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Antibacterial and cytotoxic properties of boron-containing dental composite

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Abstract: Secondary dental caries are one of the major reasons for restoration replacements. Incorporating antimicrobial properties into dental materials would limit the initiation and progression of dental caries. In the current study, dental composites having 1%, 5%, and 10% (w/w) sodium pentaborate pentahydrate were prepared and analyzed for their mechanical properties, degree of monomer conversion (DC) rate, antibacterial effects against *Streptococcus mutans*, and biocompatibility with human dental pulp stem cells (hDPSCs). Incorporation of boron into the composites significantly decreased flexural strength and DC in a dose-dependent manner, but the value for 1% boron-containing composite still remained within acceptable levels. Compressive strength and diametral tensile strength were not found to be different from those of controls. Although no inhibition zone was detected in an agar-well diffusion assay for any materials tested, significant bacterial growth inhibition was obtained in a direct contact test for boron-containing composites. Immunocytochemical and lineage-specific gene expression analysis revealed that composites with boron content increased the osteogenic and odontogenic capacity of hDPSCs. Boron-containing dental composites showed promising results for future clinical applications, displaying nontoxic, osteogenic, and odontogenic-inducing characteristics with remarkable antibacterial activity against *S. mutans*, and are hence potentially able to prevent secondary caries.

Key words: Antibacterial, boron, dental composites, biocompatibility, dental caries

1. Introduction

Secondary dental caries are referred to as diagnosed carious lesions that occur at the margins of an existing restoration and they are a worldwide health and financial problem (Tavassoli et al., 2013). Two hundred million dental restorations are applied in the United States annually, and almost half of them fail within 10 years due to secondary dental caries (Cheng et al., 2013). Tooth restoration applications impose a heavy economic burden; the cost of tooth cavity reconstructions in the United States was approximately $46 billion in 2005 (Beazoglou et al., 2007). The main reason for secondary caries is the recruitment and proliferation of cariogenic bacteria such as *Streptococcus mutans* that leak through the interface of the composite and the tooth structure (Xu et al., 2012; Özyıldız et al., 2014). Therefore, incorporating antibacterial agents into dental composites and bonding agents to prevent cariogenic microbial growth and inhibit the proliferation of remaining bacteria after the surgery is of clinical and economic importance. In this manner, several antibacterial compounds, including chlorhexidine (Leung et al., 2005), chitosan (Kim and Shin, 2013), fluoride (Wiegand et al., 2007), metal nanoparticles (Kassaee et al., 2008; Aydin Sevinç and Hanley, 2010), methacryloyloxy dodecyl pyridinium bromide (Imazato et al., 1995), and quaternary ammonium salts (Li et al., 2013), have been introduced into dental restorative materials. Although these materials have good antibacterial properties, they also have some limitations: they can reduce mechanical strength even at low concentrations, their antibacterial properties can be short-lived due to the sudden release of the material from the medium, and they can be toxic to the surrounding tissue if the dose or release is not properly arranged (Beyth et al., 2006; Weng et al., 2011). Hence, the development of novel, safe, biocompatible, durable, and effective antibacterial dental restorative materials is still the subject of international research.

Boron has been recognized as an essential element for plants since the 1900s, though its importance for animal and human bodies has not been clearly elucidated yet. In addition to plant metabolism, its essentiality for certain microorganisms is unquestionable, as boron is involved

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in the structure of the quorum-sensing signaling molecule autoinducer-2 (Chen et al., 2002) and some antibiotics, such as boromycin and aplasmomycin (Carrano et al., 2009). On the other hand, it has been found to display remarkable antimicrobial characteristics against a wide range of bacteria, yeast, and fungi (De Seta et al., 2009; Qin et al., 2010). As for higher organisms, boron has been theorized to play a role in embryonic development (Rowe and Eckhert, 1999), steroid hormone release (Naghii et al., 2011), brain function (Penland, 1994), and insulin homeostasis (Bakken and Hunt, 2003). The most prominent and well-known effect of boron on mammalian metabolism is probably its contribution to bone and teeth development. Boron deprivation in rats has resulted in delayed healing in the alveolar bone, initiated immediately after tooth extraction (Gorustovich et al., 2008a). Similarly, a boron-deficient diet reduced osteoblast surface and inhibited bone formation on both the buccal and lingual sides of the periodontal alveolar bone (Gorustovich et al., 2008b). The exact mechanism of boron on bone and teeth development still remains unclear; however, limited in vitro studies have speculated that boron has a role in progenitor cell differentiation and maturation processes. Boron supplementation increased mineralization and osteogenic differentiation of osteoblasts and bone marrow stromal cells in vitro (Hakki et al., 2010; Ying et al., 2011). In a recent work, we also showed the increase in osteogenic and odontogenic differentiation capacity of human tooth germ stem cells after they were treated with boron (Taşlı et al., 2013).

