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·基础研究·

钛表面不同去污方式的体外效果比较

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【摘要】 目的 探讨光动力疗法、喷砂、钛刮匙不同的去污方式对钛表面形貌及细菌黏附的影响，为种植体周围病的治疗提供参考。**方法** 分别将牙龈卟啉单胞菌(*Porphyromonas gingivalis*，*Pg*)和具核梭杆菌(*Fusobacterium nucleatum*，*Fn*)接种于经抛光处理的钛试样表面，培养后使用不同去污方式对钛试样表面进行处理。根据不同去污方式将钛试样分为无处理对照组、光动力组、喷砂组、钛刮匙组。原子力显微镜观测钛表面粗糙度变化，扫描电镜、活/死菌染色实验观察钛表面剩余细菌情况；在去污后的钛试样表面重新接种*Pg*和*Fn*后观察细菌再黏附情况。**结果** 原子力显微镜观测表面粗糙度结果显示钛刮匙组显著高于对照组、光动力组及喷砂组($P<0.05$)，对照组、光动力组及喷砂组组间差异均无统计学意义($P>0.05$)。接触角测量结果显示各处理组表面接触角均小于对照组($P<0.05$)。扫描电镜结果显示，钛试样表面去污后对照组表面剩余细菌数量较多，且细菌相对集中，光动力组、喷砂组、钛刮匙组表面细菌散落分布，数量较少，且光动力组表面大部分细菌菌体破裂。活/死菌染色实验结果显示光动力组表面死菌比例显著高于对照组、喷砂组及钛刮匙组($P<0.05$)，喷砂组和钛刮匙组表面剩余细菌以活菌为主；喷砂组表面剩余细菌黏附量与对照组及光动力组、钛刮匙组相比显著减少($P<0.05$)。钛试样表面细菌再黏附的扫描电镜及活/死菌染色结果显示，钛刮匙组表面牙龈卟啉单胞菌有聚集现象，其表面细菌黏附量显著高于对照组、光动力组及喷砂组($P<0.05$)。**结论** 喷砂去污效果优于光动力疗法和钛刮匙，但并不能完全清除细菌污染；光动力疗法杀菌效果优于喷砂和钛刮匙；在临床实践中可以考虑喷砂和光动力疗法相结合的方法治疗种植体周围病。

【关键词】 种植体周围炎；牙龈卟啉单胞菌；具核梭杆菌；光动力疗法；喷砂；钛刮匙；种植体；粗糙度；菌斑；去污；细菌黏附

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Effects of different debridement methods on titanium surfaces *in vitro*

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【Abstract】 Objective To investigate the effect of different decontamination methods, including photodynamic therapy, sandblasting and titanium curette, on titanium surface morphology and bacterial adhesion for the treatment of peri-

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implant disease. **Methods** *Porphyromonas gingivalis* (*Pg*) and *Fusobacterium nucleatum* (*Fn*) were inoculated on the surface of polished titanium specimens, and titanium specimen surfaces were treated with different decontamination methods after incubation. The titanium specimens were divided into a no-treatment control group, photodynamic group, sandblasting group and titanium curette group according to different decontamination methods. The changes in titanium surface roughness were observed by atomic force microscopy (AFM), and the remaining bacteria on the titanium surface were observed by scanning electron microscopy (SEM) and live/dead bacteria staining tests. After reinoculation of *Pg* and *Fn*, bacterial readhesion was observed on the surface of decontaminated titanium specimens. **Results** The AFM results showed that the surface roughness of the titanium curette group was significantly higher than that of the no-treatment control group, photodynamic group and sandblasting group ($P < 0.05$), and there was no statistically significant difference between the no-treatment control group, photodynamic group and sandblasting group ($P > 0.05$). The results of contact angle measurement showed that the surface contact angle of each treatment group was smaller than that of the no-treatment control group ($P < 0.05$). The SEM results obtained after the titanium specimen surface was decontaminated showed that the number of bacteria on the no-treatment control group surface was higher and the bacteria were relatively concentrated. The bacteria on the surface of the photodynamic group, sandblasting group and titanium curette group were scattered and distributed in small numbers, and most bacteria on the surface of the photodynamic group were ruptured. The results of the live/dead bacteria staining experiment showed that the percentage of dead bacteria on the surface of the photodynamic group was significantly higher than that of the no-treatment control group, sandblasting group and titanium curette group ($P < 0.05$). The remaining bacteria on the surface of the sandblasting group and titanium curette groups were mainly live bacteria. The remaining bacterial adhesion on the surface was significantly reduced for the sandblasting group compared to the no-treatment control group and the photodynamic and titanium curette groups ($P < 0.05$). SEM and live/dead bacteria staining results of bacterial readhesion on the surface of titanium specimens showed that there was an aggregation of *Pg* on the surface of the titanium curette group, and its surface bacterial adhesion was significantly higher than that of the no-treatment control group, photodynamic group and sandblasting group. **Conclusion** In mechanical decontamination, sandblasting machines are a better option than photodynamic therapy and titanium curettes; however, sandblasting does not remove all bacterial contamination. For sterilization, photodynamic therapy is more effective than sandblasting and titanium curettes. A combination of sandblasting and photodynamic therapy methods for the treatment of peri-implant disease may be considered in clinical practice.

