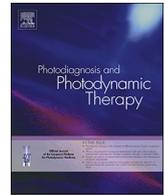




ELSEVIER

Contents lists available at ScienceDirect

## Photodiagnosis and Photodynamic Therapy

journal homepage: [www.elsevier.com/locate/pdpdt](http://www.elsevier.com/locate/pdpdt)

# Physico-mechanical and antimicrobial properties of an orthodontic adhesive containing cationic curcumin doped zinc oxide nanoparticles subjected to photodynamic therapy

Maryam Pourhajibagher<sup>a</sup>, Abbas Salehi Vaziri<sup>b</sup>, Nasrin Takzaree<sup>c</sup>, Roghayeh Ghorbanzadeh<sup>b,\*</sup>

<sup>a</sup> Dental Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran

<sup>b</sup> Department of Orthodontics, Shahed University, Tehran, Iran

<sup>c</sup> Anatomy Department, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

## ARTICLE INFO

## Keywords:

Cariogenic bacteria  
Cationic curcumin  
Orthodontic adhesive  
Zinc oxide nanoparticles  
Antimicrobial photodynamic therapy

## ABSTRACT

**Background:** Potential complications on the crown level during fixed orthodontic procedures are white spot, enamel demineralization and tooth decay. This study evaluated the antimicrobial properties of an orthodontic adhesive incorporating cationic curcumin doped zinc oxide nanoparticles (cCur/ZnONPs), which can have the highest concentration of cCur/ZnONPs and shear bond strength (SBS) value simultaneously, against cariogenic bacteria including *Streptococcus mutans*, *Streptococcus sobrinus*, and *Lactobacillus acidophilus*.

**Materials and methods:** Following synthesis and confirmation of cCur/ZnONPs, SBS and adhesive remnant index (ARI) of the test adhesives containing cCur/ZnONPs (1.2, 2.5, 5, 7.5, and 10% wt.) were measured using universal testing machine and stereomicroscope, respectively. After continuously rinsed (up to 180 day), the residual antimicrobial ability of modified adhesives which can have the highest concentration of cCur/ZnONPs and SBS value simultaneously were determined by disc agar diffusion (DAD), biofilm formation inhibition, and metabolic activity assays following photo-activation using light-emitting diode (LED) for 5 min against multi-species cariogenic biofilm-producing bacteria.

**Results:** Adhesive with 7.5% wt. cCur/ZnONPs showed the highest concentration of cCur/ZnONPs and SBS value ( $14.89 \pm 3.26$  MPa,  $P < 0.05$ ) simultaneously. No significant differences in ARI scores were found between the modified adhesive and control (Transbond XT without the cCur/ZnONPs). 7.5% wt. cCur/ZnONPs following photo-activation was not colonized by the test microorganisms and suppressed 100% metabolic activity of the test microorganisms up to 90 day compared to the control group (cCur/ZnONPs free LED irradiation;  $P < 0.05$ ). In DAD assay, the reduction of photodynamic disinfection of the 7.5% wt. cCur/ZnONPs against test bacteria was positively associated to the time, in such a way that it was decreased significantly after 60 day. From days 120 onwards, microbial biofilm formation and metabolic activity was progressively increased on 7.5% wt. cCur/ZnONPs adhesive discs compared to the control group (cCur/ZnONPs free LED irradiation).

**Conclusions:** Our findings highlight the photo-activated 7.5% wt. cCur/ZnONPs can serve as an orthodontic adhesive additive to control the cariogenic multispecies biofilm, and also to reduce their metabolic activity.

## 1. Introduction

Widespread white spot lesions (WSLs; 2–96%) and incipient tooth decay during orthodontic treatment present grave challenges to clinicians facing a dearth of effective preventive options against enamel caries around bonded orthodontic brackets [1–3]. Thus, to address these important issues, conventional antimicrobial agents such as chlorhexidine, enamel remineralization agents including fluoride and

nanoparticles of calcium phosphate containing resin composite were incorporated into the orthodontic adhesives [4,5]. However, the short-term antimicrobial effects as well as the improper mechanical properties of the modified adhesives have made this approach questionable [6]. Recent studies have shown remarkable antimicrobial activities of zinc oxide nanoparticles (ZnONPs) incorporated in adhesives agents, and dental composites [7–10]. Previous study has also shown the effects of curcumin (Cur) on *Streptococcus mutans* growth inhibition following

\* Correspondence author at: Department of Orthodontics, Shahed University, Opposite Holy Shrine of Khomeini- Khalij Fars Expressway, P.O. Box: 18155/159, Postal/ZIP 3319118651, Tehran, Iran.

E-mail addresses: [gh.ortho62@gmail.com](mailto:gh.ortho62@gmail.com), [gh.ortho62@outlook.com](mailto:gh.ortho62@outlook.com) (R. Ghorbanzadeh).

<https://doi.org/10.1016/j.pdpdt.2019.01.002>

Received 18 October 2018; Received in revised form 25 December 2018; Accepted 2 January 2019

Available online 03 January 2019

1572-1000/ © 2019 Elsevier B.V. All rights reserved.

photo-activation [11].

In addition, Cur has been reported as an excellent carrier in ZnONPs production process [12]. Because cationic antimicrobial agents disrupt the integrity of biofilms and killing microbial cells in biofilms by puncturing negatively-charged microbial cell surface [13], glycidyl-trimethylammonium chloride (GTMAC) modified Cur containing cationic moiety [14] was used in combination with ZnONPs in the present study to develop a modified orthodontic adhesive for exerting antimicrobial activities following photo-activation, without adverse effect on the quality of the bonded efficacy.

Recently, it has been proven that the Cur based photodynamic deactivation or antimicrobial photodynamic therapy (aPDT) could benefit patients undergoing orthodontic treatment [15]. In aPDT, the light sensitive material, photosensitizer, after irradiated with light with appropriate wavelength in the presence of oxygen, produces reactive oxygen species (ROS), which are able to kill microorganisms and modulate microbial virulence features [16,17]. Benefits of aPDT over conventional antimicrobials are low mutagenic potential in exposed cells, a broad spectrum of antimicrobial activity, and rapid killing of target microorganisms [18]. For the evaluation of novel anti-biofilm therapies or materials, appropriate test schemes are essential, such as multispecies cariogenic biofilm, which mimic the *in vivo* situation. According to the best of our knowledge, studies of anti-cariogenic effect of orthodontic adhesive containing antimicrobial agents have traditionally focused on single species populations especially *S. mutans*. However, given that microorganisms in tooth decay exist as multispecies consortia. The scarcity of multispecies cariogenic biofilm model in this area renders the efforts to control the enamel demineralization and the tooth decay in orthodontic patients with multibracket appliances unsuccessful.

