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Antibacterial Properties of Nano Silver-containing Orthodontic Cements in the Rat Caries Disease Model

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Abstract: The purpose of this study was to evaluate the antibacterial properties of experimental nano silver-containing cements (NSCs) using rat caries disease model. Nano silver base inorganic antibacterial powder was added to the reinforced glass ionomer cement at three different weight ratios to obtain a series of nano silver-containing cements, then two orthodontic cement products and three NSC samples were implanted into rat caries disease model, and their antibacterial properties were evaluated by the scanning electron microscope(SEM). Moreover, the rat caries disease model were established by inoculating cariogenic bacteria S mutans into antibiotics treated rat mouths and feeding with cariogenic diet. The tested materials were bonded on the surface of the buccal half crowns of the upper first premolar, and then fixed under the rats'front teeth lingual side to acquire enough retention. The SEM results indicated that the growth of streptococcus mutans was very active in group of Transbond XT. One month later, S mutans scattered on the GC Fuji ORTHO LC surface, and then the number significantly increased and arranged in chains after three months. In groups of NSC2, NSC3 and NSC4, the number of S mutans presented the downward trend and tended to disperse individually with the increase of silver nanoparticle content. We may conclude that the incorporation of silver nanoparticle enhanced GC Fuji ORTHO LC the adhesion restrain and killing effect to S mutans.

Key words: nano silver-containing orthodontic cements; rat caries disease model; antibacterial properties

1 Introduction

At present, almost all antimicrobial properties research of orthodontic adhesive materials are through the in vitro experiments[1-7]. The studies on nano silver-containing cements antibacterial durability, the bond strength, physical and chemical properties confirm that its clinical application prospect is fairly good[7]. Until now, no studies using animal model to evaluate the nano silver-containing cements antimicrobial properties have been reported. The rodent model of dental caries had made fundamental contributions to the understanding of the etiology, pathogenesis, and prevention of dental caries[8]. Therefore, animal model of caries disease may provide new methods for the research of nano silver-containing orthodontic adhesive materials, and this experiment will give an exploratory study for the methods, result evaluation and possible problems.

2 Experimental

2.1 Materials

To obtain nano silver-containing cements(NSCs), nano inorganic silver antibacterial powder was incorporated into resin-modified glass ionomer cement through physical mixing method of mechanical milling[9], among which NSC2, NSC3, and NSC4 will be used in this experiment. The following orthodontic adhesives were tested as control: reinforced glass ionomer cement(GC Fuji ORTHO LC; GC Corporation, Tokyo, Japan) and one composite resin cements (Transbond XT ; 3M Unitek Dental Products, Monrovia, Calif).

Fifty 3-week-old SD rats were randomly divided into five groups, ten each group. All rats were fed with

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cariogenic diet Keyes 2 000, whose formula including graham flour 600 g, cane sugar 5 600 g, refined milk powder 2 800 g, soybean meal 400 g, yeast 400 g, edible salt 200 g.

S mutans 8148, the primary cariogenic bacterium, provided by hospital of stomatology, wuhan university, was used as the test microorganism. S mutans was grown aerobically from frozen stock cultures in brain heart infusion (BHI, USA) broth containing 0.5% bacitracin at 37 ℃. Bacteria were used at their late-logarithmic to early stationary phase.

2.2 Rat caries disease model establishment

To control rats oral bacteria and easy to cause caries bacteria to colonize, fifty 26-day-old rats were fed with cariogenic diet Keyes 2000 added with antibiotics of ampicillin, chloramphenicol, carbenicillin each 1.0 g/kg, and drunk water added with penicillin G 4000 u/mL. After continuous feeding for three days, sampling was made with sterile swabs by wiping on rats molar tooth surface, and then inoculating was proceeded on mitis salivarius agar (MSB,USA) to ascertain no endogenous streptococcus mutans existed inside the rat mouth. Then, cariogenic bacteria S mutans 8148 was inoculated in 29-day-old rats mouths. The preparation of cariogenic bacteria was achieved by cultivation eight hours under 95% N_2, 5% CO_2, at 37 ℃ conditions, centrifugation 4000 r/min for 10min , resuspension and the addition of 20% sucrose solution with the proportion of 1:50. The rats were given bacterial colonization every 30 minutes, three times each morning and afternoon for continuous three days, by biting a germ-free cotton swabs dipped in fresh resuspended cariogenic bacteria. Three days later after inoculation, the same method was used to acquire rats oral bacteria samples and anaerobic culture for forty-eight hours on MSB. The microscopic examination was given to ascertain S mutans successful infection. The next was waiting for the formation of rat molar caries.

