

REPORT

Discussion on the development of nano Ag/TiO₂ coating bracket and its antibacterial property and biocompatibility in orthodontic treatment

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Abstract: This paper aims to explore the antibacterial property of nano Ag/TiO₂ coating bracket for the common bacteria in oral cavity, and discuss its biocompatibility. Micro morphology in the surface of nano Ag/TiO₂ coating bracket was detected by scanning electron microscope (SEM), and surface roughness of ordinary mental bracket, nano TiO₂ coating bracket and nano Ag/TiO₂ coating bracket were measured. First, antibacterial property of nano Ag/TiO₂ coating bracket on the common bacteria in oral cavity was studied by sticking membrane method. Secondly, bonding strength of nano TiO₂ coating and nano Ag/TiO₂ coating bracket in groups were detected by scratching test. The result showed that, the synthetic nano Ag/TiO₂ coating was nanogranular films with rigorous organizational structure, presenting as smooth and clean surface, and antibacterial rate of nano Ag/TiO₂ coating for the common bacteria in oral cavity for 20 min was more than 79% in the dark. All the findings suggested that, nano Ag/TiO₂ coating bracket not only has antibacterial effect but also has good biocompatibility, therefore, it can satisfy the clinical request of orthodontic treatment.

Keywords: Nano Ag/TiO₂, antibacterial property, biocompatibility.

INTRODUCTION

With the development of science of dental materials (Xu *et al.*, 2014), emergence of nano material becomes new choice for the development of new bracket material. Presently, nano antibacterial materials constitute the hotspot of dental material research at present. Combination of nano Ag and inorganic antibacterial carrier TiO₂ is an important direction for new antibacterial property. Biocompatibility refers to biological, physical and chemical reaction produced by the mutual effect between material and organism (Rui and Qingshan, 2011).

Shi Jianwei *et al.* proposed to focus on performance characteristics and application evolution of Ti and Ti alloy that are represented by Ni-Ti wire, β -Ti wire, Ni-Ti spring, titanium-screw and titanium plate (Jianwei *et al.*, 2013). In order to improve the performance of orthodontic archwire, Zhang Hao, *et al.* put forward to decrease frictional coefficient of orthodontic archwire to improve clinical treatment effectiveness and studied the development of corrosion resistance, biocompatibility and aesthetic performance of archwire (Hao *et al.*, 2013). Xie Meng *et al.* analyzed the similarity and difference of research hotspot of oral medicine at home and abroad in detail, and discussed the research hotspot distribution of

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oral medicine paper at home and abroad as well as consistency and difference of those hotspots (Meng and Jiayu, 2013).

This paper proposes a new prevention and treatment method, i.e., preparing nano Ag/TiO₂ coating bracket and improving photocatalytic rate of coating, and further studied its antibacterial property and biocompatibility, so as to promote the wide application of nano technology in the field of orthodontics materials.

MATERIALS AND METHODS

Experimental strains

S. mutans "ATCC25175"; *S. sanguis* "ATCC10556"; *A. actinomycetemcomitans* "ATCC29523"; *F. nucleatum* "ATCC25586"; *P. gingivalis* "ATCC33277"; *P. intermedia* "ATCC25611".

Experimental method

Preparation of nano Ag/TiO₂ coating bracket

Ag/TiO₂ thin film was used for coating on the surface of ordinary mental bracket with spin-on deposition to prepare nano Ag/TiO₂ coating bracket and detailed steps can refer to preliminary study (Zhaohui, 2008). The same method was used to coat nano TiO₂ thin film and Ag/TiO₂ thin film on stainless steel 17-4 in specification of 20mm×20mm×1 mm.

Analysis by scanning electron microscope (SEM)

SEM was used to observe surface appearance, microstructure and fracture surface of ordinary metal bracket processed by metal spraying, nano TiO₂ coating bracket and nano Ag/TiO₂ coating bracket.

Antibacterial activity detection on common bacteria of oral cavity

Lyophilized strains of anaerobic bacteria were incubated on blood agar plate for 48h under anaerobic condition. Then they were centrifuged, rinsed by PBS buffer solution and finally diluted with water to achieve 1×10⁶CFU/mL. A volume of 0.2mL bacterial culture broth was added on nano Ag/TiO₂ thin film and the control sample stainless steel 17-4. Every sample was triplicated and were covered by 15mm × 15 mm polyethylene film smoothly so as to make the bacterial culture broth contact with sample evenly. After keeping in the dark for 20min, the broth was separated from the samples. Then the bacterial culture broth was made up to 100 mL and diluted to 1/100 dilution. Thereafter, the solution was put on blood agar medium and incubated in bacteriological incubator at 37°C for 36h. Then observe and calculate the survival rate. Experiment on every sample was repeated thrice. Experimental steps above were repeated on nano Ag/TiO₂ thin film and nano TiO₂ thin film. After placing for 5, 10, 20, 30, 60, 120, 240 min, respectively, bacterial culture broth was separated from the samples and made up to 100mL. Then dilute them to 1/100 dilution. After that, the solution was put on BHI blood agar medium and cultured at 37°C. After 36 h, the survival rate was calculated. The formula is as follows:

$$\text{Antibacterial rate R (\%)} = \left(\frac{\text{CFU of blank control group} - \text{CFU of experimental group}}{\text{CFU of blank control group}} \right) \times 100$$

CFU: Colony Forming Units

Statistical method

SPSS 17.0 software was used for statistical analysis. Measurement data was expressed by Mean ±SD and mean was analyzed by variance. P<0.05 was considered as difference was statistically significant.

