Journal of Dentistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Dentistry

journal homepage: www.elsevier.com/locate/jdent

Influence of zinc oxide quantum dots in the antibacterial activity and cytotoxicity of an experimental adhesive resin

Isadora Martini Garcia[a], Vicente Castelo Branco Leitune[a], Fernanda Visioli[b], Susana Maria Werner Samuel[a], Fabrício Mezzomo Collares[a,*]

[a] Dental Materials Laboratory, School of Dentistry, Federal University of Rio Grande do Sul, Rua Ramiro Barcelos, 2492, Rio Branco, 90035-003, Porto Alegre, RS, Brazil
[b] Laboratory of Pathology, School of Dentistry, Federal University of Rio Grande do Sul, Rua Ramiro Barcelos, 2492, Rio Branco, 90035-003, Porto Alegre, RS, Brazil

ARTICLE INFO

Keywords:
Dentin bonding agents
Polymers
Semiconductors
Dental caries
Anti-bacterial agents
Biocompatible materials testing

ABSTRACT

Objective: To evaluate the influence of zinc oxide quantum dots (ZnO_{QDs}) into an experimental adhesive resin regarding the antibacterial activity against *Streptococcus mutans* and the cytotoxicity against pulp fibroblasts.
Materials and methods: ZnO_{QDs} were synthesized by sol-gel process and were incorporated into 2-hydroxyethyl methacrylate (HEMA). An experimental adhesive resin was formulated by mixing 66.6 wt.% bisphenol A glycol dimethacrylate (BisGMA) and 33.3 wt.% HEMA with a photoinitiator system as control group. HEMA containing ZnO_{QDs} was used for test group formulation. For the antibacterial activity assay, a direct contact inhibition evaluation was performed with biofilm of *Streptococcus mutans* (NCTC 10449). The cytotoxicity assay was performed by Sulforhodamine B (SRB) colorimetric assay for cell density determination using pulp fibroblasts. Data were analyzed by Student's *t*-test ($\alpha = 0.05$).
Results: The antibacterial activity assay indicated statistically significant difference between the groups ($p = 0.003$), with higher values of biofilm formation on the polymerized samples of control group and a reduction of more than 50% of biofilm formation on ZnO_{QDs} group. No difference of pulp fibroblasts viability was found between the adhesives ($p = 0.482$).
Conclusion: ZnO_{QDs} provided antibacterial activity when doped into an experimental adhesive resin without cytotoxic effect for pulp fibroblasts. Thus, the use of ZnO_{QDs} is a strategy to develop antibiofilm restorative polymers with non-agglomerated nanofillers.
Clinical significance: ZnO_{QDs} are non-agglomerated nanoscale fillers for dental resins and may be a strategy to reduce biofilm formation at dentin/restoration interface with no cytotoxicity for pulp fibroblasts.

1. Introduction

Desired properties for dental materials are biocompatibility, antibacterial activity, reliable mechanical properties and physical-chemical stability over time [1]. Zinc oxide (ZnO) shows a wide range of applications from materials engineering [2,3] up to health sciences [4,5]. In dental materials it presents properties such as antibacterial activity [6], bioactivity [7,8], inhibition of metalloproteinases [9,10] and biocompatibility [11,12]. Nanoscale materials could present distinct properties and some characteristics become more pronounced [13]. Previous studies indicated that the antibacterial activity [14] and the cytotoxicity [15] of ZnO is higher at smaller sizes. Although the mechanism behind these results is not well stablished, the larger surface area with smaller particles increase the interaction between materials and cells [12].

ZnO is synthesized with different sizes and morphologies even at nanometric dimension of quantum dots (QDs) [16,17]. QDs or "artificial atoms" [18] are nanoparticles at 1–10 nm range size [19] and present enhanced properties compared to material in the "bulk" form, such as photoactivity [13]. In addition, QDs do not agglomerate, as traditional nanoparticles [16], avoiding the concern about nanofillers incorporation into resins [20–22]. ZnO_{QDs} at 1.24 nm were previously synthesized and added into an experimental adhesive resin, showing increased degree of conversion and dentin/adhesive bond strength stability [23].