2.3 The implantation of material samples

Fifty orthodontic extracted maxillary premolars, whose crowns were perfect and no enamel defects, were selected for this experiment. After longitudinal incision the premolars and polish of these buccal half crowns' sharp edges, a hole would be prepared in the thick edge of each half crown.

Fifty prepared half crowns were randomly divided into five groups, and fifty bracket base plates would be bonded on the middle of these half crowns respectively by Transbond XT, GC, NSC2, NSC3, and NSC4 in accordance with the specifications. Be careful not to remove excess adhesives, and then light curing 60 seconds. In Transbond XT group, tooth surface etched area should be carried out in accordance with the brace base plate size, because too much acid corrosion area may affect the accuracy of the results observed in subsequent experiment.

In this experiment, the preparation of sample teeth had been achieved by bonding brace base plates with bonding material on the surface of the buccal half crowns of the upper first premolar, and then fixed these sample teeth under the rats'front teeth lingual side to ensure the material sample to save for a long time in the rats'mouth. Excess adhesive should not be removed for the following two reasons, one of which is the location of the acid corrosion can be covered up, and the other one is the adhesive can provide a position for us to observe the adhesion and growth of streptococcus mutans. After bonding of these base plates, these sample teeth were placed in artificial saliva at 37 ℃ to soak for twenty-four hours and hot and cold cycle experiment were conduced before putting into rat mouths, in order to fully simulate oral environment and prevent the occurrence of base plates bonding failure because of the conditions change.

Fifty streptococcus mutans inoculated rats were randomly divided into five groups, and then anesthesia was conducted by intraperitoneal injection of 7% chloral hydrate according to the standard of 1 mL/100g weight. Anaesthetic rats were fixed on the operating table and their tongue were pulled out and fixed by surgical needle. The ligature wire was penetrated with surgical needles into rat mandibular incisor base bone, and fixed the sample teeth under the rats'front teeth lingual position(Fig.1). After implantation, we checked all rats mouths every three days to observe whether there was a loss of sample teeth or bracket base plates.

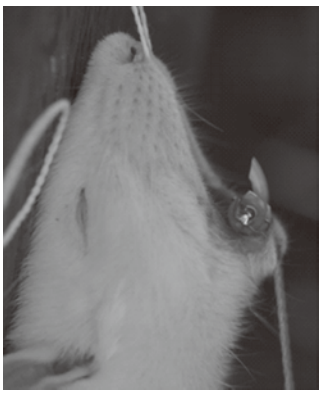

Fig.1 The location of material samples teeth in the rat mouth

2.4 The scanning electron microscope observation of bacteria growth on the surface of bonding materials

The adhesives on the sample teeth would be completely removed, dried and sprayed gold after taking out from rats' mouth under local anesthesia during one and three month of implantation respectively. Then, the scanning electron microscope observation would be conducted.

3 Results and discussion

Clinical observations have indicated that the common sites for demineralization are at the areas adjacent to the orthodontic bonding agents. For the purpose of decreasing the demineralization rate during orthodontic treatment, various antimicrobial agents have been incorporated into orthodontic adhesives. Fluoride[10-13] and chlorhexidine[14,15] are the most common preventive additives for orthodontic use. However, the effect of clinical application indicated that different degrees of caries white spot still could be seen in a lot of orthodontic patients. Despite some advances in orthodontic bonding materials and techniques in recent years, the enamel demineralization and dental caries around bracket remain unresolved. The silver ions exhibited high bactericidal activity against oral streptococci and silver bonding agent has a strong inhibitory effect on the bacterial biofilm formation and the growth of oral streptococcus mutans[16]. To keep long time antibacterial activity without affecting the bond strength, silver nanoparticles were incorporated into resin-modified glass ionomer cement in this study to acquire experimental nano-silver containing orthodontic cements[17].

In this study, we evaluated the feasibility of using rat caries disease model for orthodontic adhesives antimicrobial properties. In addition, The scanning electron microscope observation was used to estimate the antibacterial ability of five different samples. We hope that this study will lay a foundation for further application research.

3.1 Rat caries disease model establishment

MSB agar culture results showed that a lot of dark blue bacterial colonies, which constituted by catenabacterium under a microscope. As a result, we successfully built the caries disease model in rats by implanting of streptococcus mutans to the rat mouths treated with antibiotics and feeding the caries feed Keyes 2000 to them.