According unique features of Cur (cCur) and ZnONPs, the aim of the present study was therefore to evaluate potency of orthodontic adhesive containing cCur/ZnONPs as a novel composite following photo-activation against multispecies cariogenic biofilm-producing bacteria including *S. mutans*, *S. sobrinus* and *Lactobacillus acidophilus*. In this study, our hypothesis is that there is a statistically significant difference between the antimicrobial activity of the orthodontic adhesive containing cCur/ZnONPs submitted to light activation and the original orthodontic adhesive against multispecies cariogenic biofilm-producing bacteria. Under the null hypothesis, this difference is insignificant.

## 2. Materials and methods

### 2.1. Synthesis of Cur derivative

A Precipitation technique was used to fabricate of cCur, as previously described [14]. Briefly, Cur (0.92 g; 2.5 mmol; Sigma–Aldrich Co., Ltd., Dorset, United Kingdom) mixed with anhydrous sodium carbonate (0.26 g; 2.5 mmol; Merck, Darmstadt, Germany) in 15 ml of deionized water (DI water) using magnetic stirring. The solution was heated to the temperature of 60 °C. The GTMAC solution (0.76 g; 5.0 mmol in 10 ml of DI water; Sigma–Aldrich Co., Ltd., Dorset, United Kingdom) was added dropwise into the alkaline Cur solution. Temperature was kept constant at 60 °C for overnight. Then solution was cool down to 25 ± 2 °C before neutralizing with 0.5% v/v of formic acid (Merck, Darmstadt, Germany). Precipitation was down with absolute ethanol. The solid phase was then dried out at 25 ± 2 °C.

### 2.2. Synthesis of ZnONPs

ZnONPs powder was synthesized in surfactant free synthesis method following earlier report [19]. Briefly, 0.1 M zinc sulphate heptahydrate (ZnSO<sub>4</sub>·7H<sub>2</sub>O) and 0.4 M sodium hydroxide (NaOH) aqueous solutions (both purchased from Sigma-Aldrich Co., Ltd., Dorset, United Kingdom) were mixed vigorously using magnetic stirring for 15 min. The resulting mixture was put in the microwave oven for 1 min. The product was

carefully washed with DI water and dried at 60 °C for 2 h.

### 2.3. Fabrication of cCur/ZnONPs composite

The cCur/ZnONPs composite has been synthesized as previously described method [20] as follows, the cCur in ethanol was added drop wisely to the ZnONPs in DI water under sonication (Bandelin SONOP-ULS ultrasonic homogenizer, Germany) and it was continued for 4 h by ultrasonic power of 100 W and a frequency of 30 kHz. A centrifugal was used to separate reddish orange color solid materials from the liquid. The obtained solid was then dried out 25 ± 2 °C.

### 2.4. Preparation of modified adhesives

A commercially available light cure orthodontic adhesive, Transbond XT (3M Unitek, Monrovia, CA), was used as the original material for antimicrobial functionalization. Transbond XT supplemented with 1.2, 2.5, 5, 7.5, and 10% wt. cCur/ZnONPs were used as the test material. Transbond XT without the cCur/ZnONPs served as the control. To achieve modified adhesive containing 10% wt. cCur/ZnONPs, 128 mg cCur/ZnONPs was blended into 1.152 g orthodontics adhesive, using a mixing spatula on a glass slab in a moderately dark environment until a uniform consistency of modified adhesive was achieved. The 10% wt. modified adhesive was used as a bulk modified adhesive for obtain 1.2, 2.5, 5, and 7.5% wt. modified adhesive containing cCur/ZnONPs. For preparation of the 7.5% wt. modified adhesive, 300 mg of the 10% wt. blended modified adhesive was then mixed with 100 mg of the original adhesive. Two hundred mg of the 10% wt. blended modified adhesive was then mixed with 200 mg of the original adhesive to obtain 5% wt. containing cCur/ZnONPs, similarly, 100 mg of 10% wt. modified adhesive was blended with 300 mg original adhesive for the 2.5% wt. modified adhesive, as well as 40 mg of 10% wt. modified adhesive was blended with 360 mg original adhesive for the 1% wt. modified adhesive. After that the resultant modified adhesives are passed through two roll mills for five min in a mastication stage, for better dispersion of cCur/ZnONPs in the adhesive [21].

### 2.5. Grouping of the modified adhesive samples for mechanical assays

For shear bond strength (SBS) and adhesive remnant index (ARI) testing, a total of 60 modified adhesive samples were used in this study. The samples were divided into six groups (10 value samples each) according to the percentage of cCur/ZnONPs that was added to the adhesive (1.2, 2.5, 5, 7.5, and 10%) and control group (adhesive without any additive). Fig. 1 shows the study design and experimental procedures in this study.

### 2.6. Shear bond strength testing

Sixty extracted, human maxillary first premolars for periodontics and orthodontic reasons were collected, kept and preparation for SBS testing according to the ISO 29022:2013 guideline. The teeth were randomly mounted using cold-cure acrylic resin; then the teeth were polished with fluoride-free pumice using prophylactic rubber cups at low speed for 15 s and thoroughly air-dried. Orthodontic metal brackets (3SDental group, Tehran, Iran) of maxillary first premolars were used to bond all test groups of teeth as described previously [21]. The brackets bond teeth were immersed in 37 °C water bath for overnight. Before SBS testing, the brackets bond teeth subjected to thermo-cycling (according to ISO/TS 11405:2015 guideline). SBS testing was done using a universal testing machine (Zwick/Roell, Germany) with speed of 1.0 ± 0.1 mm/min in occlusal-gingival direction at the bracket-tooth interface. The SBS value in MPa was calculated as described previously [21].

