表面粘附定植，帮助成骨细胞“抢占”内植入材料表面，从而减少内植入材料相关感染的发生[16-19]。壳聚糖中氨基葡萄糖中的阳离子基团 NH3+可与大多数细菌表面的负极成分相结合引起细菌膜性改变及细胞内基质流失，从而导致细菌的死亡[20]，故具备较为广泛的抗菌谱；而在细胞毒性方面，其降解产物为细胞代谢产物的类似物，对组织细胞的毒性小，是钛合金材料表面抗菌改性较为理想的选择。Foss 等[16]应用共培育系统研究成骨细胞与金黄色葡萄球菌在钛合金表面上的相互作用关系，结果表明壳聚糖涂层后的钛合金表面更利于成骨细胞粘附。除此之外，应用壳聚糖对钛合金表面进行抗菌改性还有以下几个方面的原因：(1)为减少抗菌物质在体内的蓄积量，降低其细胞毒性。壳聚糖可同时携载多种少量抗菌物质发挥协同作用，在减少携载量的同时保证其抗菌性。Bakhshandeh 等[21]利用壳聚糖涂层将纳米银离子与万古霉素同时引入钛合金表面，虽两者的携载量较对照组(单纯携载万古霉素或银离子)少，但其抗菌性与对照组并无显著差异。(2)为避免抗生素耐药菌株的形成，壳聚糖可携载抗生素之外的抗菌物质，如纳米银离子颗粒、铜离子、锌离子及镉离子等[22-25]。(3)壳聚糖可改变自身化学结构，在保留原有特性的同时，进一步增强其抗菌性及稳定性。将壳聚糖中的氨基转化为亲水性能更强的季铵基团可得到壳聚糖季铵盐，与壳聚糖相比，在生理 PH 值条件下其水溶性及稳定性更佳，抗菌性

更强，即便是对于耐甲氧西林金黄色葡萄球菌也具备抗菌作用[26]。Yang 等[27]在钛合金材料表面制备携载有壳聚糖季铵盐的钛纳米管，并将其放入金黄色葡萄球菌感染的小鼠股骨远端中，结果表明该材料能有效抑制局部细菌生长，促进骨质愈合，并且有研究表明该材料的抗菌性可随钛纳米管的直径增大而加强[28]。(4)壳聚糖涂层可控制局部抗菌药物释放效率，延长其抗菌时间。Mohan 等[29]在携载有抗菌药物的钛纳米管上覆盖壳聚糖涂层，药物动力学研究表明钛纳米管内抗菌药物的释放可达到零级动力学释放，并且通过调整涂层厚度使得抗菌药物的释放与术后治疗时间窗相匹配。壳聚糖表面改性钛合金材料改善其抗菌性的方式总结于图 1。

4　总结与展望

应用壳聚糖对钛合金材料表面进行改性主要是将壳聚糖的特性赋予钛合金材料，除能有效弥补钛合金材料骨整合性及抗菌性差的弊端外，还可减少氧自由基对成骨细胞功能的损害[30-31]，促进骨质疏松患者术后内植入物表面骨组织的长入[32-33]，携载特定药物在局部抗肿瘤或抗炎治疗[34-35]等，可有效降低糖尿病、骨质疏松患者应用钛合金内植入材料失败的风险[36-37]，预防骨肿瘤的复发，实现了钛合金材料多元化应用，提高了其临床应用价值。有理由相信随着对壳聚糖性能及应用研究的不断深入，钛合金材料的生物学性能及应用范围还会进一步得到提升。

参考文献

[1] Liu XY, Chu PK, Ding CX. Surface modification of titanium, titanium alloys, and related materials for biomedical applications [J]. Mater Sci Eng R-Reports, 2004, 47(3-4): 49-121.

[2] Azuma K, Ifuku S, Osaki T, et al. Preparation and biomedical applications of chitin and chitosan nanofibers [J]. J Biomed Nanotechnol, 2014, 10(10): 2891-920.