Our initial observations have shown that sodium pentaborate pentahydrate (NaB) exerted less toxicity to mammalian cells (unpublished data). Therefore, NaB as a boron source was incorporated into dental composite to provide an efficient antibacterial activity against *S. mutans* along with increased osteogenic and odontogenic differentiation of human dental pulp stem cells (hDPSCs) in the current study. This is the first study, to our knowledge, reporting on the antibacterial effect of dental composite containing boron against *S. mutans*.

2. Materials and methods

2.1. Preparation of experimental composites

NaB, having 0.1832% (w/w) boron content, was kindly provided by the National Boron Research Institute-BOREN (Ankara, Turkey). Experimental ratios (1%, 5%, and 10% w/w) of boron were blended into the dental composite (Fuji G-Aenial Posterior, GC Corporation, Tokyo, Japan). A commercial formula without any additives served as a negative control. The manufacturer's compositional information is summarized in Table 1. As a comparison group, a fluoride-releasing polyacid-modified resin composite (Dyract AP, Dentsply DeTrey, Konstanz, Germany) was used in microbiological analysis. Experimental composites were prepared as described previously, i.e. in a dim room in order to prevent polymerization during blending (Beyth et al., 2006). The NaB and dental composite were mixed with a spatula inside sterile petri dishes until no powder was visible and the composite had a homogeneous consistency. After manufacturing the composites, they were UV-sterilized before use in cell culture experiments.

2.2. Flexural strength, compressive strength, diametral tensile strength

Flexural strength (FS) was determined in a three-point bending test (n = 10, each group) according to the ISO/DIN 4049: 2009 standard (ISO, 2009). Samples were prepared using rectangular molds (2 × 2 × 25 mm) compressed between 2 glass plates. Specimens were photopolymerized for 40 s by a handheld LED curing device (LED.D Curing Light, 8-mm-diameter light-guide tip, Guilin Woodpecker Medical Instrument Co., Ltd., China; 1000 mW/cm^2) and then 20 s with a light curing oven (Estenia CS-110 light and heat curing unit, Kuraray Dental, Japan) from both sides. After curing, excess irregular edges were trimmed away using sandpaper (grit size: 320). All specimens were then stored in distilled water at 37 °C for 24 h prior to testing. FS was determined using a universal testing machine (Shimadzu AGS-X, Japan) at 0.5 mm/min crosshead speed with 10 mm of distance between supports.

Compressive strength (CS) was determined on cylindrical specimens 8 mm in height and 4 mm in diameter. Specimens (n = 10, each group) were light-cured from both sides with the LED curing device for 40 s. The

Table 1. Composite (Fuji G-Aenial) product details.

Resin matrix	Urethane dimethacrylate and dimethacrylate comonomers
Filler	Fluoroaluminosilicate glass, Sr glass, silica, lanthanide fluoride
Filler content by weight	81%

Source: GC Dental Products Corp.

samples were placed with the flat end on the supporting plate of the universal testing machine and a compressive load was applied axially at 0.5 mm/min crosshead speed.

Diametric tensile strength (DTS) was determined on cylindrical specimens 3 mm in height and 6 mm in diameter cured in the same manner as the CS specimens. The samples (n = 10, each group) were placed with the outside surface of the cylinder contacting the supporting plates of the universal testing machine and a compressive load was applied at 0.5 mm/min crosshead speed.

2.3. Degree of conversion

Degree of monomer conversion (DC) was measured using a FT-IR spectrometer (Thermo Scientific, Nicolet iS10, USA). The FT-IR spectra were recorded with 1 scan at a resolution of 4 cm^{-1}. Samples from each experimental group (n = 3) and controls were analyzed in a mold (2 mm in width and 4 mm in diameter). First, the spectrum of the unpolymerized sample was measured. Samples were then irradiated with the same LED curing device for 40 s. The sample was scanned for its FT-IR spectrum every 4 s for 1 min after the beginning of irradiation. The percentages of monomer conversion were calculated using intensities of C-O bond absorbance peak at 1320 cm^{-1} with a modified equation described before (Sideridou et al., 2002):

$$(\%)\ DC = ((A_0 - A_t) / (A_0 - A_b)) \times 100,$$

where A_0 is the absorbance intensity before polymerization, A_t is the absorbance intensity at time t, and A_b is the baseline absorbance intensity.