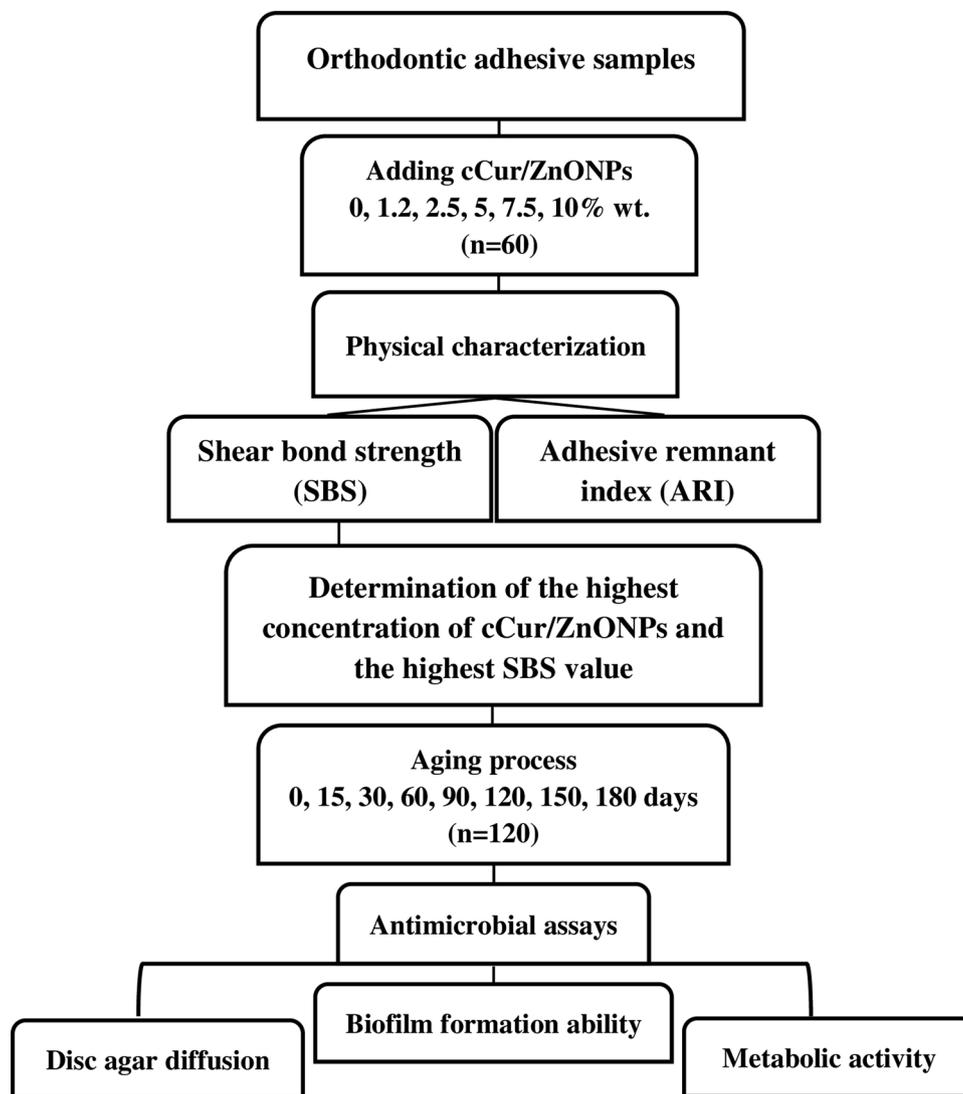


Fig. 1. Flowchart of the study design and experimental procedures.

## 2.7. Adhesive remnant index

After debonding, the quantitative analysis and interpretation criteria of residual adhesive adhering to the enamel surface were determined using the ARI under a stereomicroscope (SMZ800, Nikon, Tokyo, Japan) at  $\times 10$  magnification according to previous study [22].

## 2.8. Antimicrobial testing

### 2.8.1. Bacterial strains and culture conditions

Standard strains of *S. mutans* (ATCC 35668), *S. sobrinus* (ATCC 33478), and *L. acidophilus* (ATCC 314) were used in this study. Fresh brain heart infusion (BHI) broth (Merck, Darmstadt, Germany) bacterial cultures in an anaerobic atmosphere at 37 °C in the logarithmic growth phase (4–5 h old), were adjusted to a concentration of  $1.5 \times 10^8$  colony forming units (CFU)/ml, as verified by both spectrophotometry (optical density [OD] 600 nm: 0.08–0.13) and colony counting.

### 2.8.2. Molds construction for preparation the modified adhesive samples for antimicrobial assays

Metallic round molds were used in fabricating adhesive discs of uniform size- five mm diameter by two mm thickness for antimicrobial assays as described previously [23].

### 2.8.3. Modified orthodontic adhesives samples preparation

The original and modified orthodontic adhesives were condensed in the molds, which were then placed on glass slides according to each group. Molds containing adhesives were covered with celluloid strips and pressed with another glass slide. Both sides of all samples were exposed to light cure (Demetron, Kerr, Orange, CA, USA) for 40 s. The samples were kept in DI water prior to artificial aging progress as follow.

### 2.8.4. Artificial aging progress of discs

Following gamma-irradiation sterilization, the 120 discs with the highest concentration of cCur/ZnONPs and the highest SBS value simultaneously were placed in a homemade aging progressor device (Fig. 2), containing 75 ml of sterile artificial saliva (0.2 g NaCl, 0.2 g KCl, 0.453 g  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.345 g  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ , 0.0025 g  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ , 0.5 g urea in 1000 ml of DI water; pH 7) (time = T0), and subjected to a constant flow of sterile artificial saliva at  $7.5 \pm 0.5$  ml/h using the pumps (EMC. Co, Tehran, Iran) at 37 °C for up to 180 day. At the desired time, 0, 15, 30, 60, 90, 120, 150, and 180 days, 5 discs from each group were retrieved from the tank with sterile forceps, allowed to dry inside a Class II Biological Safety Cabinet (Besat Industrial Complex Co. Qom, Iran), and subjected to the following antimicrobial assays [24].



Fig. 2. Homemade aging progressor device.

#### 2.8.5. Light source

Light-emitting diode (DY400-4, Denjoy Dental Co., Ltd., Shenzhen, China) as the corresponding light source with output intensity of 1000–1400 mW/cm<sup>2</sup> at the wavelength of 435 ± 20 nm, a dose of 300–420 J/cm<sup>2</sup> and an exposure time of 5 min was used to activation of cCur/ZnONPs composite.

#### 2.8.6. Antimicrobial activity effects of modified adhesive

The influence of adding cCur/ZnONPs on antimicrobial properties of orthodontic adhesive following photo-activation were determined by prevention of biofilm formation of multispecies cariogenic biofilm-producing bacteria in crystal violet assay, reduction of metabolic activity of biofilms of these strains using XTT reduction assay and increase of zones of growth inhibition (ZGI) around each of samples through the agar medium in disc agar diffusion (DAD) testing. Biofilm formation, metabolic activity and ZGI in the control group (orthodontic adhesive containing cCur/ZnONPs samples without LED irradiation) was considered 100%, and biofilm formation, metabolic activity and ZGI in the treated groups (orthodontic adhesive containing cCur/ZnONPs samples following LED irradiation) were compared with that in the control group. There was no change in biofilm formation, metabolic activity, and ZGI following LED irradiation in test bacterial cells.

#### 2.8.7. Crystal violet assay

The biofilm formation assay was performed according to previous study [25]. Briefly, aliquots of bacterial suspension in BHI broth medium (200 µl; 1.0 × 10<sup>5</sup> CFU/ml, final concentration of each test strain) were placed into each well in 96-well microplates (TPP; Trasadingen, Switzerland). Orthodontic adhesive disc samples of each group were placed singly in an each well containing bacterial suspension. Following LED irradiation for 5 min, microplates were incubated at 37 °C for 48 h in an anaerobic atmosphere and then samples were rinsed in phosphate-buffered saline (PBS; pH 7.4) for 1 min to remove planktonic cells. Each orthodontic adhesive disc sample was then stained with 0.1% (w/v) crystal violet for 20 min at room temperature, and then washed twice with distilled water. Biofilm was solubilized using 1000 µl of 95% ethanol for 20 min. The optical density of each well was quantified at wavelength of 570 nm using an automatic microplate reader (Thermo Fisher Scientific, US). Orthodontic adhesive containing cCur/ZnONPs samples placed in the medium without LED irradiation and processed along with other samples constituted the control.