At present, experimental caries disease animal model is only a try, but provides us with a simulated mouth although there is no ready-made experience. According to the similarity of rat with human mouth in the flora composition and caries disease occurrence, we were able to make orthodontics materials related research. It broke the the status quo of orthodontic adhesive materials research only limited to in vitro study, and would be a very meaningful try for the new research method.

Although rats caries disease model was built successfully, there are still many uncertain factors to impact experiments results, such as the cariogenic ability of Streptococcus mutans in rats mouth, the subsequent experimental methods, and the observation time, and so on.

3.2 The implantation of material samples

In addition to the individual rats died of anesthesia, the material samples were implanted in the most rats' mouths. Nearly no material samples fell off during the whole experiment process for the reason of eating soft food and material samples' position without affecting their normal eating. We observed that part of the rat molars decayed in the pit and fissure region in the 80 days of rat caries disease model establishment.

The success of this experiment is mainly manifested in the following respects. Firstly, how to keep the tested material in the rats mouth for a long time. It is almost impossible to bind the material directly on rat tooth surface because the rat tooth is too small to provide enough space. And even rat tooth can provide space for material bonding, it is easy to fall off in the later process of eating or as the tooth abrasion. In this experiment, samples material teeth were fixed in the jaw by orthodontic ligature wire to obtain enough retention, thereby avoiding the disadvantages mentioned above. At the same time, this method also brings another problem - the cariogenic bacteria can make the human tooth demineralize or decay in the rat mouth? The second respect is the determination of observation time and antibacterial effect. Normally, the time of rat tooth decay in caries disease model appears in 20 days or so, which mainly depends on the cariogenic ability and the concentration of implanted bacteria. At present, however, the vitality of cariogenic bacteria saved in many laboratories shows a downward trend. So the observation time should last at least more than two months, which also brings great difficulty to experiment, such as, increased animal death, sample material falling-off risk and animal feed costs, and so on.

3.3 SEM analysis

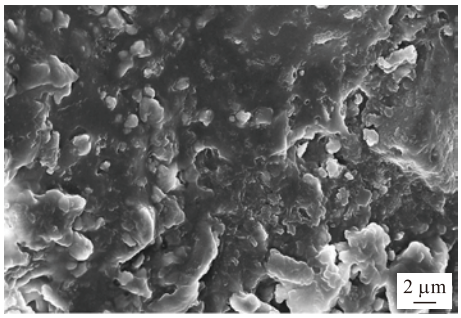

Fig.2 3M Transbond XT SEM (before the experiment)

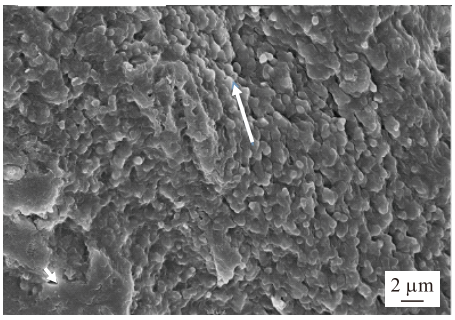

Fig.3 3M Transbond XT SEM (after one month) the arrow point to S. mutans

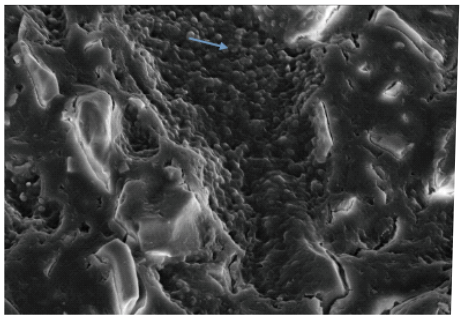

Fig.4 3M Transbond XT SEM (after three month)

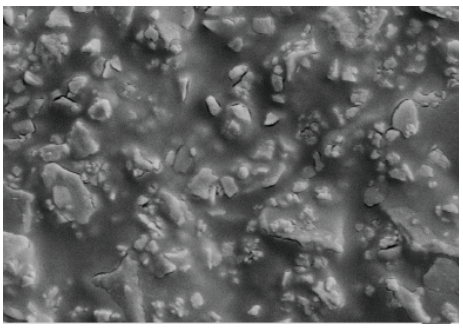

Fig.5 GC Fuji ORTHO LC SEM (before the experiment)

The scanning electron microscope results of five tested material's surface before implantation into rats mouths were shown in Figs.2, 5, 8,11, and 15. As a result, in the one month group of Transbond XT(Fig.3), which has not any antibacterial ability, the growth of streptococcus mutans was very active, layer upon layer. Three months later(Fig.4), A large number of streptococcus mutans were arranged closely, piling up, in the resin cracks. On the one month GC Fuji ORTHO LC surface(Fig.6), streptococcus mutans scattered or a small amount of them gathered into a cluster , and the number significantly increased and arranged in chains on the three months material surface(Fig.7). In groups of NSC2, NSC3, and NSC4, the number of streptococcus mutans presented the downward trend and tended to disperse individually with the increase of nano silver content(Figs.9, 10, 12, 13, 14, 16, and 17). The results showed that the incorporation of silver nanoparticles enhanced GC Fuji ORTHO LC the effect on adhesion restrain and killing effect to streptococcus mutans.