【Key words】 peri-implantitis; *Porphyromonas gingivalis*; *Fusobacterium nucleatum*; photodynamic therapy; sandblasting; titanium curette; implant; roughness; plaque; debridement; bacterial adhesion

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随着口腔种植修复治疗技术的不断发展，影响种植体长期稳定性的生物学并发症成为当代口腔医学的一个重要课题[1]。种植体生物学并发症主要指种植体周围病，包括种植体周围黏膜炎和种植体周围炎。种植体周围黏膜炎是指没有支持骨或没有持续性边缘骨丧失的种植体周围软组织的炎症性病变[2]，是程度较轻的种植体周围疾病；而种植体周围炎以种植体周围结缔组织的炎症及支持骨组织的进行性吸收为特征[3]，往往导致种植体预后不佳；一项meta分析显示，种植体周围黏膜炎的患病率为23.9%～88.0%，种植体周围炎患病率为8.9%～45%[4]，这一方面表明种植体周围病的患病率较高，另一方面也表明因不同人群、检测标准、主客观条件等影响其差异较大。Monje等[5]的一项横断面研究发现，在种植后平均46个月的治疗随访期中，坚持进行维护治疗的患者比依从性差的患者被诊断为种植体周围炎的可能性更小。因此，菌斑控制是预防和治疗种植体周围病的关键[6]。

种植体周的菌斑主要由革兰阳性需氧菌和革兰阴性厌氧菌等组成，当种植体周围组织存在炎症时，厌氧菌明显增多[7]。此外，细菌定植主要与种植体表面特性有关[8]，因此，探索种植体表面清洁去污方式是否会改变种植体表面特性从而影响

细菌黏附对于控制种植体早期黏膜炎症以及加强日常定期维护尤为重要。目前治疗种植体周围病的方法主要分为非手术治疗和手术治疗，非手术治疗包括机械清创、喷砂处理、激光治疗、光动力疗法等。然而，上述治疗方式是否改变种植体表面特性及后续细菌黏附数量仍需要更多研究，目前种植体周围病的处理仍缺乏公认的标准化治疗方案。本实验拟通过评估光动力疗法、龈下喷砂以及钛刮匙3种目前常用的去污清洁方式对种植体钛材表面特性的影响，并进行细菌清除效率及后期细菌再次黏附的比较，为临床预防及治疗种植体周围病提供实验依据。