#### 2.8.8. Assessment of treated biofilm cells metabolic activity using XTT reduction assay

The metabolic activity of the biofilm cells was determined using the XTT (2,3-bis [2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide) (Sigma-Aldrich Co., Ltd., Dorset, United Kingdom)

reduction assay, as described previously [25]. Following treatments under identical conditions to those described as biofilm formation assay section, then samples were removed from each well and planktonic cells were deducted by washing three times with PBS. Following samples of each group were placed singly in an each well of 24-well microplates (TPP; Trasadingen, Switzerland) containing 1 ml of culture medium and 0.1 ml of 5 mg/ml MTT solution, then incubated at 37 °C for 2 h. The medium was then removed and dimethyl sulfoxide was added (1 ml/each well) to solubilize the MTT for 3 h in the dark at 37 °C. Following incubation, 100 µl of the solution was transferred to fresh wells of 96-well microplates and the color intensity was measured by an automatic microplate reader at 570 nm.

#### 2.8.9. Disc agar diffusion test

The Clinical Laboratory Standards Institute (CLSI) guideline [26] for Disc agar diffusion test (DAD) used to assess the susceptibility of the test cariogenic bacteria to the orthodontic adhesive containing cCur/ZnONPs samples. The DAD method was performed by applying the bacterial inoculums of approximately 1–2 × 10<sup>8</sup> CFU/ml to the surface of BHI agar (Merck, Darmstadt, Germany) plates. Samples were placed on the inoculated agar surface with 2 cm distance from each other. Following LED irradiation for 5 min, plates were incubated for 24 h at 37 °C in an anaerobic condition. The ZGI around each of samples were measured to the nearest mm using a manual caliper. The diameter of the zone is related to the susceptibility of the test bacteria and to the diffusion rate of activated cCur/ZnONPs through the agar medium. Orthodontic adhesive containing cCur/ZnONPs samples placed on the plates without LED irradiation and processed along with other samples constituted the control.

#### 2.9. Statistically analysis

Two-way analysis of variance (ANOVA) was performed to detect the significant effects of the variables (different concentration of addition nanoparticles to orthodontics adhesive, i.e. material type, and aging status) on DAD test, biofilm formation inhibition assays and bacterial metabolic activity, while Tamhane multiple comparison test was used for comparison between the means of any two groups. One-way ANOVA and post-hoc tests were used to determine significant differences in SBS values and ARI score among test groups. Statistical significance for all statistical tests was predetermined at P < 0.05.

### 3. Results

#### 3.1. Confirmation of synthesized cCur/ZnONPs

Successful synthesis of cCur/ZnONPs was confirmed via the SEM. As shown in Fig. 3, the cCur/ZnONPs was a nano-sized particle around 30–80 nm in diameter with a uniform shape.

#### 3.2. Shear bond strength

Table 1 shows the effect of the incorporation of different concentration of cCur/ZnONPs in orthodontic adhesive on SBS. Modified orthodontic adhesive sample with 1% wt. cCur/ZnONPs showed the highest SBS value (25.82 ± 3.81 MPa, P > 0.05). According to one-way ANOVA, modify orthodontic adhesive samples with 10% wt. cCur/ZnONPs showed a significantly lowest SBS value (5.42 ± 0.86 MPa, P < 0.05) in comparison with the control group. Table 1 also shows the reduction in SBS was positively associated to the cCur/ZnONPs concentration. The results revealed that despite lower SBS value in 7.5% wt. cCur/ZnONPs (P < 0.05), 5% wt. cCur/ZnONPs (P > 0.05), 2.5% wt. cCur/ZnONPs (P > 0.05) and 1.2% wt. cCur/ZnONPs (P > 0.05) groups than the control group; these differences did not reach statistical significance. Since the highest concentration of cCur/ZnONPs and the highest SBS value were shown simultaneously after

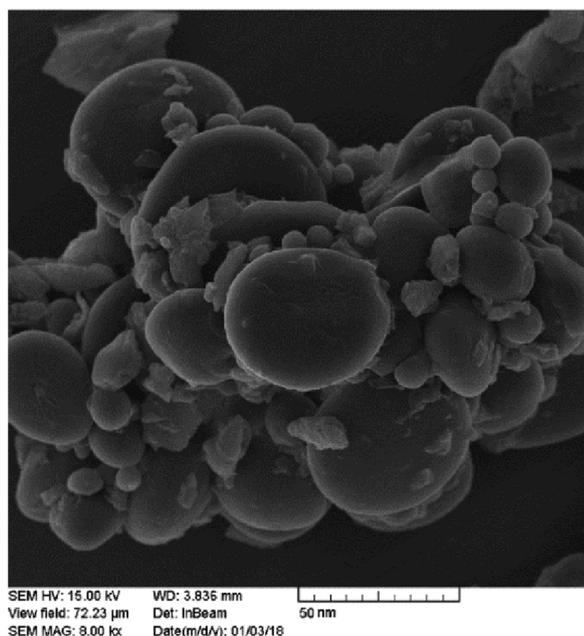


Fig. 3. Scanning electron microscope image of synthesized cCur/ZnONPs (scale bar represents 50 nm).

Table 1

The mean of shear bond strength (SBS) of bracket to enamel in the test groups (MPa).

% wt. cCur/ZnONPs modified adhesive (n = 60)	Minimum	Maximum	Mean ± SD <sup>a</sup>	P value
0.0	16.37	39.22	27.33 ± 5.17	–
1.2	14.61	35.19	25.82 ± 3.81	P > 0.05
2.5	13.73	32.65	21.61 ± 4.68	P > 0.05
5.0	11.87	28.18	17.53 ± 4.12	P > 0.05
7.5	6.40	19.30	14.89 ± 3.26	P < 0.05
10.0	2.35	8.43	5.42 ± 0.86	P < 0.05

<sup>a</sup> Standard deviation.

7.5% wt. cCur/ZnONPs modification, we selected this modification for antimicrobial analysis.

### 3.3. Adhesive remnant index

Table 2 shows the comparison between the mean ARI scores of tested groups. The significant differences were not found in the ARI scores between the modified orthodontic adhesive samples with different concentration of cCur/ZnONPs and the original orthodontic adhesive, as control, when tested after thermocycling (P > 0.05).

Table 2

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

% wt. cCur/ZnONPs modified adhesive (n = 60)	ARI scores					P value
	0.00	1.00	2.00	3.00	4.00	
0.0	0	2	3	3	4	–
1.2	1	2	3	3	3	P < 0.05
2.5	0	3	3	3	3	P < 0.05
5.0	1	3	3	3	2	P < 0.05
7.5	1	4	2	3	2	P < 0.05
10.0	1	5	3	2	1	P < 0.05

### 3.4. Antimicrobial testing

#### 3.4.1. Effects of treatments on biofilm formation ability

The viable numbers of *S. mutans*, *S. sobrinus*, and *L. acidophilus* cells in the biofilm after 48 h were approximately 6.97, 6.64, and 6.31 logs CFU/ml, respectively. Photo-activated 7.5% wt. cCur/ZnONPs were not colonized by the test microorganisms, up to 90 day of rinsing, and from days 90 onwards, photo-activated 7.5% wt. cCur/ZnONPs were colonized by a progressively increasing number of microorganisms, although the number of biofilm former microorganisms was always significantly lower than that detected at the surface of cCur/ZnONPs free LED irradiation (the negative control), except at day 180 (Table 3). The *S. mutans* and *S. sobrinus* in biofilm were more susceptible to photo-activated 7.5% wt. cCur/ZnONPs than the *L. acidophilus* in biofilm (P < 0.05). 7.5% wt. cCur/ZnONPs following photo-activation using LED were colonized only by *L. acidophilus* at day 90. The reduction of *L. acidophilus* biofilm former was reduced significantly by 99.98% at photo-activated 7.5% wt. cCur/ZnONPs surface at days 90 comparisons with control group (cCur/ZnONPs free LED irradiation; P < 0.05).