2.4. Microbiological analysis

Three different concentrations of boron-containing solid disk composites (1%, 5%, and 10% w/w), a commercial composite without any additive as a control, and a polyacid-modified resin composite (Dyract) were evaluated for their antibacterial effects against *S. mutans* via an agar-well diffusion assay and a direct contact test.

2.4.1. Agar-well diffusion assay

The agar-well diffusion assay was conducted according to the protocol as described previously (Lai et al., 2001; Chanda et al., 2013). *S. mutans*, provided from the culture collection of the Microbiology Laboratory of Yeditepe University, was grown in brain heart infusion broth (BHIB, Fluka, USA) at 36 ± 1 °C with 5% CO_2 for 16 h. The suspension density was set to be equivalent to McFarland 0.5, and then 100 µL of bacterial suspension was spread onto the BHIB. Wells with a diameter of 6 mm and depth of 4 mm were punched in an inoculated agar plate and filled with freshly prepared solid disk composites. The plates were incubated at 36 ± 1 °C with 5% CO_2 for 48 h. Antimicrobial activity was evaluated by measuring the zone of inhibition around the composites (Kalaycı et al., 2013).

2.4.2. Direct contact test

The direct contact test was carried out according to the protocol described previously with minor modifications (Beyth et al., 2006). Briefly, 10 µL of freshly prepared bacterial suspension (10^6 CFU/mL) was put on the surface of each composite tested in a 48-well plate (TPP, Switzerland). The plate was incubated vertically at 36 ± 1 °C with 5% CO_2 for 1 h to provide the evaporation of suspension liquid and direct contact between bacteria and the composites. The plate was then placed horizontally and 70 µL of BHIB was added to each composite surface. The specimens were incubated for 24 h followed by washing of experimental composite surfaces with 10 mL of sterile phosphate buffered saline (PBS). PBS washing solution aliquots, 100 µL in volume, were inoculated onto BHIB in 2 replicates. Bacterial growth inhibition percentage was calculated by counting colony-forming units after 48 h of incubation.

2.5. Biocompatibility analysis of dental composites

2.5.1. Characterization of hDPSCs

The composite materials' biocompatibility with hDPSCs was evaluated. They were isolated from the wisdom teeth of a 20-year-old healthy male donor after written informed consent of the patient and ethical approval from İstanbul University, Turkey, were obtained. The hDPSCs were characterized as described previously by our group (Yalvac et al., 2009; Doğan et al., 2012). In short, separated pulp tissues were rinsed, minced, and seeded on 6-well plates (TPP). Cells were maintained in Dulbecco's modified essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin-amphotericin (Invitrogen, USA) solution. As cells reached confluence (80%), they were trypsinized by 0.05% trypsin/EDTA (Invitrogen, UK) and transferred to the tissue culture flasks. For the characterization of hDPSCs, propagated cells were removed from flasks by trypsinization and incubated with primary antibodies against CD45 (sc-70686), CD90 (sc-53456), CD105 (sc-71043), CD166 (sc-53551), and CD14 (sc-7328) (Santa Cruz Biotechnology, USA); CD29 (BD556049) and CD73 (BD550256) (Zymed, USA); and CD44 (NBP1-41266) (Novus Biologicals, UK). Cells were washed twice with PBS and incubated with fluorescein isothiocyanate-conjugated chicken antimouse secondary antibodies (sc-2989, 200 µg/0.5 mL; Santa Cruz Biotechnology), except for CD29, against which a chromophore-containing phycoerythrin protein-conjugated monoclonal antibody was used. The characterization of stem cells was evaluated using a Becton Dickinson FACSCalibur (Becton Dickinson, USA) flow cytometry system. Freshly isolated and characterized hDPSCs were used for osteogenic and odontogenic differentiation experiments to test the biocompatibility of the materials.