Efforts have been made to develop materials with extra therapeutic effect, capable of reducing biofilm accumulation at tooth/restoration interface as an aid to prevent the recurrence of caries [24–27]. ZnO_{QDs} could be a nanofiller for restorative materials with antibacterial activity, since ZnO presents known activity against *Streptococcus mutans*

* Corresponding author.
E-mail addresses: isadora.garcia@ufrgs.br (I.M. Garcia), vicente.leitune@ufrgs.br (V.C.B. Leitune), fernanda.visioli@ufrgs.br (F. Visioli), susana.samuel@ufrgs.br (S.M.W. Samuel), fabricio.collares@ufrgs.br (F.M. Collares).

https://doi.org/10.1016/j.jdent.2018.04.003
Received 5 February 2018; Received in revised form 28 March 2018; Accepted 7 April 2018

[6,28], which could be enhanced with ZnO_{QDs} due to higher surface area [14]. On the other hand, as the particle size decreases, the cell cytotoxicity increases, mainly for nanoparticles [29]. Despite the positive previous results about the incorporation of ZnO_{QDs} in an experimental adhesive resin, there are no reports on the assessment of the biological properties. Thus, the aim of this study is to evaluate the influence of ZnO_{QDs} into an experimental adhesive resin regarding the antibacterial activity against *Streptococcus mutans* and the cytotoxicity against pulp fibroblasts.

2. Materials and methods

All reagents were purchased from Aldrich Chemical Company (St. Louis, Missouri, USA). All components were weighed using an analytical balance (AUW220D, Shimadzu, Kyoto, Kyoto, Japan). The samples were polymerized using a light-emitting diode (Radii Cal, SDI, Australia) at 1200 mW/cm^2 for all tests.

2.1. Synthesis of ZnO_{QDs}

The ZnO_{QDs} was synthesized by a sol-gel route [16,23]. The solutions were formulated by mixing 0.24 mmol (0.0548 g) of zinc acetate dihydrate with 42 mL of isopropanol (PA) and 0.7 mmol (0.0167 g) of lithium hydroxide monohydrate with 8 mL of ethanol (PA). The solutions were ultrasonicated and cooled to 4 °C for one hour to be incorporated to 125 mL of isopropanol (PA) at 4 °C by stirring. The formulated solution was heated at 40 °C in water bath until ZnO_{QDs} stability and analyzed by UV–vis spectroscopy (Cary 7000, Agilent Technologies, Santa Clara, CA, USA) as previous studies [16,23].

2.2. Experimental adhesive resins formulation

The isopropanol was replaced by 2-hydroxyethyl methacrylate (HEMA) using the high vacuum device at low temperature previously reported [23]. Two experimental adhesive resins were formulated: the control group by mixing 66.6 wt.% bisphenol A glycol dimethacrylate (BisGMA) and 33.3 wt.% HEMA; the test group by mixing 66.6 wt.% BisGMA and 33.33 wt.% HEMA with ZnO_{QDs}. A photoinitiator system composed by Camphorquinone (CQ) and ethyl 4-dimethylaminobenzoate (EDAB) was added to both experimental resins at 1 mol%, according to the monomer moles, and 0.01 wt.% Butylated hydroxytoluene (BHT). The samples were photopolymerized for 30 s on each side.

2.3. Antibacterial activity evaluation

For the antibacterial activity assay, a direct contact inhibition evaluation was performed against *Streptococcus mutans* (NCTC 10449) using five samples of experimental adhesive resins per group (4 mm X 1 mm). The samples were attached on the lid of a test plate and the assembly was submitted to hydrogen peroxide sterilization to be evaluated 24 h after the photopolymerization. Each well contained 900 mL of brain-heart infusion broth (Aldrich Chemical Co. (St. Louis, Missouri, USA)) with 1% sucrose and 100 µL of a suspension of an overnight broth culture of *Streptococcus mutans* adjusted to optical density of 0.453 (550 nm) corresponding to 7.2×10^7 CFU/mL. The 48-well plate was incubated with the assembly (lid and samples) at 37 °C for 24 h. The samples were removed from the lid and vortexed in 1 mL of saline solution (0.9%) to be diluted until 10^{-6} dilution. Two 25-µL drops of each dilution were platted in brain-heart infusion agar Petri dishes and incubated at 37 °C for 48 h. The number of colony forming units (CFUs) was counted using optical microscopy and transformed to log CFU/mL.