Although photo-activated 7.5% wt. cCur/ZnONPs containing discs were colonized by all test bacterial strains, after 120 day of rinsing. At day 90, the number of colonized *L. acidophilus* was lower than that detected at the surface of photo-activated 7.5% wt. cCur/ZnONPs at days 120, 150 and 180 (99.98, 98.76 and 36.90% reduction, respectively; Table 3). The populations of *S. mutans*, *S. sobrinus*, and *L. acidophilus* biofilm cells were lower significantly by 99.89%–99.99% at photo-activated 7.5% wt. cCur/ZnONPs surface at days 120 and 150 in comparisons with control group (cCur/ZnONPs free LED irradiation; P < 0.05). Statistical analysis test rejected the null hypothesis that there is no statistically significant difference between the antimicrobial activity of the orthodontic adhesive containing 7.5% wt. cCur/ZnONPs at days 150 submitted to light activation and the original orthodontic adhesive against multispecies cariogenic biofilm-producing bacteria (P < 0.05).

#### 3.4.2. Metabolic activity

Photo-activated 7.5% wt. cCur/ZnONPs suppressed 100% metabolic activity of the test microorganisms up to 90 day of rinsing (P < 0.05). From day 90 onwards, there was distinct a progressively increasing metabolic activity of biofilm cells, although, in day 180, photo-activated 7.5% wt. cCur/ZnONPs were colonized by all test bacteria as the same as negative control (P > 0.05), but the metabolic activities of biofilm former microorganisms were significantly lower (59%; P < 0.05) than that detected at the surface of the negative control; suggesting that photo-activated 7.5% wt. cCur/ZnONPs have a significant effect on the metabolic oxidative activity of the treated biofilm cells.

#### 3.4.3. Disc agar diffusion test

The DAD assays showed that photo-activated 7.5% wt. cCur/ZnONPs had antimicrobial activity toward all test microorganisms during the early part of the 180 day experimental period. The photo-activated 7.5% wt. cCur/ZnONPs retained antimicrobial properties against *S. mutans* and *S. sobrinus* up to 180 day of rinsing during experimental period, although from day 90 onwards, significant differences were observed in the ZGI compared with T0 (P < 0.05; Table 4). The reduction of antimicrobial activity of the photo-activated 7.5% wt. cCur/ZnONPs against *L. acidophilus* was positively associated to the time, in such a way that it was decreased significantly after 60 day of continuous rinsing. Between 60 and 180 days, photo-activated 7.5% wt. cCur/ZnONPs had completely lost their antimicrobial activity against *L. acidophilus*, so that bacterial growth extended under the discs. Analysis by ANOVA, of the obtained data from DAD assays showed that microorganisms and aging progress were significantly associated with the size of the ZGI. According to the post-hoc test, *L. acidophilus* was the most resistance microorganism, whereas the two streptococci species

**Table 3**  
Quantitative evaluation of biofilm (formed by the 3 test bacteria) to photo-activated 7.5% wt. cCur/ZnONPs orthodontic adhesive pretreated by incubation in a flow of sterile artificial saliva for 1–180 days.

Strains	Days									Control
	1	15	30	60	90	120	150	180		
<i>S. mutans</i>	NG	NG	NG	NG	NG	2.12 ± 2.2*	3.25 ± 2.4*	5.01 ± 3.3	6.97 ± 4.7	
<i>S. sobrinus</i>	NG	NG	NG	NG	NG	1.85 ± 1.4*	3.73 ± 2.6*	5.64 ± 3.8	6.64 ± 5.4	
<i>L. acidophilus</i>	NG	NG	NG	NG	2.47 ± 1.3*	3.32 ± 2.4*	4.40 ± 3.2*	6.11 ± 4.1	6.31 ± 4.2	

Values are given as mean number of adherent bacteria per disc ± SD.

NG: non-growth.

\* Significant difference (P ≤ 0.05) vs. values obtained at the same time point for original orthodontic adhesive (the negative control).

**Table 4**  
Comparison zone growth inhibition (mm) of photo-activated 7.5% wt. cCur/ZnONPs orthodontic adhesive against test microorganisms in disc agar diffusion assay.

Strains	Days							
	1	15	30	60	90	120	150	180
<i>S. mutans</i>	13	13	13	11	7	7	6	6
<i>S. sobrinus</i>	13	12	12	11	7	7	6	6
<i>L. acidophilus</i>	10	10	10	5	5	5	5	5

In this assay, 5 × 2-mm discs of photo-activated 5% wt. cCur/ZnONP orthodontic adhesive, pre-incubated for 1–180 days in a continuous flow of sterile artificial saliva, were placed on plate cultures of the 3 test cariogenic strains and the plates were incubated at 37 °C for an overnight. Data are reported as overall means of five determinations for each tested strain. The SD values were always ≤ 10%.

tested demonstrated comparable susceptibilities.

#### 4. Discussions

Potential complications during fixed orthodontic procedures are enamel demineralization, WSLs, and incipient tooth decay around bonded orthodontic brackets. Lack of the antimicrobial activity of conventional orthodontic adhesive and bonding agents justify the efforts of the different research groups on approaches that may potentiate the antimicrobial activity of orthodontic adhesive against cariogenic bacteria to effectively inhibit the formation of WSLs [4,5,27–33]. Recent reports have declared that modification of material due to adding antimicrobial agents can affect the bonding systems and the failure rates of orthodontic brackets. In this study we evaluated the effect of incorporation of cCur/ZnONPs on mechanical properties including SBS and ARI as well as antimicrobial effects of orthodontic adhesive. ZnONPs have an antimicrobial effect, biocompatibility, minimum cytotoxicity and white color which are less likely to alter its esthetic [34]. cCur was used because it has improved antimicrobial, mechanical and physical properties as well as excellent biocompatibility, higher solubility, better pass through the cell membrane, and greater efficacy in lower dose compared to Cur [34]. Both ZnONPs and cCur were reported to be better than single additives due to the size differences between both items.