1　材料和方法

1.1　主要试剂及仪器

牙龈卟啉单胞菌（*Porphyromonas gingivalis*，*Pg*）（ATCC33277，空军军医大学口腔医院微生物实验室）；具核梭杆菌（*Fusobacterium nucleatum*，*Fn*）（ATCC10953，空军军医大学口腔医院微生物实验室）；纯钛试件（10 mm × 10 mm × 1.5 mm，弘森，中国）；厌氧产气袋（MGC，日本）；脑心浸出液（BHI）培养基（海博，中国）；脱纤维羊血（鸿泉，中国）；氯化血红素（海博，中国）；维生素K1（海博，中国）；活/死菌染色试剂盒（LIVE/DEAD BacLight™ L7012，Invitrogen，美国）。恒温培养箱（IFA-32-8，ESCO，新加坡）；生物安全柜（CB 800 V，苏洁，苏州）；原子力显微镜（5500，Keysight，中国），接触角测量仪（DSA25，Kruss，德国）；场发射扫描电镜（S4800，日立，日本）；激光共聚焦显微镜（FV3000，Olympus，日本）；酶标仪（Infinite200，Tecan，瑞士）；光动力仪（HHL-1000，Periowave，中国）；喷砂机（FT-200，EMS，瑞士）；钛刮匙（IMPLG1/2T，Hu-Friedy，美国）。

1.2　试样处理及制备

将纯钛钛片依次使用800、1 200、1 500、2 000、3 000、5 000、7 000目砂纸逐级打磨抛光至光滑镜面，使用丙酮、无水乙醇、去离子水依次超声荡洗15 min，干燥消毒备用。

1.3　细菌培养和菌液准备

将*Pg*和*Fn*分别接种于BHI培养基中（每100 mL培养基中含BHI 5.2 g，脱纤维羊血10 mL，氯化血红素0.5 mL，维生素K 100 μL），37 ℃厌氧培养48 h，利用比浊仪测定细菌光密度值，倍比稀释至10^7 CFU/mL。将无菌钛试样放入24孔板中，分别将稀释后的*Pg*菌液和*Fn*菌液接种于材料表面（1 mL/孔），置于37 ℃恒温箱中厌氧培养48 h。

1.4　实验分组

将24孔板中钛试样使用PBS缓冲液冲洗3次，随机分为4组，每组每种菌12个试样，处理组按以下方式进行清洁和器械处理，所有操作均由同一名实验员进行。①钛刮匙组（titanium curette，TiC）：使用钛刮匙刮除钛片表面细菌，力量与去除天然牙表面牙石相当（约20 ~ 25 g），作用60 s；②光动力组（photodynamic，PTD）：钛片表面注射光敏剂1.5 μL，导光头呈15 °照射60 s；③喷砂组（sand-blasting，SB）：使用perio模式，功率及水位调节至“Max”，距离钛片表面约2 mm，作用30 s[9-10]；④对照组（NC）：表面不作任何去污处理。

1.5　钛试样表征

1.5.1　原子力显微镜测量粗糙度

每组每种菌任选3个试样临界点干燥，利用原子力显微镜测量试样表面粗糙度，每个试样任取3个测试点，结果取均值。

1.5.2　接触角测量仪测量接触角

每组每种菌任选3个试样临界点干燥，湿度45%条件下，每次滴加2 μL超纯水于试样上，待液滴形态趋于稳定，利用接触角测量仪测量水滴与试样表面间的接触角。每个试样任取3个测试点，结果取均值。

1.6　细菌黏附检测

1.6.1　扫描电镜观察去污后的钛表面形貌和细菌黏附形态

每组每种菌任选3个试样移至无菌24孔板，2.5%戊二醛溶液固定4 h，PBS冲洗，梯度乙醇（30%、50%、70%、90%、95%、100%）脱水，每个梯度7 min。干燥、喷金后置于扫描电镜下观察。

1.6.2　活/死菌染色法观察细菌活性

每组每种菌任选3个试样移至无菌24孔板，参照微生物细胞活性检测试剂盒说明书，配制检测试剂，将试剂加入放置试样的24孔板中避光孵育15 min后吸出，即刻置于激光共聚焦显微镜下拍摄荧光图像，使用Image J软件将荧光图像转换成灰度图，设定分析参数后获得Mean（荧光强度）值。

1.7　细菌再黏附实验

将实验1.4中去污处理后的钛试样放入2 mL PBS中使用震荡机震荡5 min，丙酮、无水乙醇、去离子水依次超声荡洗15 min，高压灭菌后分组放入24孔板中，再次接种*Pg*菌液和*Fn*菌液（1 mL/孔），浓度为10^7 CFU/mL，置于37 ℃恒温箱中厌氧培养