2.5.2. Differentiation of hDPSCs

A Transwell system was used to investigate the effects of boron-incorporated dental materials on the osteogenic and odontogenic differentiation capacity of hDPSCs. hDPSCs at passage 3 were seeded in the lower part of the Transwell system (24-well size, pore size 0.4 μm; Costar, Corning Incorporated, USA) at a density of 15,000 cells/well. Experimental composites (4 mm in depth and 6 mm in diameter) were placed on the upper surface of the Transwell culture plate to provide indirect contract between cells and dental materials. hDPSCs were induced to differentiate into osteogenic and odontogenic cell lineages according to the protocol previously used by our group (Taşlı et al., 2013). Briefly, cells were treated with an osteogenic medium composed of growth medium supplemented with 0.1 mmol/L dexamethasone, 10 mmol/L β-glycerolphosphate, and 50 mmol/L ascorbate as well as an odontogenic medium composed of growth medium supplemented with 10 nmol/L dexamethasone, 10 mmol/L β-glycerol-phosphate, and 50 mmol/L ascorbate (Sigma Chemical Co., USA). Cells were then incubated in a humidified incubator supplemented with 5% CO_2 at 37 °C for 7–10 days. The differentiation media were replaced every other day.

2.5.3. Immunocytochemistry analysis

Differentiated cells were fixed with paraformaldehyde and incubated with the following primary antibodies: anticollagen type I (sc-59772) and antiosteocalcin (sc-30044) for osteogenic differentiation, and antidentin sialophosphoprotein (DSPP, sc-33586) for odontogenic differentiation. After labeling, cells were incubated with Alexa Fluor-488 goat antirabbit or goat antimouse immunoglobulin G secondary antibodies (Invitrogen, USA), and 4',6-diamidino-2-phenylindole (AppliChem, Germany). Cells were then observed under a fluorescence microscope (Nikon Eclipse TE200, Nikon, Japan).

2.5.4. Real-time PCR analysis

Primers for collagen type I, osteonectin, osteocalcin, and dentin matrix acidic phosphoprotein 1 (DMP1) were used to evaluate lineage-specific gene expression levels of differentiated hDPSCs treated with various boron-containing specimens. β-Actin was used as a housekeeping gene for normalization of the data. The sequences of the primers used in RT-PCR analysis are given in Table 2. Total RNAs from osteogenically and odontogenically differentiated hDPSCs at the end of the 10-day incubation period were isolated using a High Pure RNA isolation kit (Roche, USA) and cDNA was synthesized by using High Fidelity cDNA synthesis kit (Roche) according to the manufacturer's recommendations. RT-PCR was conducted to quantify mRNA levels of the desired genes by using the SYBR Green staining method. cDNAs were mixed with primers and SYBR Green PCR Master Mix (Applied Biosystems, cat. No. 4309155) in a final volume of 20 μL. The results were evaluated using the delta delta CT method. All RT-PCR experiments were done using an iCycler RT-PCR system (Bio-Rad, USA).

2.5.5. von Kossa Staining

von Kossa staining was performed to characterize the mineralization and calcium deposition of osteogenic and odontogenic cells derived from hDPSCs. After fixation with paraformaldehyde, cells were washed with distilled water and stained with a von Kossa kit (Bio Optica, Italy) according to the manufacturer's instructions. Cells were observed under a light microscope (Zeiss, Germany).

2.6. Statistical analysis

Results were statistically analyzed using one-way ANOVA and Tukey's post hoc test using SPSS 20 for Windows. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Characterization of dental composites

Dental composites containing different concentrations of boron were evaluated for their mechanical properties to determine the effect of boron on the FS, CS, and DTS of modified materials. The overall findings for the mechanical analysis are summarized in Table 3. Results revealed that boron had a striking dose-dependent effect on the FS of the composites ($P < 0.001$). In comparison with the control group, approximately 23%, 36%, and 40% decreases in

Table 2. The sequences of primers used in RT-PCR assay.

Marker	Sense (5'-3')	Antisense (5'-3')
Osteocalcin	GTGCAGAGTCCAGCAAAGGT	TCAGCCAACTCGTCACAGTC
DMP1	CCCAGAGGCACAGGCAAATA	TCCTCCCCAATGTCCTTCTT
Col1	CCTGCTGGTGCTGCTGGTCC	GGGCCGGGTTCTCCTTTGGC
Osteonectin	ATGAGGGCCTGGATCTTCTT	CTGCTTCTCAGTCAGAAGGT
β-actin	CTTCCAGCCTTCCTTCCTGG	AATGCCTGGGTACATGGTGG

DMP1: Dentin matrix acidic phosphoprotein 1, Col1: Collagen type I.

Table 3. Experimental composite flexural strength (FS), compressive strength (CS), and diametral tensile strength (DTS) values.