2.4. Cytotoxicity evaluation

For the cytotoxicity evaluation of experimental resins, human fibroblasts were collected from a third molar after extraction (approved by the local Ethics Committee n° 1.739.340). Three samples per group (1 mm-thick x 4 mm diameter) were immersed in 1 mL of Dulbecco's Modified Eagle Medium (DMEM) for 24 h to prepare the eluates. The fibroblasts were placed at 5×10^3 in 96-well plates and treated with 100 µL of eluate from each sample. After 72 h, the cells were fixed with trichloroacetic acid (Sigma-Aldrich Chemical Co, St. Louis, Missouri, USA) at 10% incubated at 4 °C for one hour, washed six times with running water for 30 s and dried at room temperature. Sulforodamide B (Sigma-Aldrich Chemical Co, St. Louis, Missouri, USA) at 4% was added. The plate was incubated for 30 min at room temperature. Plates were washed four times with 1% acetic acid and dried at room temperature. Trizma solution was added and the plate incubated for 1 h. The microplates were read at 560 nm and the cell viability was normalized against viability of cells in wells without treatment and the cell viability was expressed in percentage [30].

2.5. Statistical analysis

Data distribution was evaluated by Shapiro-Wilk test. Student's *t*-test was used to compare the control group and the ZnO_{QDs} group at a level of 0.05 of significance.

3. Results

The results of antibacterial evaluation of the experimental adhesive resins are presented in Fig. 1. The experimental adhesive resin with ZnO_{QDs} showed decreased biofilm formation compared to control group ($p = 0.003$). The addition of ZnO_{QDs} reduced biofilm formation in experimental adhesive resin surface at about 50%, with 2.32 (± 0.34) log CFU/mL for the test group and 4.48 (± 0.47) log CFU/mL for the control group.

The results of cytotoxicity evaluation are shown in Fig. 2. It was observed that there was no difference between the groups ($p = 0.482$). The cell viability in percentage was 110.26 (± 17.27) for ZnO_{QDs} group and 100.65 (± 12.83) for control group. In this way, the viability of pulp fibroblasts was higher than 70% for both groups.

4. Discussion

Antimicrobial resins with bactericidal or antifouling effect have been developed to may reduce the biofilm formation at tooth-restoration interface [31]. In this study, an experimental adhesive resin was formulated with ZnO_{QDs} and evaluated regarding the antibacterial

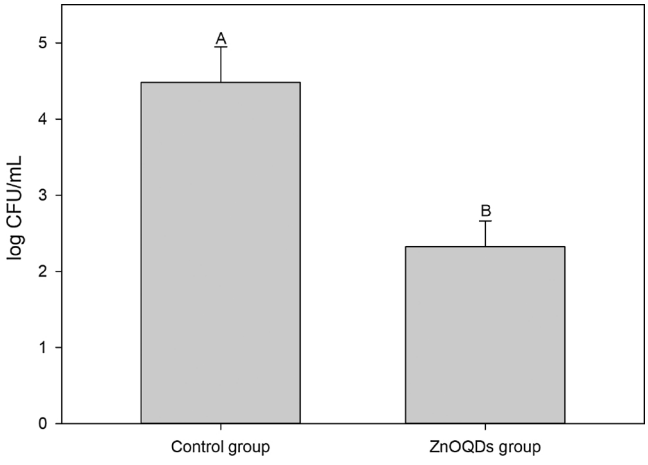

Fig. 1. Graph of mean and standard deviation values of colony forming units per milliliter after logarithmic transformation (log CFU/mL) of antibacterial activity evaluation of the experimental adhesive resins. Different capital letters indicate statistical difference between the groups ($p < 0.05$).