48 h后按方法1.6进行实验，每组每种菌任选3个试样，重复3次。

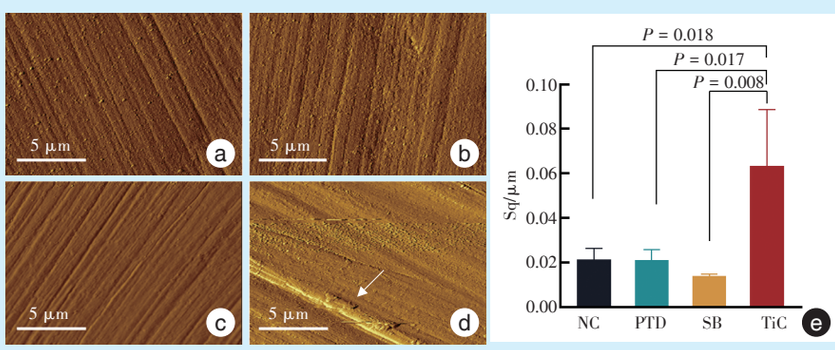
1.8　统计学分析

使用SPSS19.0统计软件进行统计学分析，检测各组数据均符合正态性，采用单因素方差分析比较不同组间整体差异，经Levene's方差齐性检验，方差齐性者采用Bonferroni进行两两分析比较，若方差不齐者，采用Tamhane's T2进行两两分析比较，检测水准为双侧α=0.05。

2　结　果

2.1　钛试样经去污处理后表面粗糙度及接触角比较

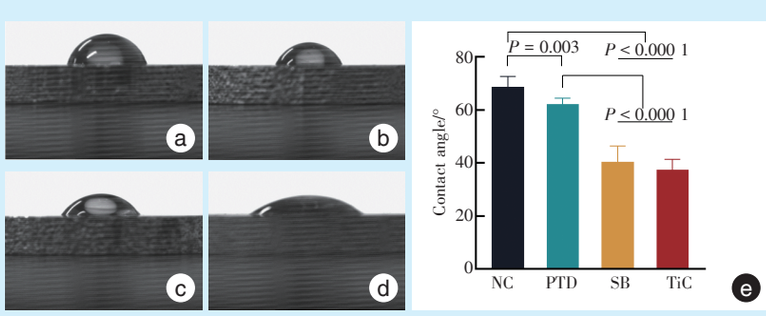
原子力显微镜观测结果显示，钛刮匙组表面刮痕明显，粗糙度显著高于对照组、光动力组及喷砂组（$P<0.05$），对照组、光动力组及喷砂组组间差异均无统计学意义（$P>0.05$）（图1）。接触角测量结果显示，各处理组表面接触角均小于对照组，差异具有统计学意义（$P<0.05$）（图2）。



The titanium specimens were divided into a no-treatment control group (NC group), photodynamic group (PTD group), sandblasting machine group (SB group) and titanium curette group (TiC group) according to different decontamination methods. Bar = 5 μm. a: NC group, smooth surface of titanium specimens with uniform scratches; b: PTD group, no significant change was observed in the titanium surface after photodynamic decontamination treatment; c: SB group, no significant change was observed in the titanium surface after erythritol sandblasting treatment; d: TiC group, titanium curette leaves deep scratches on titanium surface after decontamination treatment (as arrow showed); e: roughness measurement results: Sq: the root mean square height

Figure 1　Surface roughness change of titanium surfaces observed under atomic force microscopy after different decontamination treatments