RESULTS

Observing surface appearance of nano Ag/TiO₂ coating bracket by SEM

Figure 1 demonstrated that, the surface of metal bracket was still rough and had depression in the shape of gully and pit though it had been polished; nano TiO₂ coating is granular film composed of many tiny spherical particles and the particle size of TiO₂ was 10~30 nm; also, nano TiO₂ coating was granular film composed of many tiny spherical particles, with rigorous organizational structure; TiO₂ particles was uniform; Ag particle with size of 50~100 nm was evenly distributed on thin film with rare reunion, as shown in fig. 1.

Detection result of antibacterial activity of nano TiO₂ coating, nano Ag/TiO₂ coating on the common bacterial in oral cavity

Table 1 demonstrates that, nano Ag/TiO₂ had antibacterial activity to all experimental bacteria in the dark, and the antibacterial activity after 20 min could be more than 79%. Table 2 demonstrates that, nano Ag/TiO₂ in the dark after 30~60 min could completely kill the common bacteria from oral cavity in bacterial culture broth at the concentration of 1×10⁶CFU/mL; however, nano TiO₂ coating did not show antibacterial activity even in the dark for 240 min. Antibacterial activities of nano Ag/TiO₂ on different bacteria were different. Antibacterial activity to *S. mutans*, *S. sanguis* and *P.g.* was the strongest, with killing time of 20~30 min while to *A. actinomycetemcomitans*, *F. nucleatum* and *P. intermedia* was relatively weak, with killing time of 30~60 min.

DISCUSSION

Nano coating technology employs physical and chemical methods to form coating with different properties on the surface of material, in order to improve the performance of matrix material surface. Common preparation methods include sol-gel method (Dersot, 2010; Lina *et al.*, 2011), liquid phase deposition (Yu *et al.*, 2013), physical vapor deposition and electrochemical preparation method (Di, 2011; Yan, 2013). Sol-gel method includes dissolving precursor such as metal alkoxide and inorganic salt into water or organic solvent to form homogeneous solution; then solute generated nanoparticle and formed sol after hydrolysis reaction; sol reunites into gel and then gel was dried and heated so as to remove organic component; finally, materials in molecular level and even nano substructure was obtained. This method is the most common method for preparing nano TiO₂ powder, thin film and coating, with the most industrial prospect. Compared to the traditional method, it offers a lot of advantages: the obtained sample can reach molecular and atomic level; it is expected to prepare multi-functional multiplex photo-catalyst through doping; the temperature is low; the technology and equipment are simple; the process has good reproducibility; obtained nano TiO₂ thin film has high purity, good uniformity, small particle size and narrow distribution.

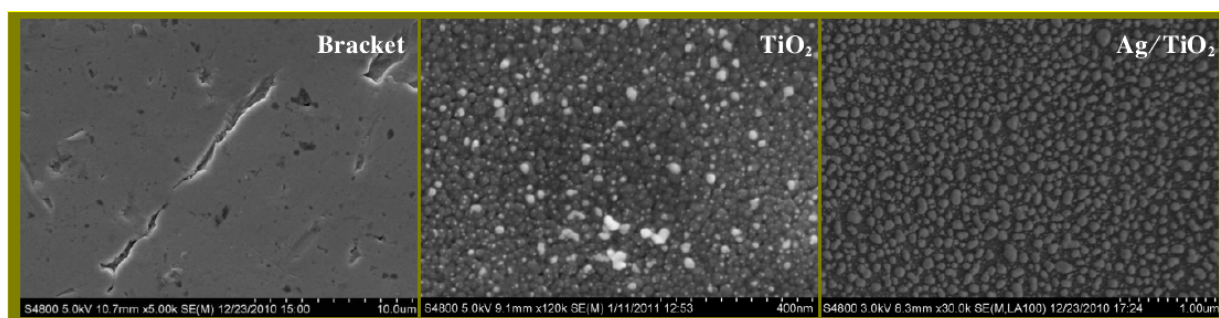
Rough surface is the initial factor of plaque adhesion (Xueying, 2010), since it can provide adhesion place for bacteria and produce shielding effect to prevent from the influence of flow of saliva, chew, swallow and some oral cavity cleaning measurement; in addition, rough surface relatively increases the superficial area for bacterial adhesion. Through observing the surface appearance of nano Ag/TiO₂ coating bracket by SEM, it was found that, coating had rigorous structure and even particle distribution, and the thickness of coating and size of particle were both controlled in nano level. It could be

