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Original Article

Physico-Mechanical Properties, Antimicrobial Activities, and Anti-Biofilm Potencies of Orthodontic Adhesive Containing Cerium Oxide Nanoparticles against *Streptococcus mutans*

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Abstract

Introduction: White spot lesions around orthodontic brackets may lead to the formation of dental caries during and following fixed orthodontic treatment.

Aim: This study aimed to evaluate the physico-mechanical properties and antimicrobial potencies of orthodontic adhesive doped with cerium oxide nanoparticles (CeO_2-NPs) against *Streptococcus mutans*.

Materials and methods: After synthesis and conformation of CeO_2-NPs by transmission electron microscope (TEM), shear bond strength (SBS) and adhesive remnant index (ARI) of modified orthodontic adhesive containing different concentrations of CeO_2-NPs (0, 1, 2, 5, and 10 wt%) were measured. The antimicrobial effects of modified orthodontic adhesive were evaluated by disk agar diffusion method and biofilm formation inhibition assay.

Results: The pseudo-spherical shapes of CeO_2-NPs were observed in TEM micrographs. The physico-mechanical finding showed that 5 wt% CeO_2-NPs showed the highest concentration of CeO_2-NPs and SBS value (18.21±9.06 MPa, $p<0.05$) simultaneously with no significant differences in ARI compared with the control group ($p>0.05$). There was a significant reduction in cell viability of *S. mutans* with increasing CeO_2-NPs concentration. The 3.1 Log_{10} and 4.6 Log_{10} reductions were observed in the count of treated *S. mutans* with 5 and 10 wt% CeO_2-NPs, respectively ($p<0.05$).

Conclusions: Overall, an orthodontic adhesive containing 5 wt% CeO_2-NPs had antimicrobial properties against *S. mutans* without adverse effects on SBS and ARI.

Keywords

cerium oxide, cariogenic bacteria, orthodontic adhesive, shear bond strength, *Streptococcus mutans*

INTRODUCTION

The composite resin bonding method in orthodontics is mainly used to attach the bracket to the tooth surface.[1] In direct orthodontic bonding, the brackets are placed directly and individually on the enamel of each tooth. This method requires more time than the indirect bonding method. The most important advantage of direct bonding is to make sure that the brackets are properly attached and placed on the tooth.[2,3] Unfortunately, despite the advantages of bonding such as high beauty and easy technique, this method has some disadvantages such as plaque accumulation, white lesions, and broken band.[4] These disadvantages prolong treatment, increase the duration of clinical work and the cost of treatment. Several methods have been introduced to prevent biofilm formation and tooth decay. One method is to add antimicrobials to the composite resin.[5]

Nanoparticles have high antimicrobial properties due to their small size. In addition to knowing the antimicrobial effects of nanoparticles, their effects on the bond strength between the bracket and the composite or bond strength of orthodontic cement are also important.[6]

Oral streptococci, especially *Streptococcus mutans* as the most important member of Viridans streptococci, are known to cause tooth decay and subsequent diseases by synthesizing extracellular polymers and forming biofilms on dental surfaces.[7]

Recently, the antimicrobial properties of cerium oxide nanoparticles (CeO_2-NPs) and their applications in the field of medicine and other sciences have been considered, and the decision to combine these nanoparticles with resin composites used in orthodontics was taken.[8] To the best of our knowledge, no studies are available regarding the antimicrobial efficacy of orthodontic adhesive doped with CeO_2-NPs against any cariogenic bacteria.

AIM

Therefore, this study aimed to investigate the antimicrobial and anti-biofilm properties of orthodontic adhesive doped with CeO_2-NPs, as well as maintenance of sufficient shear bond strength (SBS) of orthodontic light-curing composite toward the eradication of *S. mutans*. Therefore, we tested the hypothesis that CeO_2-NPs can act as an antimicrobial and anti-biofilm agent against *S. mutans* biofilm culture.