图1　原子力显微镜下观测经不同去污处理后钛试样表面粗糙度改变



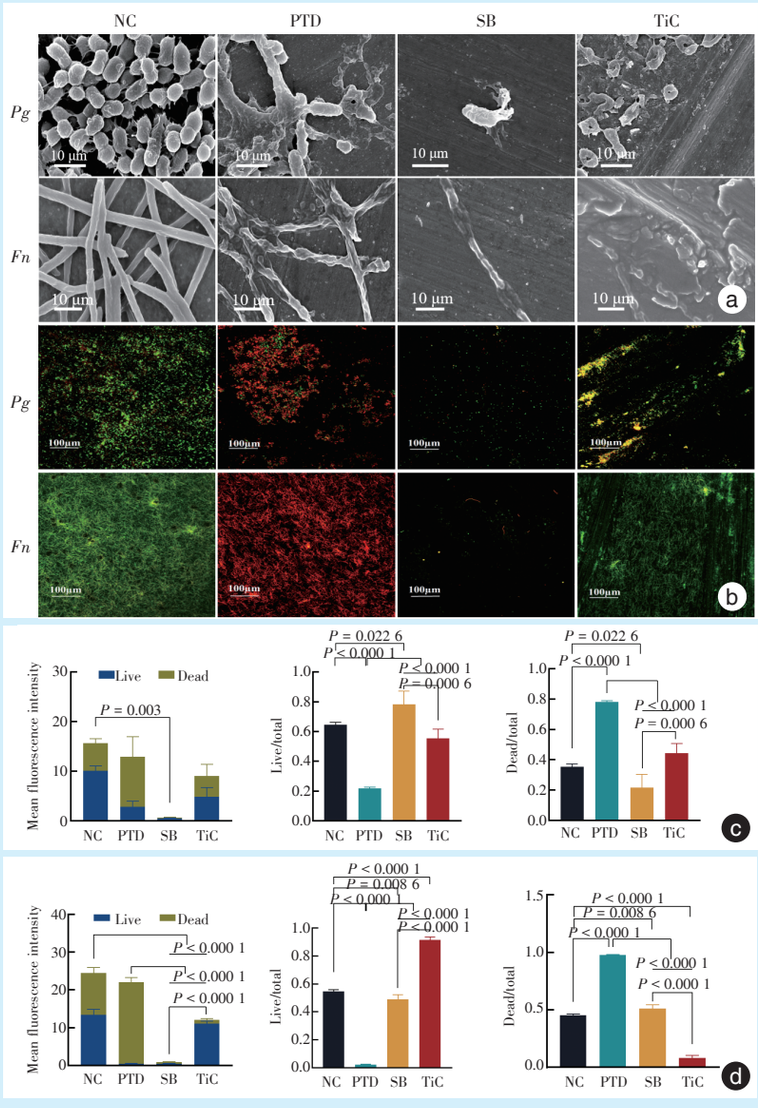
The titanium specimens were divided into a no-treatment control group(NC group), photodynamic group (PTD group), sandblasting machine group (SB group) and titanium curette group (TiC group) according to different decontamination methods. a: NC group; b: PTD group; c: SB group; d: TiC group. Increased hydrophilicity of titanium surface after decontamination treatment. e: contact angle measurement results

Figure 2　Contact angle change observed by contact angle measuring instrument on titanium surface after different decontamination treatment

图2　接触角测量仪观测经不同去污处理后钛表面接触角改变

2.2　钛试样经去污处理后扫描电镜及荧光染色分析

扫描电镜结果显示，对照组钛试样表面细菌数量较多，且细菌相对集中，光动力组、喷砂组、钛刮匙组表面细菌散落分布，数量较少，且光动力组表面大部分细菌菌体破裂（图3a）。在激光共聚焦显微镜下观察各组钛试样表面黏附活菌量、死菌量及黏附总量用平均荧光强度，对照组表面视野内有大片被荧光标记的活菌，光动力组表面死菌比例显著高于对照组及喷砂组、钛刮匙组，差异具有统计学意义（$P<0.05$），喷砂组和钛刮匙组表面剩余细菌以活菌为主，喷砂组表面细菌黏附量与对照组及光动力组、钛刮匙组相比显著减少，差异具有统计学意义（$P<0.05$）（图3b～3d）。