[3] Martin HJ, Schulz KH, Bumgardner JD, et al. XPS study on the use of 3-aminopropyltriethoxysilane to bond chitosan to a titanium surface [J]. Langmuir, 2007, 23(12): 6645-6651.

[4] Valverde A, Perez-Alvarez L, Ruiz-Rubio L, et al. Antibacterial hyaluronic acid/chitosan multilayers onto smooth and micropatterned titanium surfaces [J]. Carbohydr Polym, 2019, 207: 824-833.

[5] Liu P, Hao Y, Zhao Y, et al. Surface modification of titanium substrates for enhanced osteogenetic and antibacterial properties [J]. Colloids Surf B Biointerfaces, 2017, 160: 110-116.

[6] Suo L, Jiang N, Wang Y, et al. The enhancement of osseointegration using a graphene oxide/chitosan/hydroxyapatite composite coating on titanium fabricated by electrophoretic deposition [J]. J Biomed Mater Res B Appl Biomater, 2019, 107(3): 635-645.

[7] Zhao L, Chu P K, Zhang Y, et al. Antibacterial coatings on titanium implants [J]. J Biomed Mater Res B Appl Biomater, 2009, 91(1): 470-480.

[8] Junter GA, Thebault P, Lebrun L. Polysaccharide-based antibiofilm surfaces [J]. Acta Biomater, 2016, 30: 13-25.

[9] Bumgardner JD, Chesnutt BM, Yuan Y, et al. The integration of

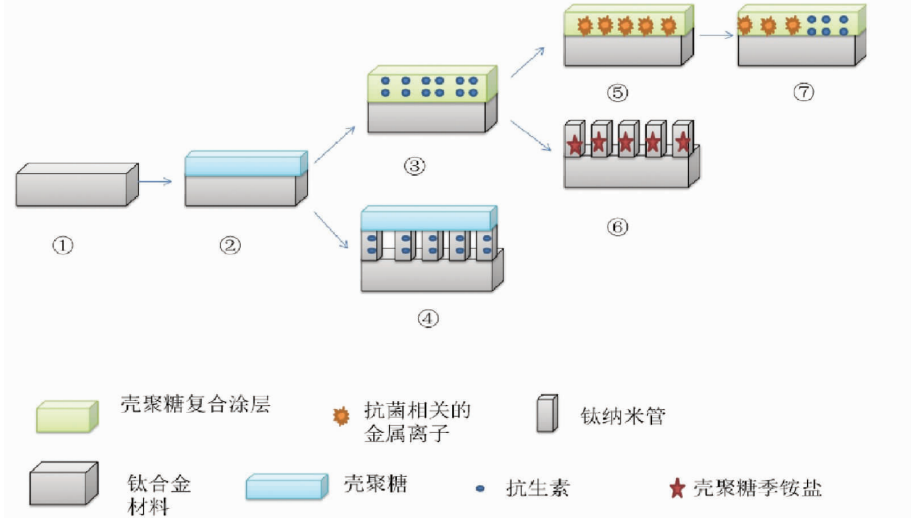

图 1　壳聚糖表面改性钛合金材料改善其抗菌性的方式。①未经任何处理的钛合金材料；②在钛合金表面制备壳聚糖涂层；③钛合金表面制备携载抗生素的壳聚糖复合涂层；④在钛纳米管表面覆盖壳聚糖涂层以控制抗生素的释放率；⑤钛合金表面制备携载有抗菌离子以减少抗生素耐药菌株的发生；⑥壳聚糖的衍生物-壳聚糖季铵盐通过钛纳米管引入钛合金表面；⑦制备同时携载有抗菌离子及抗生素的壳聚糖复合涂层，以减少抗菌物质的携载量，降低抗菌药物的细胞毒性