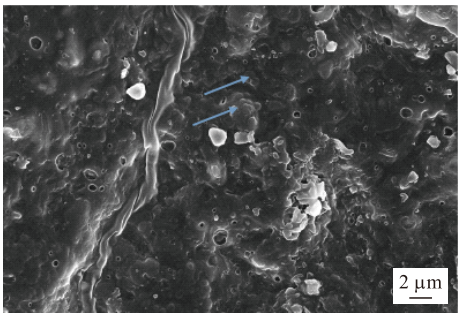

Fig.6 GC Fuji ORTHO LC SEM (after one month)

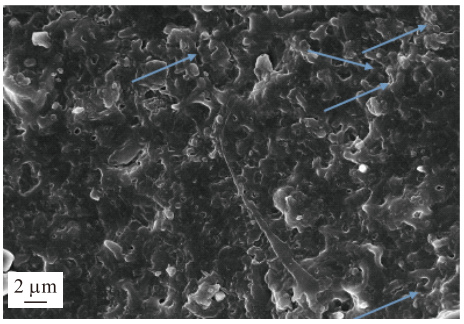

Fig.7 GC Fuji ORTHO LC SEM (after three month)

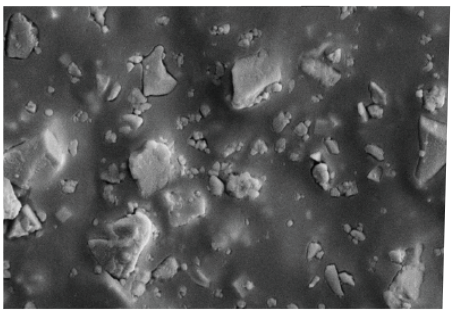

Fig.8 NSC2 SEM (before the experiment)

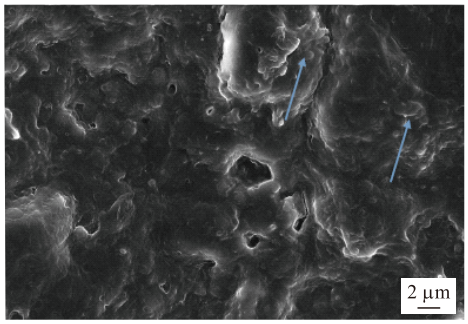

Fig.9 NSC2 SEM (after one month)

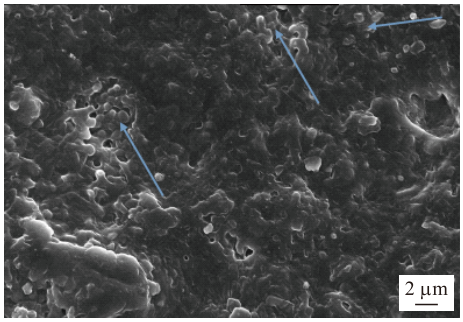

Fig.10 NSC2 SEM (after three month)

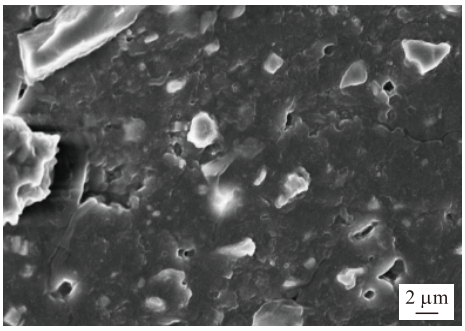

Fig.11 NSC3 SEM (before the experiment)

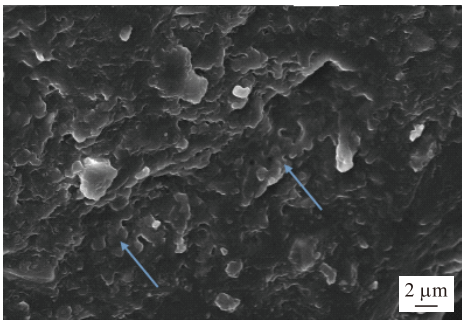

Fig.12 NSC3 SEM (after one month)