The titanium specimens were divided into a no-treatment control group (NC group), photodynamic group (PTD group), sandblasting machine group (SB group) and titanium curette group (TiC group) according to different decontamination methods. a: adhesion morphology of *Pg* and *Fn* on titanium samples, as observed by scanning electron microscopy, bar = 10 μm, NC group: dense bacteria and intact morphology, PTD group: loss of bacterial integrity and cell membrane rupture on titanium surfaces; SB group: significant reduction in bacterial density on titanium surfaces; TiC group: reduction in the number of bacteria on titanium surfaces. b: the adhesion of *Pg* and *Fn* on the titanium surface was observed by CLSM, bar = 100 μm, green: live bacteria, red: dead bacteria. A large area of live bacteria was visible on the titanium surface of the NC group, and a large amount of dead bacteria was observed on the surface of the PTD group. The number of bacteria in the SB group was significantly reduced, and live bacteria were predominant. c & d: the total number of adherent bacteria and the ratio of live/dead bacteria of *Pg* (c) and *Fn* (d) on the titanium surface. *Pg*: *Porphyromonas gingivalis*; *Fn*: *Fusobacterium nucleatum*

Figure 3　Comparison of the remaining bacterial adhesion morphology and the number of live/dead bacteria on the titanium surfaces after different decontamination treatments

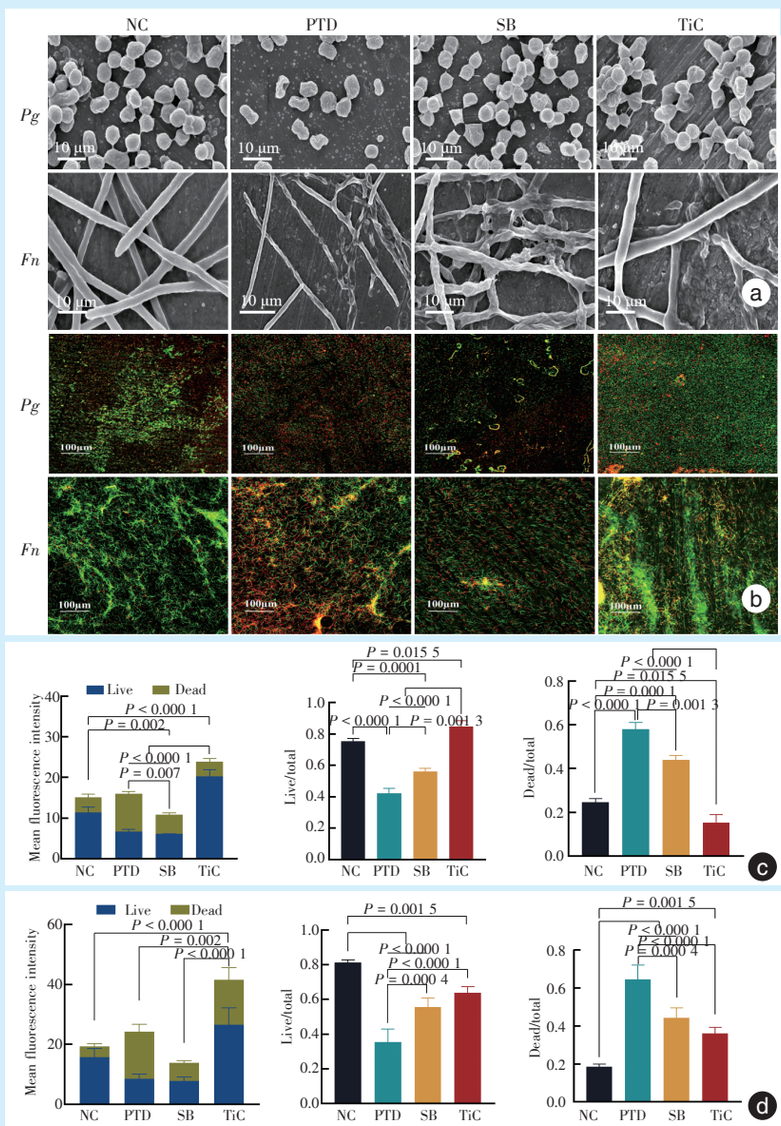
图3　经不同去污处理后钛试样表面剩余细菌黏附形态及活/死菌数量比较

2.3　钛试样表面细菌再黏附的扫描电镜及荧光染色分析

扫描电镜结果显示，钛刮匙组表面牙龈卟啉单胞菌有聚集现象（图4a）。在激光共聚焦显微镜下观察，钛刮匙组表面细菌黏附量显著高于对照组及其余处理组，差异具有统计学意义（$P<0.05$），光动力组表面黏附死菌比例显著高于对照组及其余处理组，差异具有统计学意义（$P<0.05$）（图4b～4d）。