The results of our study showed that by addition up to 7.5% wt. cCur/ZnONPs, the SBS of Transbond XT composite to enamel did not change significantly in comparison with the control group and was within the clinically acceptable range (6–8 MPa). It should be noted that SBS can be 40% less when measured real clinical cases than *in vitro* [35] and this makes our SBS results in the acceptable score what will be in *in vivo*, adding 7.5% wt. cCur/ZnONPs can maintained the SBS for optimum clinical applications, further *in vivo* studies are needed. In this study, addition of 10% wt. cCur/ZnONPs, the SBS of orthodontic adhesive, Transbond XT, to enamel change significantly. This clinically unacceptable SBS of cCur/ZnONPs incorporated modified orthodontic

adhesive is in consistent with the research conducted by Sodagar et al. [36]; they reported that the SBS in 10% CurNPs group was significantly lower than that in the control group; however, they used CurNPs only instead of cCur/ZnONPs. In the other study, Akhavan et al. [37] reported a significant reduction in bond strength following addition of 5% and 10% silver nanoparticles (AgNPs)/hydroxyapatite. In the dose dependent manner, their results were in line with ours. In the current study, we have observed a high SBS (25.82 ± 3.81 MPa) in 1% wt. cCur/ZnONPs incorporated orthodontic adhesive in agreement with other studies [21,36] which showed orthodontic adhesive with 1% wt. zirconium dioxide- titanium dioxide (ZrO<sub>2</sub>-TiO<sub>2</sub>) and 1% wt. CurNPs had the SBS 20.05 ± 0.2 and 23.03 ± 6.15 MPa, respectively [21,36]. However, Akhavan et al. [37] results regarding increase SBS in 1% AgNPs/hydroxyapatite group were different from Sodagar et al. [36] and our findings, which are attributed to the main differences between the studies including the type of teeth and the type of nanoparticles used. Akhavan et al. [37] conducted that increased SBS in 1% AgNPs/hydroxyapatite group was due to the ability of hydroxyapatite to enhance reinforcing the supporting structures and increasing the mechanical strength of the adhesive layer. However, it should be considered that excessive increased SBS is not always desirable and if it exceeds an optimal amount, it can cause enamel damage at the time of bracket debonding [37].

In the current study, no significant difference was noted among the test groups in terms of ARI scores, which agreed with the results of Sodagar et al. [36] and Asiry et al. [38] studies. Sodagar et al. [36] reported no significant difference in ARI scores among Transbond XT containing silver/hydroxyapatite nanoparticles and conventional Transbond XT adhesive. Similarly, according to Asiry et al. [38] study, comparison of ARI scores based on the type of adhesive and presence/absence of yttrium fluoride nanoparticles showed no significant difference in this regard. ARI evaluation showed a higher number of scores 2, which has been considered favorable by some authors [39], since there is less adhesive to remove from the enamel surface, thus, less risk of iatrogenic damage to enamel during polishing after debonding. Studies have been conducted over this matter, since the literature contains conflicting reports of whether low ARI scores are optimal or not [37,39,40].

Data obtained from our experiments confirm that addition of cCur/ZnONPs and activation following photo-activation is a valid solution to prolong antimicrobial activity of conventional orthodontic adhesive through the local inhibition of bacterial growth in biofilm conditions. In this study, the antimicrobial activity of the modified orthodontic adhesive showed a progressive reduction with the length of time that the material was rinsed with sterile artificial saliva up to 180 day. As expected, original orthodontic adhesive without adding any antimicrobial substance was grouped alone as the worst performer in antimicrobial tests. Such antimicrobial activities are possibly a consequence of the release of photo-activated cCur/ZnONPs, in the test conditions adopted, the effectiveness decreased gradually following exposure to artificial saliva.

Several studies [15,41,42] demonstrated that aPDT can be used as a

convenient adjunct strategy to promote the oral decontamination, prevention, and treatment of gingivitis and delay undesired side effects including WSLs, and incipient tooth decay during fixed orthodontic treatment. Recent reports have declared during aPDT, adjacent tissues also receive light and thus further effects could be expected due to the sum of the antimicrobial activity, photobiomodulation and photo stimulatory effects. Moreover, it has been reported that LED light may have potential in stimulating periodontal tissue repair and decrease inflammation as well as inhibitory effects on orthodontically induced root resorption [43,44]. Some authors reported that LED-mediated-photobiomodulation therapy method after multibanding can accelerate orthodontic tooth movement and reduce the pain associated with it [44,45]. It has been shown that LED-mediated photobiomodulation associated with accelerating bone formation and improves rapid maxillary expansion in the midpalatal suture as well as mandibular growth in cases with mandibular hypoplasia [46–48].

Generally, our findings are consistent with recent Passariello et al. study [49] that shows reduction of antimicrobial activity, as detected by DAD assay, was paralleled by loss of the ability of modified commercial test material to inhibit biofilm formation of cariogenic strains at its surface through the time. However, they used mono species biofilm formation model instead of multispecies biofilm formation model, which was used in this study. As already noted, the multitude of bacterial species inhabiting in dental plaque, and it take into account the effect of the oral microbiome, their metabolisms and virulence factors on the evaluating the antimicrobial activity of the dental material. In our study, contrary to the study of Passariello et al. [49], this notice was considered, so that the results of our work are closer to reality and *in vivo* situation. The effect of oral *in vivo* conditions on this activity cannot be resulted based on the obtained information of these *in vitro* assays. However, contrary to their report, all the test streptococci, in the present study, were more susceptible to modified orthodontic adhesive containing photo-activated cCur/ZnONPs than their test material. Although Passariello et al. [49] demonstrated that *L. acidophilus* yielded significantly larger inhibition halos and poorer biofilm former compared with the test streptococci in DAD and biofilm formation assays, respectively. Passariello et al. [49] in tests evaluating the residual inhibitory activity of the modified commercial materials, during 180 day of rinsing with saline, on biofilm formation by the cariogenic bacterial strains, showed that from days 30 and 60 onwards, fluoride-enriched test materials (Glass ionomer, Light-cured adhesive and Light-cured resin-modified glass ionomer + 23% ZnO) were colonized by a progressively increasing number of test strains, although glass ionomer + fluoride + 10% chlorhexidine was not colonized by the test bacteria, even following rinsing for 180 day.

In current study, despite the use of multispecies cariogenic biofilm-producing bacteria model, artificial saliva and ageing process of modified test material, in interpreting the obtained data it must be emphasized that the study design adopted did not take into account the effects of all variability of the physico-chemical conditions of the environment, mechanical disturbance, the complex dental microbiota, and a multitude of other subjective aspects that may influence the antimicrobial activity and clinical effectiveness of the selected materials *in vivo*. Polymerization shrinkage and color stability of adding cCur/ZnONPs on orthodontics adhesives could be studied in the future. In spite of the debated limitations of our study, the results provide sufficient information to support *in vitro* significant activity against sessile bacteria without adverse effect on mechanical properties, which will be improved clinician confidence in the choice of materials that can be mediated compatible with different clinical expectations and application. Consequently, further clinical trial studies are needed to confirm that orthodontic adhesive containing photo-activated cCur/ZnONPs is associated with a reduced incidence of white spotting and incipient caries and increase remineralization.