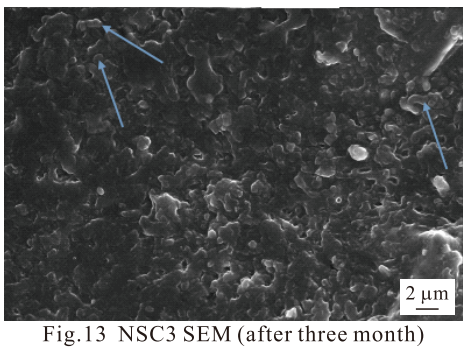

Fig.13 NSC3 SEM (after three month)

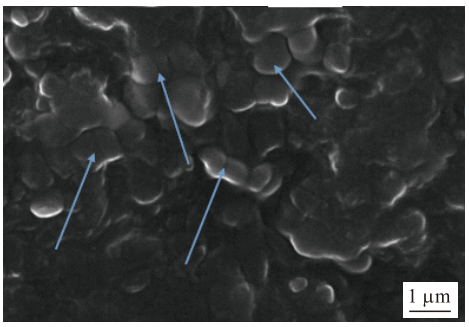

Fig.14 Fig.12 partial enlargement

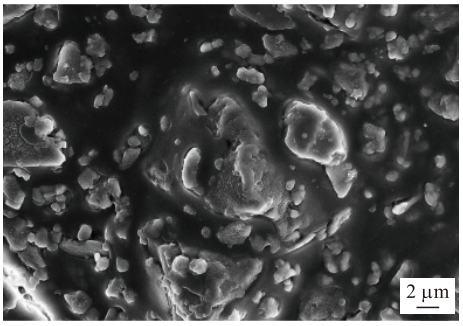

Fig.15 NSC4 SEM (before the experiment)

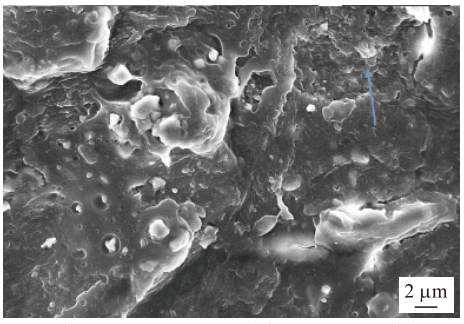

Fig.16 NSC4 SEM (after one month)

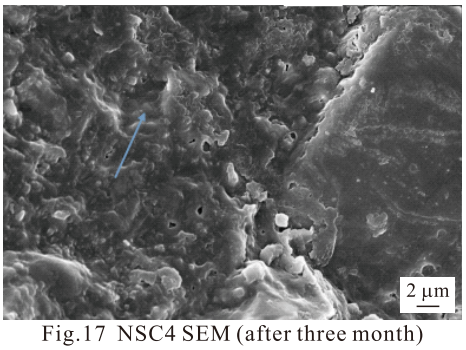

Fig.17 NSC4 SEM (after three month)

Although the antibacterial mechanism of silver nanoparticles is not very clear yet, it has very strong sterilization effect on several oral pathogenic bacteria, such as streptococcus mutans, lactobacillus, sticky actinomycetes and candida albicans[9,18,19]. The bactericidal effect of silver nanoparticles show concentration dependence, and nano silver of different concentrations will present obvious difference to bacterial adhesion inhibition and killing effect[20,21]. In

this experiment, streptococcus mutans actively adhered and bred on the surface of Transbond XT. In the early stage, GC Fuji ORTHO LC could significantly inhibit the adhesion of streptococcus mutans, and showed strong bactericidal effect, which was explained indirectly by not formation of long chains between them. As the the loss of fluorine in the material, its adhesion restrain and killing effect on bacterial decreased significantly. For lack of exogenous fluorine, GC Fuji ORTHO LC could not obtain the fluoride supplements, so the results of this study did not reflect the actual circumstances in the human mouth. According to the change of streptococcus mutans' number and morphology between one and three months after five tested materials being implanted into rat mouth, we drew the conclusion that the incorporation of silver nanoparticles enhanced the adhesion inhibition and killing effect of GC Fuji ORTHO LC on streptococcus mutans, and showed a concentration dependence. Due to the limitation of conditions, the further experiment is needed to determine the long-term bacterial colonization change tendency.

4 Conclusions

In short, though there are certain inadequacies in the experiment, we provided a new method for material antibacterial property research. It's a significant attempt and accumulates certain experience for further research.

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