3　讨　论

种植体周围病是导致种植失败的重要因素之一，有学者通过20年随访的纵向研究对种植体存活率进行了评估，发现虽然种植义齿平均累计存活率高达94.6%[11]，但种植体周围病仍是最常见的生物学并发症。种植体周围黏膜炎表现为探诊时出血、红肿和化脓，但仅累及黏膜而无边缘骨的丢失，具有可逆性，被认为是种植体周围炎的前兆；种植体周围炎除黏膜炎症还表现为支持骨的丧失[2-3]。大量研究已经证实，种植体周围病很多是由于菌斑生物膜的堆积引起，通过细菌增殖和生物膜的形成导致种植体周围黏膜发生炎症，随后引起支持骨的逐渐吸收[12]。有研究显示，没有进行定期维护的患者患种植体周围病的风险可能会增加4.25倍[13]。健康种植体周围定植的细菌与天然牙类似，大多为革兰阳性微需氧菌还有少量的革兰阴性厌氧菌[14]，但当种植体周围发生炎症时，革兰阴性厌氧菌及产黑色素厌氧菌、螺旋体等数量开始增多[7]；Sahrmann等[15]通过比较健康种植体和炎症种植体周围微生物图谱发现，炎症种植体



The titanium specimens were divided into a no-treatment control group (NC group), photodynamic group (PTD group), sandblasting machine group (SB group) and titanium curette group (TiC group) according to different decontamination methods. a: readhesion morphology of *Pg* and *Fn* on titanium samples observed by scanning electron microscopy, bar = 10 μm, *Pg* clustered on the titanium surface in the TiC group; b: readhesion of *Pg* and *Fn* on the titanium surface was observed by CLSM, bar = 100 μm, green: live bacteria, red: dead bacteria. c & d: the total number of readherent bacteria and the ratio of live/dead bacteria of *Pg* (c) and *Fn* (d) on the titanium surface. *Pg*: *Porphyromonas gingivalis*; *Fn*: *Fusobacterium nucleatum*

Figure 4　Comparison of bacterial re-adhesion morphology and the number of live/dead bacteria on the titanium surfaces after different decontamination treatments

图4　经不同去污处理后钛试样表面细菌再黏附形态及活/死菌数量比较

周围菌群中牙龈卟啉单胞菌、具核梭杆菌的检出率明显增加，金黄色葡萄球菌在黏膜炎周围尤其是化脓位点检出率较高[16]。因此，破坏及清除黏附在种植体表面的菌斑生物膜是治疗种植体周围病的有效方法。

由于种植体周围病与牙周病相似的临床特征和病因，治疗种植体周围疾病的方法大多由牙周炎的治疗方式迁延而来，临床常用于治疗种植体周围病的去污方式包括机械清创（如金属刮匙、钛刮匙、钛刷、超声洁治器等）、喷砂处理、激光治疗、光动力治疗等，但由于种植体周围菌群的复杂性和种植体表面特性，理想的治疗方式应最大限度清除种植体表面黏附的菌斑生物膜，还要避免破坏种植体表面结构，以期获得良好的预后效果。大多数研究都基于对钛种植体表面黏附生物膜的清除效率来评估去污结果，并未对后期细菌再次黏附钛表面后形态及量的变化进行分析比较，因此，本研究重点在于对不同去污方式是否使钛表面性能发生改变，以及对细菌再附着的可能影响来进行对比性评估，使临床医师了解不同去污方式的作用特点及参考选用。

金属刮匙、超声洁治器等传统的机械清创方式被大量研究证实容易对钛表面造成损伤，且清洁效率较低[17-18]；Larsen等[19]发现钛刷能够明显减少种植体表面牙龈卟啉单胞菌的数量，但也有研究认为钛刷可能导致种植体表面微形貌发生显著改变[20]。Huang等[21]研究发现钛刮匙会在钛表面产生明显划痕，使钛表面粗糙度及亲水性显著增