Groups	FS [MPa]	CS [MPa]	DTS [MPa]
Control (composite without NaB) (n = 10)	105.97 ± 8.82	226.9 ± 56.46	34.38 ± 8.29
Composite + 1% NaB (w/w) (n = 10)	82.1 ± 10.64[a]	204.57 ± 41.69	31.5 ± 4.62
Composite + 5% NaB (w/w) (n = 10)	68 ± 8.02[a,b]	230.72 ± 28	27.92 ± 5.18
Composite + 10% NaB (w/w) (n = 10)	63.9 3± 6.07[a, b]	191.41 ± 16.22	28.87 ± 4.7
P	P < 0.001	P < 0.05	P < 0.05

Values are given as mean and standard deviation.
NaB: Sodium pentaborate pentahydrate, composite: GC.
[a, b]: Tukey multiple comparison results, [a]: Significantly different from control (P < 0.001), [b]: Significantly different from composite + 1% NaB (w/w) (P < 0.001).

FS values were obtained for the 1%, 5%, and 10% boron groups, respectively. On the other hand, although boron supplementation slightly lowered the CS and DTS values of dental materials, the differences were not found to be significant, even for the dental composite with the highest boron concentration.

DC in boron-containing dental composites was investigated using an FT-IR spectrometer. The results revealed that while adding boron to composites reduced the monomer conversion in a dose-dependent manner, there was no significant difference between the 1% NaB (51.2%) and the control group (54.8%) (Figure 1). However, monomer conversion rates with 5% and 10% NaB composites were significantly lower compared to the control group (44.6% and 44.7%, respectively).

3.2. Microbiological analysis

Dental materials incorporated with different boron concentrations (1%, 5%, and 10% w/w) were investigated for their antibacterial activities against *S. mutans* by agar-well diffusion assay and direct contact test. In the agar-well diffusion assay, no inhibition zones were detected around the tested specimens on the agar medium, even around the Dyract AP and 10% boron-containing composite (data not shown).

Conversely, bacterial growth was significantly suppressed on boron-incorporated dental materials according to the direct contact test results. Bacterial growth inhibition rates of 77.81 ± 4.23%, 94.47 ± 2.14%, 99.38 ± 3.64%, and 33.79 ± 6.12% were obtained for 1%, 5%, and 10% boron-containing dental composites and

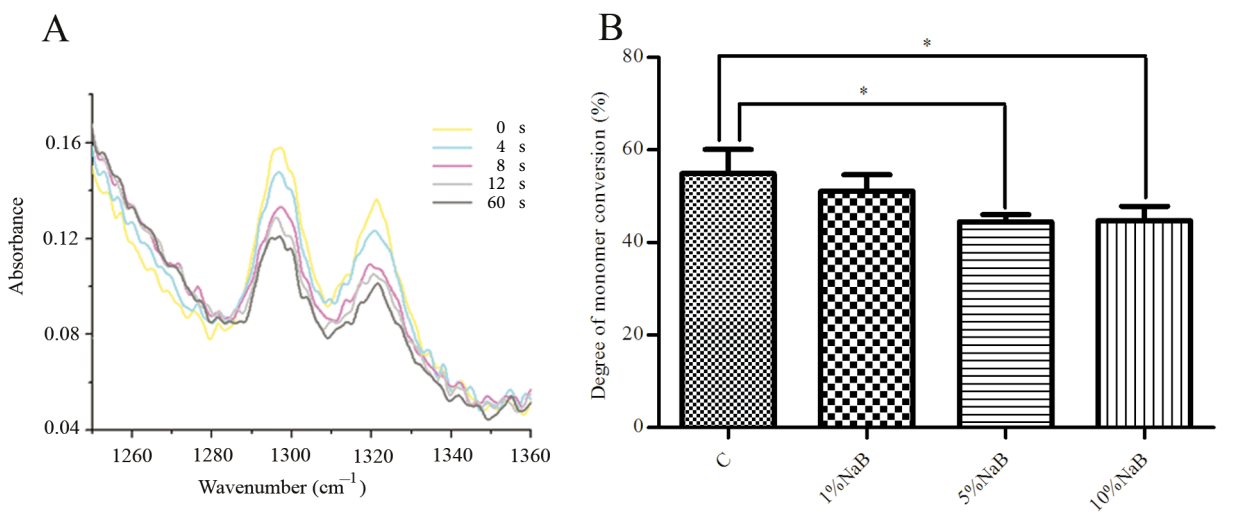

Figure 1. A) Differences in the absorption peaks at 1296 and 1320 cm^{-1} before irradiation (0 s) and at 4, 8, 12, and 60 s after the beginning of irradiation. **B)** Monomer conversion of dental composites with or without NaB, NC: Dental composites without NaB, NaB: Sodium pentaborate pentahydrate, *: Comparison with NC (*P < 0.05).