Fig.1　The way to improve the antibacterial property of titanium alloy by chitosan surface modification. ① Titanium alloy materials without any treatment; ② chitosan coating on titanium alloy surface; ③ chitosan composite coating with antibiotics on titanium alloy surface; ④ chitosan coating on titanium nanotube surface to control the release rate of antibiotics; ⑤ antibacterial ions on titanium alloy surface to reduce the occurrence of antibiotic resistant strains; ⑥ Chitosan Derivative Chitosan Sugar quaternary ammonium salt is introduced into the surface of titanium alloy through titanium nanotubes; ⑦ chitosan composite coating with antibacterial ions and antibiotics is prepared to reduce the carrying capacity of antibacterial substances and the cytotoxicity of antibacterial drugs

chitosan-coated titanium in bone: an in vivo study in rabbits[J]. Implant Dent, 2007, 16(1): 66–79.

[10] Poth N, Seiffart V, Gross G, et al. Biodegradable chitosan nanoparticle coatings on titanium for the delivery of BMP–2[J]. Biomolecules, 2015, 5(1): 3–19.

[11] Venkatesan J, Anil S, Kim SK, et al. Chitosan as a vehicle for growth factor delivery: Various preparations and their applications in bone tissue regeneration[J]. Int J Biol Macromol, 2017, 104(Pt B): 1383–1397.

[12] Meng YB, Li X, Li ZY, et al. microRNA–21 promotes osteogenic differentiation of mesenchymal stem cells by the PI3K/beta-catenin pathway[J]. J Orthop Res, 2015, 33(7): 957–964.

[13] Wang Z, Hu Z, Zhang D, et al. Silencing tumor necrosis factor-alpha in vitro from small interfering RNA-decorated titanium nanotube array can facilitate osteogenic differentiation of mesenchymal stem cells[J]. Int J Nanomedicine, 2016, 11: 3205–3214.

[14] Chen Y, Frith JE, Dehghan-Manshadi A, et al. Biocompatible porous titanium scaffolds produced using a novel space holder technique[J]. J Biomed Mater Res B Appl Biomater, 2018, 106(8): 2796–2806.

[15] Wang Z, Wu G, Feng Z, et al. Microarc-oxidized titanium surfaces functionalized with microRNA-21-loaded chitosan/hyaluronic acid nanoparticles promote the osteogenic differentiation of human bone marrow mesenchymal stem cells[J]. Int J Nanomedicine, 2015, 10: 6675–6687.

[16] Foss BL, Ghimire N, Tang R, et al. Bacteria and osteoblast adhesion to chitosan immobilized titanium surface: A race for the surface[J]. Colloids Surf B Biointerfaces, 2015, 134: 370–376.

[17] Han L, Wang M, Sun H, et al. Porous titanium scaffolds with self-assembled micro/nano-hierarchical structure for dual functions of bone regeneration and anti-infection[J]. J Biomed Mater Res A, 2017, 105(12): 3482–3492.

[18] Sutrisno L, Hu Y, Shen X, et al. Fabrication of hyaluronidase-responsive biocompatible multilayers on BMP2 loaded titanium nanotube for the bacterial infection prevention[J]. Mater Sci Eng C Mater Biol Appl, 2018, 89: 95–105.

[19] Ghimire N, Foss BL, Sun Y, et al. Interactions among osteoblastic cells, Staphylococcus aureus, and chitosan-immobilized titanium implants in a postoperative coculture system: An in vitro study[J]. J Biomed Mater Res A, 2016, 104(3): 586: 594.

[20] Raafat D, Sahl HG. Chitosan and its antimicrobial potential–a critical literature survey[J]. Microb Biotechnol, 2009, 2(2): 186–201.

[21] Bakhshandeh S, Gorgin Karaji Z. Simultaneous delivery of multiple antibacterial agents from additively manufactured porous biomaterials to fully eradicate planktonic and adherent staphylococcus aureus[J]. ACS Appl Mater Interfaces, 2017, 9(31): 25691–25699.

[22] Huang D, Ma K, Cai X, et al. Evaluation of antibacterial, angiogenic, and osteogenic activities of green synthesized gap-bridging copper-doped nanocomposite coatings[J]. Int J Nanomedicine, 2017, 12: 7483: 7500.