MATERIALS AND METHODS

Synthesis of CeO_2-NPs

CeO_2-NPs were synthesized using a modified hydrothermal method.[9] Briefly, 0.22 g of cerium(III) nitrate hexahydrate (Sigma-Aldrich, Steinheim, Germany) was dissolved in 75 µL of trisodium phosphate dodecahydrate (0.02 g/mL) (Sigma-Aldrich, Steinheim, Germany) and 20 mL of deionized water and stirred vigorously for 4 h at room temperature. The mixture was then placed into a stainless steel autoclave at 170°C for 12 hours. The mixed solution was cooled and a white precipitate was isolated by centrifugation at 10000 rpm for 10 min. The supernatant was decanted away and the white CeO_2 was then washed repeatedly with deionized water and ethanol. Finally, the product was freeze-dried overnight. Synthesized CeO_2-NPs morphology was observed and photographed using transmission electron microscopy (TEM; Zeiss EM10C, Germany) with an accelerating voltage of 100 kV.

Fabrication of CeO_2-NPs adhesives

For the preparation of modified adhesive containing 1, 2, 5, and 10 wt% CeO_2-NPs, 12.5, 25, 62.5, and 125 mg of CeO_2-NPs, respectively, were blended into 0.11, 0.22, 0.55, and 1.1 g of Transbond™ XT primer (3M Unitek, Monrovia, CA) as an orthodontic adhesive. The prepared samples were then de-molded, polished, and sterilized according to ISO 11135:1994 for medical devices[10] before the tests.

Determination of physico-mechanical properties of modified orthodontic adhesive samples

Shear bond strength (SBS) testing

Twenty freshly extracted bovine incisors with intact buccal enamel and with no cracks or any lesions were collected, immersed in 0.5% of chloramine T trihydrate (Sigma Aldrich Chemie GmbH, Taufkirchen, Germany) at 4°C for seven days, and embedded in cold-cure acrylic resin according to ISO/TS 11405:2003. The surfaces of all teeth were cleaned, polished, and rinsed with air-water spray for 10 s and air-dried for 10 s. Thirty-five percent of phosphoric acid gel (Ultra etch; Ultradent Products Inc., South Jordan, UT, USA) was used to etch the buccal surfaces of all teeth. After 20 s, the teeth were rinsed with water for 10 s, and dried with air for 10 s. Then, the etched area of the buccal surface of all teeth was covered with a thin layer of CeO_2-NPs at the different concentrations (1, 2, 5, and 10 wt%) and cured with a LED light-curing unit (Demetron, Kerr, Orange, CA, USA). According to the Felemban and Ebrahim study[11], the orthodontic metal brackets were used to bond all teeth. Based on the ISO/TS 11405:2015 guideline, all teeth were stored in distilled water at 37°C for 24 hours, thermocycled 3000 times in a water bath between +5°C and +55°C, and remained in each reservoir for 30 s after the bonding procedures. Zwick/Roell, Germany with a speed of 1.0±0.1 mm/min in occlusal-gingival direction at the bracket-tooth interface was used as a mechanical testing machine for SBS testing **(Fig. 1)**. Finally, the values of SBS were calculated in MPa as described previously. [11]

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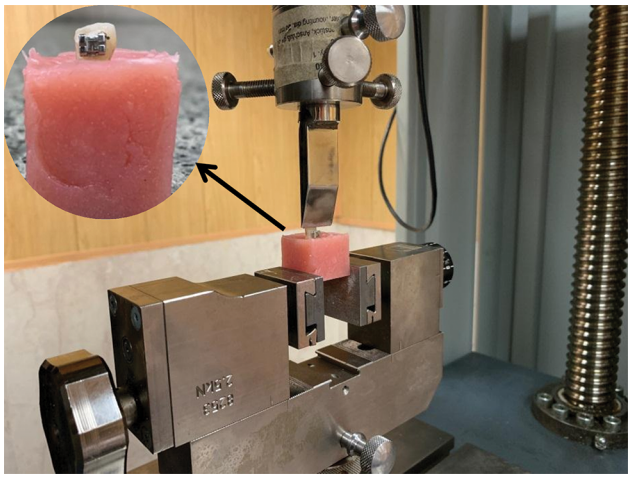

Figure 1. Zwick/Roell testing machine.

Adhesive remnant index (ARI)

ARI score in debonding of stainless-steel brackets from enamel surface was assessed by a stereomicroscope (SMZ800, Nikon, Tokyo, Japan) at ×10 magnification based on the Oliver and Griffiths study.[12]

Microorganism and growth conditions

S. mutans (ATCC 35668) was cultured in brain heart infusion (BHI) broth (Merck, Darmstadt, Germany) supplemented with 0.1% sucrose and incubated in an aerobic atmosphere with 5% CO_2 at 37°C. A 0.5 McFarland standard bacterial suspension (1.5×10^8 colony forming units (CFUs)/mL) was prepared to examine the antimicrobial efficacy of orthodontic adhesive doped with CeO_2-NPs.