Table 1: Antibacterial activity of nano Ag/TiO₂ coating after 20 min (Mean ±SD)

Bacteria	Colony counting of blank control group	Colony counts of experimental group	Antibacterial rate (%)
<i>S. mutans</i>	403.12±15.13	37.73±8.12	90.57
<i>S. sanguis</i>	398.25±10.23	35.62±6.44	93.56
<i>A. actinomycetemcomitans</i>	282.66±16.16	56.73±10.13	79.92
<i>F. nucleatum</i>	321.63±22.15	60.38±11.21	81.22
<i>P. gingivalis</i>	348.61±19.18	40.17±6.12	88.47
<i>P. intermedia</i>	252.16±12.14	28.37±16.16	88.74

Table 2: Antibacterial activity of nano TiO₂ and nano Ag/TiO₂ coatings on the common oral cavity bacteria at different time points

		5min	10min	20min	30min	60min	120min	240min
<i>S. mutans</i>	TiO ₂	+	+	+	+	+	+	+
	Ag/TiO ₂	+	+	+	-	-	-	-
<i>S. sanguis</i>	TiO ₂	+	+	+	+	+	+	+
	Ag/TiO ₂	+	+	-	-	-	-	-
<i>A. actinomycete</i>	TiO ₂	+	+	+	+	+	+	+
	Ag/TiO ₂	+	+	+	±	-	-	-
<i>F. nucleatum</i>	TiO ₂	+	+	+	+	+	+	+
	Ag/TiO ₂	+	+	+	±	-	-	-
<i>P. gingivalis</i>	TiO ₂	+	+	+	+	+	+	+
	Ag/TiO ₂	+	+	+	-	-	-	-
<i>P. intermedia</i>	TiO ₂	+	+	+	+	+	+	+
	Ag/TiO ₂	+	+	+	+	-	-	-

**Fig. 1:** Surface appearance of mental bracket, nano TiO₂ coating bracket and Ag/TiO₂ coating bracket

seen that, nano Ag/TiO₂ had characteristics of nano materials, and with high degree of finishing, thereby laying foundation for enhancing the clearance rate of plaque on the surface of bracket and preventing or reducing the occurrence of enamel caries and periodontal inflammation around bracket.

Structure of bracket is complex, therefore, self-cleaning ability of patients is weakened and the residue and soft dirt are easy to stay. As a result, plaque index of dental face after splice of bracket increases greatly compared to before splice of bracket. Besides, the bracket has certain influence on the micro-ecological environment of oral cavity, leading to the imbalance of microorganisms within plaque (Lixia and Lin, 2014). It was found that, proportion of *Streptococcus mutans* in plaque around

bracket had increased, and showed the trend of straight upward as the treatment time prolonged; *Streptococcus mutans* and *Lactobacillus* in saliva also increased, thereby increasing the risk coefficient of enamel demineralization (Jing *et al.*, 2011); ecological environment of sub gingival plaque (Xiaofei, 2011) also changed, which was beneficial to the growth of invasive organisms. At the first three months of correction, detection rate of *P. gingivalis*, *F. nucleatum*, *A. actinomycetemcomitans* obviously increased, and then decreased; within 6 months, the detection rate restored to the level before correction, and the positive rate of bacteria was positively correlated to plaque index (Hui *et al.*, 2010).

Biocompatibility, biomechanics adaptability and antithrombotic effects are the indispensable basic factors

of biological materials. Biological materials with single component are hard to satisfy the requirement of environment of human body because of its structure. Nano Ag/TiO₂ has improved performance compared to single biological coating bracket. Mechanical behavior of nano Ag/TiO₂ coating bracket implant on skeletal system under different stress state was studied; structure design and processing of nano biological ceramics was explored in the aspect of biomechanics; moreover, nano biological material suitable for clinical application of orthopedics was developed through studying nano Ag/TiO₂ coating structure and bone formation and its influence factors of friction and injury. Accordingly, nano Ag/TiO₂ coating bracket for bone formation is a direction for the development of biological materials, thereby providing a good prospect for clinical application of oral cavity.

CONCLUSION

All in all, the nano Ag/TiO₂ coating bracket developed in the present study has even thickness, high degree of finish and enough matrix combination strength, therefore, it can satisfy clinical need of orthodontic treatment. In addition, its antibacterial property and biocompatibility are also satisfactory. With the deepening of nanotechnology and nano materials, nano Ag/TiO₂ materials highlights its advantages and its strength, toughness, hardness and biocompatibility all significantly improve. However, antibacterial property of nano Ag/TiO₂ coating against different bacteria is different. Therefore, antibacterial activity is waiting to be further studied in clinical orthodontic treatment.

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