## 5. Conclusions

Since there were no significant differences in the SBS and ARI score among the 7.5% wt. cCur/ZnONPs adhesive and conventional orthodontic adhesive, photo-activated 7.5% wt. cCur/ZnONPs can serve as an orthodontic adhesive additive with antimicrobial properties for control cariogenic multispecies biofilm formation.

## Conflicts of interest

The authors declare that they have no conflict of interests with regard to this study.

## References

- [1] J.A. Chapman, W.E. Roberts, G.J. Eckert, K.S. Kula, C. González-Cabezas, Risk factors for incidence and severity of white spot lesions during treatment with fixed orthodontic appliances, *Am. J. Orthod. Dentofac. Orthop.* 138 (2010) 188–194.
- [2] A. Tiro, Orthodontic Treatment-Related Risks and Complications: part I dental complications, *South. Eur. J. Orthod. Dentofac. Res.* 4 (2017) 43–47.
- [3] J. Kreth, J. Merritt, W. Shi, F. Qi, Competition and coexistence between *Streptococcus mutans* and *Streptococcus sanguinis* in the dental biofilm, *J. Bacteriol.* 187 (November) (2005) 7193–7203.
- [4] E. Ionescu, E. Teodorescu, A. Badarau, R. Grigore, M. Popa, Prevention perspective in orthodontics and dento-facial orthopedics, *J. Med. Life* 1 (2008) 397–402.
- [5] A. Borzabadi-Farahani, E. Borzabadi, E. Lynch, Nanoparticles in orthodontics, a review of antimicrobial and anti-caries applications, *Acta. Odontol. Scand.* 72 (2014) 413–417.
- [6] J.R. Jedrychowski, A.A. Caputo, S. Kerper, Antibacterial and mechanical properties of restorative materials combined with chlorhexidines, *J. Oral. Rehabil.* 10 (1983) 373–381.
- [7] I. Bay, G. Rølla, Plaque inhibition and improved gingival condition by use of a stannous fluoride toothpaste, *Scand. J. Dent. Res.* 88 (1980) 313–315.
- [8] M. Cierech, J. Wojnarowicz, D.B. Szmigiel, B. aczkowski, A. Grudniak, I. Wolska, et al., Preparation and characterization of ZnO-PMMA resin nanocomposites for denture bases, *Acta. Bioeng. Biomech.* 18 (2016) 31–41.
- [9] M. Cierech, A. Kolenda, A. Grudniak, J. Wojnarowicz, B. Woźniak, M. Goła's, et al., Significance of polymethylmethacrylate (PMMA) modification by zinc oxide nanoparticles for *funFagal* biofilm formation, *Int. J. Pharm.* 510 (2016) 323–335.
- [10] P. Popovic, R. Bobovnik, S. Bolka, M. Vukadinovic, V. Lazic, R. Rudolf, Synthesis of PMMA/ZnO nanoparticles composite used for resin teeth, *Mater. Technol.* 51 (2017) 871–878.
- [11] D.A. Cusicanqui Méndez, E. Gutierrez, E.J. Dionisio, M.A. Rabelo Buzalaf, R.C. Oliveira, M.A. Andrade Moreira Machado, et al., Curcumin-mediated Antimicrobial Photodynamic Therapy reduces the viability and vitality of infected dentin caries microcosms, *Photodiagn. Photodyn. Ther.* 1000 (2018) 30207.
- [12] R.N. Moussawi, D. Patra, Nanoparticle Self-Assembled Grain Like Curcumin conjugated ZnO: curcumin conjugation enhances removal of perylene, fluoranthene, and chrysenes by ZnO, *Sci. Rep.* 6 (2016) 24565.
- [13] F. Li, Z.G. Chai, M.N. Sun, F. Wang, S. Ma, L. Zhang, et al., Anti-biofilm effect of dental adhesive with cationic monomer, *J. Dent. Res.* 88 (2009) 372–376.
- [14] S. Boonroeng, K. Srikulkit, J.H. Xin, L. He, Preparation of a novel cationic curcumin and its properties evaluation on cotton fabric, *Fib. Polym.* 16 (2015) 2426–2431.
- [15] M.A. Paschoal, C.M. Moura, F. Jeremias, J.F. Souza, V.S. Bagnato, J.S. Giusti, et al., Longitudinal effect of curcumin-photodynamic antimicrobial chemotherapy in adolescents during fixed orthodontic treatment: a single-blind randomized clinical trial study, *Lasers Med. Sci.* 30 (2015) 2059–2065.
- [16] S. Rajesh, E. Koshi, K. Philip, A. Mohan, Antimicrobial photodynamic therapy: an overview, *J. Indian Soc. Periodontol.* 15 (2011) 323.
- [17] M. Pourhajibagher, L. Beytollahi, R. Ghorbanzadeh, A. Bahador, Analysis of glucosyltransferase gene expression of clinical isolates of *Streptococcus mutans* obtained from dental plaques in response to sub-lethal doses of photoactivated disinfection, *Photodiagn. Photodyn. Ther.* 24 (2018) 75–81.
- [18] N. Chiniforush, M. Pourhajibagher, S. Shahabi, A. Bahador, Clinical Approach of High Technology Techniques for Control and Elimination of Endodontic Microbiota, *J. Lasers Med. Sci.* 6 (2015) 139–150.
- [19] D. Sharma, J. Rajput, B.S. Kaith, M. Kaur, S. Sharma, Synthesis of ZnO nanoparticles and study of their antibacterial and antifungal properties, *Thin Solid Films* 519 (2010) 1224–1229.
- [20] D. Raman, R. Jothi, J. Rajendhran, M. Rajasekaran, J. Annaraj, pH responsive curcumin/ZnO nanocomposite for drug delivery, *Adv. Mater. Lett.* 6 (2015) 505–512.
- [21] N.H. Felemban, M.I. Ebrahim, The influence of adding modified zirconium oxide-titanium dioxide nano-particles on mechanical properties of orthodontic adhesive: an *in vitro* study, *BMC Oral Health* 17 (2017) 43.
- [22] T.A. Al-Musallam, C.A. Evans, J.L. Drummond, C. Matasa, C.D. Wu, Antimicrobial properties of an orthodontic adhesive combined with cetylpyridinium chloride, *Am. J. Orthod. Dentofacial Orthop.* 129 (2006) 245–251.
- [23] S.E. Bishara, R. Ajlouni, J.F. Laffoon, Effect of thermocycling on the shear bond strength of a cyanoacrylate orthodontic adhesive, *Am. J. Orthod. Dentofacial Orthop.* 123 (2003) 21–24.