Preparation of modified orthodontic adhesives samples

Disk-shaped modified orthodontic adhesive patterns were prepared using metal molds 5 mm in diameter and 1 mm thick. Modified orthodontic adhesive disks containing different concentrations of CeO_2-NPs were made by the fabrication of CeO_2-NPs adhesives section. According to the ISO 11135:1994, prepared disks were then exposed to light cure for 40 s, de-molded, polished, and sterilized.[10]

Antimicrobial testing

Disk agar diffusion test

Disk agar diffusion test by the Kirby-Bauer method was done to determine the antimicrobial features of orthodontic adhesive by zones of growth inhibition around each of the samples.[13] 1.5×10^8 CFU/mL of *S. mutans* was swabbed onto the surface of Mueller-Hinton agar (Merck, Darmstadt, Germany) plates (i.e., 100-mm plate diameter). The size of the growth inhibition zone around the disks was measured after overnight incubation at 37°C in an aerobic atmosphere with 5% CO_2.

Biofilm inhibition test

Twenty-five orthodontic adhesive disks with different concentrations of CeO_2-NPs were placed in flat-bottomed 48-well microtiter plates containing *S. mutans* suspension with a concentration of 1.5×10^8 CFU/mL. To form biofilms on the disks, the microtiter plate was incubated under the aerobic atmosphere with 5% CO_2 at 37°C for 72 hours. Afterwards, disks were rinsed in 1 mL of sterile deionized water for 1 min to remove planktonic microbial cells. Orthodontic adhesive disks were then vortexed severely in 1 mL of BHI broth for 30 s. The obtained bacterial suspensions were serially diluted, cultured in mitis salivarius agar (Merck, Darmstadt, Germany), and the microbial colony counts were determined as mentioned in the previous study.[14,15]

Statistical analysis

The microbial experiments were done in triplicate and the data were analysed using one-way analysis of variance (ANOVA). Statistical analysis was performed using SPSS for windows version 23.0 (SPSS Inc., Chicago, IL, USA). *P* values <0.05 were considered statistically significant.

RESULTS

The morphologies of synthesized CeO_2-NPs

TEM analysis has confirmed the general structure of the synthesized CeO_2-NPs **(Fig. 2)**. The presence of aggregates with pseudo-spherical shapes was observed in TEM micrographs.

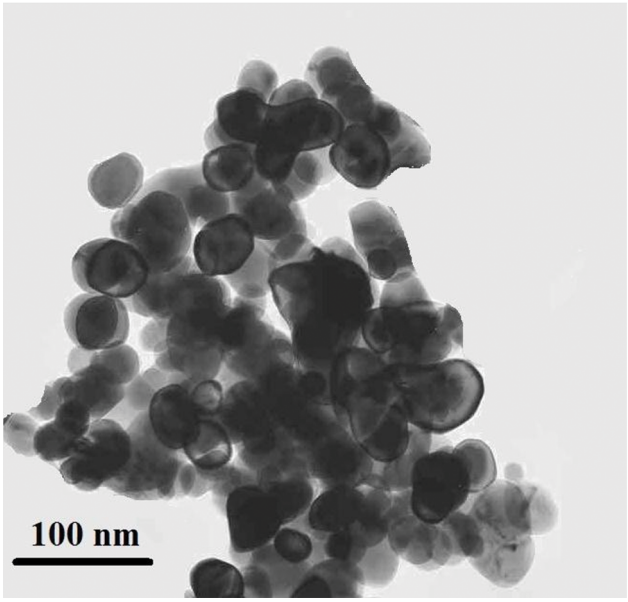

Figure 2. Transmission electron microscopy image of CeO_2-NPs.

SBS test

The data of SBS of orthodontic adhesive doped with different concentrations of CeO_2-NPs are presented in **Table 1**. As shown, 1 wt% CeO_2-NPs revealed the highest value of SBS (30.42±11.15 MPa, $p>0.05$). Also, the lowest SBS value (7.75±2.43 MPa, $p<0.05$) was reported in 10 wt% CeO_2-NPs. According to the results, the SBS values decreased following an increase in the concentration of CeO_2-NPs.