加，这与本研究结果一致，这些变化可能有利于细菌黏附。在本研究的细菌再次黏附结果中显示，即使是使用与种植体同质的钛刮匙去污处理后，钛材表面的细菌黏附量仍然是最高的。综上，机械去污使钛表面结构发生改变的几率最高，可能会影响后期细菌黏附及骨结合，因此，在常规临床治疗中，应谨慎应用。

喷砂的清洁效率受到喷砂粉的影响。Matsubara等[22]对碳酸氢钠喷砂粉、甘氨酸喷砂粉及赤藓糖醇喷砂粉的去污能力和对种植体表面粗糙度影响进行了体外评估，结果显示碳酸氢钠喷砂粉清洁能力最强，但会显著增加钛种植体表面粗糙度，甘氨酸及赤藓糖醇喷砂粉对种植体表面粗糙度无明显影响，但清洁能力有限。本研究选用了最新改进的赤藓糖醇喷砂粉，结果显示赤藓糖醇喷砂粉处理相对于其他处理组而言，其对钛表面细菌生物膜清除率最高，且对其表面粗糙度无明显影响，但会增加其表面亲水性，这有利于后期细胞与材料表面结合[23]，但也可能会使细菌更容易定植。因此本研究同时对细菌再次黏附在材料表面进行了探讨，结果显示，黏附量与对照组相比无明显差异。这一现象可能与种植体结构有关，钛种植体的螺纹结构可能使清洁效率降低，但新型龈下喷砂嘴能够深入袋内进行清洁，这对种植体周围疾病的治疗将具有积极意义。

光动力作为一种新颖的光化学疗法，通过激光与光敏剂发生的光化学反应，产生的活性氧（reactive oxygen species，ROS）具有杀灭细菌，灭活细菌内毒素等效果[24]，已被用于牙周病和种植体周围病的治疗。一项使用光动力疗法作为机械去污的辅助治疗种植体周围炎的临床研究结果显示，光动力疗法能够有效改善种植体周围菌斑评分、探诊深度、探诊出血和临床附着丧失，减轻种植体周围炎症，其中牙龈卟啉单胞菌的数量显示出明显下降[25]，本研究同样发现光动力对于牙龈卟啉单胞菌和具核梭杆菌的杀灭作用显著，这与Sayar等[26]的研究结果相同，Huang等[27]解释为在病变的种植体周围部位加入光敏剂有助于通过靶向细菌的细菌膜来减少细菌含量，这表明光动力疗法是一种有效的种植体周感染辅助治疗手段。

本研究对光动力、喷砂、钛刮匙三种常用的去污清洁方式对细菌定植的钛表面形貌、清除效率及细菌再次黏附能力进行了对比性评价，结果显示，喷砂在机械去污方面表现较好，能够去除钛表面大部分细菌，但在细菌再次黏附的结果中，笔者发现牙龈卟啉单胞菌与具核梭杆菌在钛材表面的黏附力有一定差异，这提示下一步应深入比较不同细菌间的生物学差异。光动力在杀菌方面具有优越性，且细菌再次黏附于钛表面后发现为死菌黏附量增多，其内在机制值得进一步深入研究探讨；钛刮匙对细菌清除效率较低，并且会使钛表面性能改变，增加细菌后期黏附效果，同时笔者也注意到了牙龈卟啉单胞菌与具核梭杆菌在各组的表现不尽一致，这可能与不同细菌本身的一些特性有关，后续计划下一步从扩大样本量及不同细菌生物学分析等不同方面进行研究和证实。本实验结果提示喷砂处理联合光动力疗法可能对种植体周围疾病的治疗更为有效，但本研究为体外实验，未来仍需更多体内实验验证。

【Author contributions】 Ge XT performed the experiments and wrote the article. Ye QY, Wang JJ, Zhang XG, Wang YZ, Wang XY and Ji JY performed the experiments and revised the article. Wang QT designed the study and revised the article. All authors read and approved the final manuscript as submitted.

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