- [24] A.P. Desbois, V.J. Smith, Disk diffusion assay to assess the antimicrobial activity of marine algal extracts, *Methods Mol. Biol.* 1308 (2015) 403–410.
- [25] M. Pourhajibagher, E. Boluki, N. Chiniforush, B. Pourakbari, Z. Farshadzadeh, R. Ghorbanzadeh, et al., Modulation of virulence in *Acinetobacter baumannii* cells surviving photodynamic treatment with toluidine blue, *Photodiagnosis Photodyn. Ther.* 15 (2016) 202–212.
- [26] Clinical and Laboratory Standards Institute, *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*; approved standard, 10th ed., M07-A11 Clinical and Laboratory Standards Institute, Wayne, PA, 2018.
- [27] N. Arhun, A. Arman, S.B. Cehreli, S. Arikan, E. Karabulut, K. Gülşahi, Microleakage beneath ceramic and metal brackets bonded with a conventional and an anti-bacterial adhesive system, *Angle Orthod.* 76 (2006) 1028–1034.
- [28] M. Amasyali, S. Enhos, T. Uysal, I. Saygun, A. Kilic, O. Bedir, Effect of a self-etching adhesive containing an antibacterial monomer on clinical periodontal parameters and subgingival microbiologic composition in orthodontic patients, *Am. J. Orthod. Dentofacial Orthop.* 140 (2011) 147–153.
- [29] T. Uysal, M. Amasyali, S. Ozcan, A.E. Koyuturk, D. Sagdic, Effect of antibacterial monomer-containing adhesive on enamel demineralization around orthodontic brackets: an in-vivo study, *Am. J. Orthod. Dentofacial Orthop.* 139 (2011) 650–656.
- [30] K. Saito, T. Hayakawa, R. Kawabata, D. Meguro, K. Kasai, In vitro antibacterial and cytotoxicity assessments of an orthodontic bonding agent containing benzalkonium chloride, *Angle Orthod.* 79 (2009) 331–337.
- [31] M.M. Farret, E.M. de Lima, E.G. Mota, H.M. Oshima, V. Barth, S.D. de Oliveira, Can we add chlorhexidine into glass ionomer cements for band cementation? *Angle Orthod.* 81 (2011) 496–502.
- [32] C.G. Spencer, P.M. Campbell, P.H. Buschang, J. Cai, A.L. Honeyman, Antimicrobial effects of zinc oxide in an orthodontic bonding agent, *Angle Orthod.* 79 (2009) 317–322.
- [33] S.J. Ahn, S.J. Lee, J.K. Kook, B.S. Lim, Experimental antimicrobial orthodontic adhesives using nanofillers and silver nanoparticles, *Dent. Mater.* 25 (2009) 206–213.
- [34] F.L. Yen, T.H. Wu, C.W. Tzeng, L.T. Lin, C.C. Lin, Curcumin nanoparticles improve the physicochemical properties of curcumin and effectively enhance its antioxidant and antihepatoma activities, *J. Agric. Food Chem.* 58 (2010) 7376–7382.
- [35] L. Eslamian, A. Borzabadi-Farahani, N. Mousavi, A. Ghasemi, A comparative study of shear bond strength between metal and ceramic brackets and artificially aged composite restorations using different surface treatments, *Eur. J. Orthod.* 34 (2012) 610.
- [36] A. Sodagar, A. Akhavan, E. Hashemi, S. Arab, M. Pourhajibagher, K. Sodagar, et al., Evaluation of the antibacterial activity of a conventional orthodontic composite containing silver/hydroxyapatite nanoparticles, *Prog. Orthod.* 17 (2016) 40.
- [37] A. Akhavan, A. Sodagar, F. Mojtahedzadeh, K. Sodagar, Investigating the effect of incorporating nanosilver/nanohydroxyapatite particles on the shear bond strength of orthodontic adhesives, *Acta Odontol. Scand.* 71 (2013) 1038–1042.
- [38] M.A. Asiry, I. Alshahrani, N.D. Alqahtani, B. Durgesh, Efficacy of yttrium (III) fluoride nanoparticles in orthodontic bonding, *J. Nanosci. Nanotechnol.* 19 (2019) 1105–1110.
- [39] F.S. Henkin, É.O. Macêdo, K.D. Santos, M. Schwarzbach, S.M. Samuel, K.S. Mundstock, In vitro analysis of shear bond strength and adhesive remnant index of different metal brackets, *Dental Press J. Orthod.* 21 (2016) 67–73.
- [40] É.M. Faria-Júnior, R.D. Guiraldo, S.B. Berger, A.B. Correr, L. Correr-Sobrinho, E.F. Contreras, et al., In-vivo evaluation of the surface roughness and morphology of enamel after bracket removal and polishing by different techniques, *Am. J. Orthod. Dentofacial Orthop.* 147 (2015) 324–329.
- [41] C. Gómez, R. Abellán, J.C. Palma, Efficacy of photodynamic therapy vs ultrasonic scaler for preventing gingival inflammation and white spot lesions during orthodontic treatment, *Photodiagn. Photodyn. Ther.* 24 (2018) 377–383.
- [42] V.H. Panhóca, F.L. Esteban Florez, T.Q. Corrêa, F.R. Paolillo, C.W. de Souza, V.S. Bagnato, Oral decontamination of orthodontic patients using photodynamic therapy mediated by blue-light irradiation and curcumin associated with sodium dodecyl sulfate, *Photomed. Laser Surg.* 34 (2016) 411–417.
- [43] P.D. Fonseca, F.M. de Lima, D.T. Higashi, D.F. Koyama, O. Togninho Filho Dde, I.F. Dias, et al., Effects of light emitting diode (LED) therapy at 940 nm on inflammatory root resorption in rats, *Lasers Med. Sci.* 28 (2013) 49–55.
- [44] A. Ekizer, T. Uysal, E. Güray, D. Akkuş, Effect of LED-mediated-photobiomodulation therapy on orthodontic tooth movement and root resorption in rats, *Lasers Med. Sci.* 30 (2015) 779–785.
- [45] I.Z. Figueira, A.P.C. Sousa, A.W. Machado, F.A.L. Habib, L.G.P. Soares, A.L.B. Pinheiro, Clinical study on the efficacy of LED phototherapy for pain control in an orthodontic procedure, *Lasers. Med. Sci.* (2018), <https://doi.org/10.1007/s10103-018-2617-3>.
- [46] C.B. Rosa, F.A. Habib, T.M. de Araújo, J.N. Dos Santos, M.C. Cangussu, A.F. Barbosa, et al., Laser and LED phototherapy on midpalatal suture after rapid maxilla expansion: raman and histological analysis, *Lasers Med. Sci.* 32 (2017) 263–274.
- [47] T. El-Bialy, A. Alhadlaq, New therapeutics in promoting and modulating mandibular growth in cases with mandibular hypoplasia, *Biomed Res. Int.* 2013 (2013) 789679.
- [48] T. El-Bialy, A. Alhadlaq, N. Felemban, J. Yeung, A. Ebrahim, A.H. Hassan, The effect of light-emitting diode and laser on mandibular growth in rats, *Angle Orthod.* 85 (2015) 233–238.
- [49] C. Passariello, G. Sannino, S. Petti, P. Gigola, Intensity and duration of in-vitro antibacterial activity of different adhesives used in orthodontics, *Eur. J. Oral Sci.* 122 (2014) 154–160.