Table 1. The mean of shear bond strength (SBS) of orthodontic adhesive doped with CeO_2-NPs

Orthodontic adhesive doped with CeO_2-NPs (%)	SBS (MPa)		
	Minimum	Maximum	Mean ± SD
0	18.52	40.65	30.42±11.15
1	16.26	38.12	25.02±11.55
2	13.38	30.40	20.56±8.81
5	10.37	28.14	18.21±9.06
10	5.28	10.14	7.75±2.43*

SD: standard deviation; *P value <0.05

ARI score

The frequencies of ARI scores in the test groups are shown in **Table 2**. There was no significant difference in the ARI scores between different concentrations of CeO_2-NPs in orthodontic adhesive disks and the control group ($p<0.05$).

Disk agar diffusion assay

The antimicrobial property of orthodontic adhesive disks containing different concentrations of CeO_2-NPs was assessed using the release of nanoparticles from the disks. No growth inhibition zone was observed around any of the disks. This indicates that the CeO_2-NPs were not able to be released at the plate surface.

Effects of CeO_2-NPs on biofilm formation inhibition

As shown in **Fig. 3A**, there was a considerable decrease in cell viability of *S. mutans* with increasing CeO_2-NPs concentration. The results exhibited that 5 and 10 wt% CeO_2-NPs significantly reduced *S. mutans* to 3.1 Log_{10} and 4.6 Log_{10}, respectively ($p<0.05$). The effect of different concentrations of CeO_2-NPs on cell viability of *S. mutans* is shown in **Fig. 3B**.

DISCUSSION

Bonding brackets to teeth is one of the common methods in orthodontic treatment.[16] One of the important complications of fixed orthodontic appliances, such as brackets,

Table 2. The frequency of adhesive remnant index (ARI) scores in the test groups

Orthodontic adhesive doped with CeO_2-NPs (%)	ARI scores				
	0.00	1.00	2.00	3.00	4.00
0	0	2	2	2	3
1	1	2	2	3	3
2	0	2	2	3	3
5	1	2	3	3	3
10	1	3	4	5	2

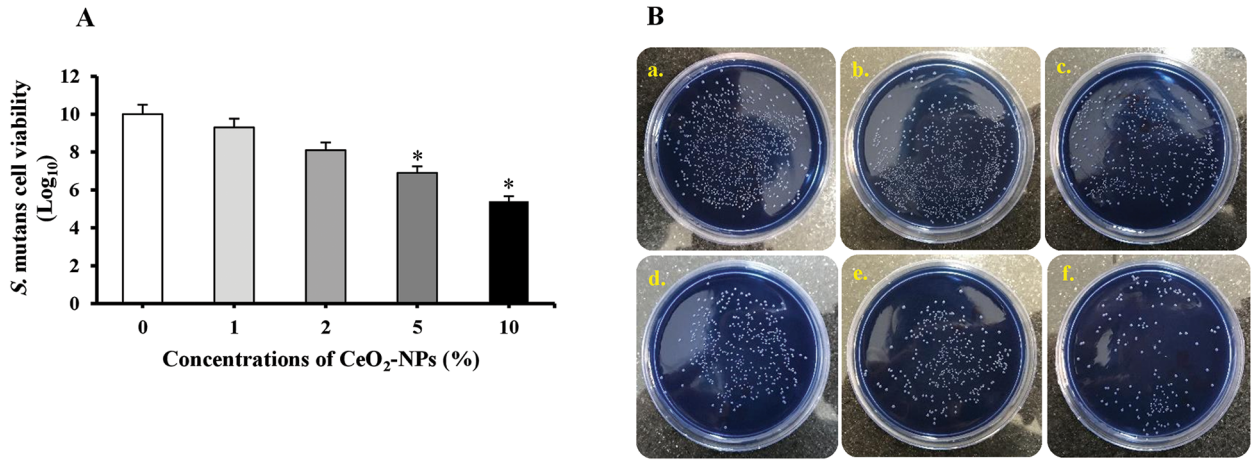

Figure 3. Cell viability of *S. mutans* according to the percentages of CeO_2-NPs incorporated into orthodontics adhesive: **A**) different concentrations of CeO_2-NPs against *S. mutans*; **B**) Colonies of treated *S. mutans* following different concentrations of CeO_2-NPs: **a**. Control (untreated *S. mutans*); **b**. 0 wt% CeO_2-NPs; **c**. 1 wt% CeO_2-NPs; **d**. 2 wt% CeO_2-NPs; **e**. 5 wt% CeO_2-NPs; **f**. 10 wt% CeO_2-NPs.