[23] Huang P, Ma K, Cai X, et al. Enhanced antibacterial activity and biocompatibility of zinc-incorporated organic-inorganic nanocomposite coatings via electrophoretic deposition[J]. Colloids Surf B Biointerfaces, 2017, 160: 628–638.

[24] Bonifacio MA, Cometa S, Dicarlo M, et al. Gallium-modified chitosan/poly(acrylic acid) bilayer coatings for improved titanium implant performances[J]. Carbohydr Polym, 2017, 166: 348–357.

[25] Cheng YF, Zhang JY, Wang YB, et al. Deposition of catechol-functionalized chitosan and silver nanoparticles on biomedical titanium surfaces for antibacterial application[J]. Mater Sci Eng C Mater Biol Appl, 2019, 98: 649–656.

[26] Tan HL, Ao HY, Ma R, et al. In vivo effect of quaternized chitosan-loaded polymethylmethacrylate bone cement on methicillin-resistant Staphylococcus epidermidis infection of the tibial metaphysis in a rabbit model[J]. Antimicrob Agents Chemother, 2014, 58(10): 6016–6023.

[27] Yang Y, Ao H, Wang Y, et al. Cytocompatibility with osteogenic cells and enhanced in vivo anti-infection potential of quaternized chitosan-loaded titania nanotubes[J]. Bone Res, 2016, 4: 16027.

[28] Lin WT, Zhang YY, Tan HL, et al. Inhibited bacterial adhesion and biofilm formation on quaternized chitosan-loaded titania nanotubes with various diameters[J]. Materials (Basel), 2016, 9(3): 155.

[29] Mohan L, Anandan C, Rajendran N. Drug release characteristics of quercetin-loaded TiO2 nanotubes coated with chitosan[J]. Int J Biol Macromol, 2016, 93(Pt B): 1633–1638.

[30] Lee YH, Kim JS, Kim JE, et al. Nanoparticle mediated PPARgamma gene delivery on dental implants improves osseointegration via mitochondrial biogenesis in diabetes mellitus rat model[J]. Nanomedicine, 2017, 13(5): 1821–1832.

[31] Ma XY, Feng YF, Wang TS, et al. Involvement of FAK-mediated BMP–2/Smad pathway in mediating osteoblast adhesion and differentiation on nano-HA/chitosan composite coated titanium implant under diabetic conditions[J]. Biomater Sci, 2017, 6(1): 225–238.

[32] Mu C, Hu Y, Huang L, et al. Sustained raloxifene release from hyaluronan-alendronate-functionalized titanium nanotube arrays capable of enhancing osseointegration in osteoporotic rabbits[J]. Mater Sci Eng C Mater Biol Appl, 2018, 82: 345–353.

[33] Huang L, Luo Z, Hu Y, et al. Enhancement of local bone remodeling in osteoporotic rabbits by biomimic multilayered structures on Ti6Al4V implants[J]. J Biomed Mater Res A, 2016, 104(6): 1437–1451.

[34] Krukiewicz K, Zak JK. Biomaterial-based regional chemotherapy: Local anticancer drug delivery to enhance chemotherapy and minimize its side-effects[J]. Mater Sci Eng C Mater Biol Appl, 2016, 62: 927–942.

[35] Pawlik A, Jarosz M, Syrek K, et al. Co-delivery of ibuprofen and gentamicin from nanoporous anodic titanium dioxide layers[J]. Colloids Surf B Biointerfaces, 2017, 152: 95–102.

[36] Yi C, Hao K Y, Ma T, et al. Inhibition of cathepsin K promotes osseointegration of titanium implants in ovariectomised rats[J]. Sci Rep, 2017, 7: 44682.

[37] Chen W, Shen X, Hu Y, et al. Surface functionalization of titanium implants with chitosan-catechol conjugate for suppression of ROS-induced cells damage and improvement of osteogenesis[J]. Biomaterials, 2017, 114: 82–96.

（收稿日期：2019–11–12　本文编辑：连智华）