in which dental composites are used to attach them, is the breaking of the composite bond due to the forces applied to the bracket, as well as increasing the risk of plaque accumulation and expansion and subsequent caries around the brackets.[17] Therefore, it is necessary to have a composite which, while having suitable mechanical properties, can reduce the amount of dental plaque microorganisms and reduce dental demineralization.

Another complex problem in fixed orthodontic treatments is the control of enamel demineralization around the brackets used during treatment. Brackets and various tools used in orthodontic treatments make oral hygiene more difficult and, according to some studies, increase the number of oral bacteria.[18] Previous studies have also shown that the rate of demineralization of enamel, white spots, and tooth decay are much higher in people who have received orthodontic treatment.[19-22]

It should be noted that the composites used in orthodontics have a polymer matrix that is involved in the accumulation of aerobic and anaerobic microorganisms. The formation of supragingival biofilm is mainly seen around orthodontic attachments in clinical studies. The main cause of dental caries is *S. mutans*. *S. mutans* is a Gram-positive, optional anaerobic coccus that is the oral cavity flora in humans.[23,24] Any imbalance of the oral microflora will lead to oral diseases. *S. mutans* causes damage to tooth enamel by fermenting sucrose and producing lactic acid. The bacterium also uses sucrose to make dental plaque. Dental plaque is made from dextran, a type of polysaccharide.[25] Chin et al.[26] found that *S. mutans* and *Lactobacillus acidophilus* have the ability to bind to orthodontic bonding agents and colonization during orthodontic treatment, and with increasing the load of these bacteria, the prevalence of caries increases.

Nanotechnology can be used effectively to maintain oral health. In particular, nanoparticles are useful antimicrobial agents for bonding and orthodontic appliances. They can also be used in dental restorations such as cavities, sealants, and root canals. Its antimicrobial ability reduces plaque around the brackets, which can prevent decay during treatment.[27,28]

Due to the problem of decalcification and decay around orthodontic brackets, various research studies have been done on the effect of using antimicrobial and anti-decay materials.[29-32] Due to the variety of studies performed so far, no study has been performed on the antimicrobial effect of CeO_2-NPs on reducing the rate of *S. mutans* in patients using fixed orthodontic appliances.

The relevant literature shows that CeO_2-NPs are widely used as a catalyst in industry and as an antioxidant in applied nanomedicine. The antimicrobial mechanism of CeO_2-NP action probably occurs via oxidative stress of components in the microbial cell membrane and accumulation of oxygen reactive species in microbial cells.[33-35] In the present study, the effect of CeO_2-NPs on physico-mechanical properties including SBS testing and ARI were determined. Based on the results, by addition of CeO_2-NPs up to 5%, the SBS of Transbond XT composite to enamel was within the clinically acceptable score without remarkable changes. In contrast, the SBS in 10% CeO_2-NPs was significantly lower than that in the control group. As previously reported, the SBS in clinical conditions can be 40% less in vitro conditions.[36] Therefore, this makes our SBS results in the acceptable score that will be in vivo, adding 5 wt% CeO_2-NPs can maintain the SBS for optimum clinical applications. Also, there is no considerable difference between the different concentrations of CeO_2-NPs in terms of ARI scores, which was in agreement with the other studies.[36-38]

Moreover, in this study, the antimicrobial potential of orthodontic adhesive doped with different concentrations of CeO_2-NPs was evaluated. Similar to previous studies[36-41], a progressive increase in the inhibition of microbial biofilm growth was revealed with increasing the concentration of CeO_2-NPs. Although concentrations higher than 5 wt% CeO_2-NPs could considerably decrease the growth and biofilm formation of *S. mutans*, the mean of SBS decreased.

Pelletier et al.[39] evaluated the growth and viability of *Escherichia coli* and *Bacillus subtilis* as the Gram-negative and Gram-positive species, respectively, relative to CeO_2-NP. Bactericidal effects of CeO_2-NP were determined using the minimum inhibitory concentrations (MIC) and CFU/mL measurements, disk agar diffusion test, and live/dead assays. Their results showed that the bacterial growth rates depended on CeO_2-NP concentrations in the range of 50 to 150 mg/L. Also, the growth inhibition of *E. coli* and *B. subtilis* was observed. There was no significant difference in inhibiting the microbial growth of bacteria by CeO_2-NPs at 50 μg/mL. In another study, the efficient and antibacterial application of CeO_2-NP against Gram-positive and Gram-negative pathogens was assessed by Pop et al.[40] According to their findings, the growth inhibition toward all five pathogens tested including *E. coli*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *B. cereus* was observed with notable results. The MICs of CeO_2-NPs against pathogenic bacteria were 2.15, 1.07, 1.07, 10, and 4.3 mg/mL for *E. coli*, *S. typhimurium*, *L. monocytogenes*, *S. aureus*, and *B. cereus*, respectively. Several other studies have reported the antimicrobial effect of CeO_2-NPs against *E. coli* and *S. aureus*.[8,34,35,41,42] The results of these studies showed that CeO_2-NPs with the lowest concentration are able to inhibit significantly the growth of these microorganisms.

One of the important aspects of using nanoparticles is their toxicity. Although the evidence is insufficient, nanoparticles do not appear to be more toxic than conventional materials. Due to the wide scope of nanotechnology and the lack of studies on the effects of nanoparticles on SBS of orthodontic adhesives, as well as the physical properties of orthodontic acrylics, further studies are proposed to clarify these aspects. Also, clinical trial studies should

be performed to confirm the anti-caries properties of orthodontic adhesive doped with CeO_2-NPs.

CONCLUSIONS

In overview, this study demonstrated that 5 wt% CeO_2-NPs as an orthodontic adhesive with a clinically acceptable score of SBS and ARI had antimicrobial and anti-biofilm activities against *S. mutans*. However, we acknowledged that further evaluation of these activities of cerium oxide nanoparticles against cariogenic bacteria in multi-species biofilm structure is warranted.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Физико-механические свойства, антимикробная активность и антибиоплёночная активность ортодонтического клея, содержащего наночастицы оксида церия, в отношении *Streptococcus mutans*

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Резюме

Введение: Белые пятна вокруг ортодонтических брекетов могут привести к образованию кариеса во время и после фиксированного ортодонтического лечения.

Цель: Это исследование было направлено на оценку физико-механических свойств и антимикробной активности ортодонтического клея, легированного наночастицами оксида церия (НЧ CeO_2), в отношении *Streptococcus mutans*.

Материалы и методы: После синтеза и конформации НЧ CeO_2 с помощью просвечивающего электронного микроскопа (ПЭМ) определяли прочность соединения с надрезом при сдвиге (SBS) и показатель адгезионного остатка (ARI) модифицированного ортодонтического адгезива, содержащего различные концентрации НЧ CeO_2 (0, 1, 2, 5 и 10 wt%). Антимикробное действие модифицированного ортодонтического клея оценивали методом диффузии в дисковом агаре и методом ингибирования образования биоплёнки.

Результаты: Псевдосферические формы НЧ CeO_2 наблюдались на микрофотографиях ПЭМ. Физико-механические данные показали, что 5 wt% НЧ CeO_2 показали самую высокую концентрацию НЧ CeO_2 и значение SBS (18.21±9.06 MPa, $p<0.05$) одновременно без существенных различий в ARI по сравнению с контрольной группой ($p>0.05$). Наблюдалось значительное снижение жизнеспособности клеток *S. mutans* при увеличении концентрации НЧ CeO_2. Снижение 3.1 Log_{10} и 4.6 Log_{10} наблюдалось при подсчёте обработанных *S. mutans* с 5 и 10 wt% CeO_2-NPs соответственно ($p<0.05$).

Заключение: В целом, ортодонтический клей, содержащий 5 wt% НЧ CeO_2, обладал антимикробными свойствами в отношении *S. mutans* без неблагоприятного воздействия на SBS и ARI.

Ключевые слова

оксид церия, кариесогенные бактерии, ортодонтический адгезив, прочность соединения с надрезом при сдвиге, *Streptococcus mutans*