Dyract AP, respectively, indicating that boron provided contact antibacterial activity after polymerization rather than releasing the content into the medium. Data and statistical analyses are presented in Table 4.

3.3. Biocompatibility analysis

To test the biocompatibility, osteogenesis, and odontogenesis of the test specimens, hDPSCs characterized for their stem cell surface marker profile using flow cytometry (data not shown) were differentiated in the presence of boron-incorporated dental composites in an indirect contact system. As dental material with 10% boron content was found to be physically unacceptable according to mechanical analysis, biocompatibility tests were carried out using composites with 1% and 5% boron levels. Immunocytochemical and RT-PCR analysis revealed that 1% and 5% boron-incorporated composite treatments increased the levels of osteogenic markers, collagen type I, and osteonectin in hDPSCs compared to control group cells (Figure 2). However, there was not a significant difference between boron groups. In concurrence with these findings, von Kossa staining results showed that there were higher levels of calcium depositions in cells treated with boron-incorporated materials. In odontogenic-differentiated cells, while osteocalcin gene levels were found to be superior in boron-containing composites, there was no significant differences in DMP1 gene levels among the test groups (Figure 3). Moreover, protein expression levels of DSPP were found to be greater in hDPSCs exposed to boron-incorporated composites, especially 1% boron-containing material, compared to controls.

4. Discussion

Development of secondary caries due to the penetration of bacteria into the cavity between the tooth and the dental composite formed during polymerization remains a major hurdle in dental applications because of leading to a myriad of restoration failures (Huang et al., 2012). Incorporating effective and durable antimicrobial properties into dental composites to be a future solution could bridge this gap. However, new biological properties in these materials should not result in inadequate physical or chemical characteristics and biocompatibility in order to be clinically applicable. The findings of the current study revealed that while incorporation of boron into the composites significantly decreased FS in a dose-dependent manner, the 1% boron-containing composite still maintained the minimal 80 MPa necessary to be used as a restorative agent according to ISO standards (ISO, 2009). On the other hand, DTS was not found to be different from those of the controls. FS and DTS values of materials were found to decrease as the boron concentration increased, but CS values did not differ accordingly. Similar to the mechanical analysis results, adding boron to the experimental composites resulted in a decrease in DC rate. However, the addition of 1% boron to the dental composite had no adverse impact on monomer conversion. Therefore, 1% boron was found to be the ideal concentration achieved from our current experimental setup, which provided minimal decreases in DC, CS, and DTS as well as an acceptable level of fracture resistance with significant antibacterial effects. Nevertheless, as commercial composites have been designed optimally for their mechanical properties, further studies should be conducted to develop optimized formulations containing boron, other than just blending the chemical into the composites.

Antimicrobial activity of dental materials is conventionally evaluated by using agar diffusion tests (Attin et al., 2001) or determining minimum inhibition concentrations (Yoshida et al., 1999), which are based on water solubility and diffusion rates of antimicrobial agents. Therefore, the use of these assays in determining dental and medical materials with antibacterial properties is questionable (Beyth et al., 2008). Dental composites with sudden content-releasing characteristics are undesirable in clinics due to their short-term effectiveness that do not fulfill the medical requirements. Hence, to maintain antibacterial structure for a long time, high amounts of bioactive molecules should be incorporated into the resin monomers, which may exert toxicity to the host tissue. In the current study, no antibacterial activity against *S. mutans* was detected in the agar-well diffusion test, which indicates low or nonreleasing characteristics of the boron-incorporated dental composites into the surrounding

Table 4. Antimicrobial activities of experimental composites against *S. mutans*.

Groups	Composite + 1% NaB (w/w) (n = 3)	Composite + 5% NaB (w/w) (n = 3)	Composite + 10% NaB (w/w) (n = 3)	Polyacid-modified composite (n = 3)	P
Antimicrobial activity (%)	$77.81 \pm 4.23^{a,b}$	$94.47 \pm 2.14^{a,b,c}$	$99.38 \pm 3.64^{a,b,c}$	33.79 ± 6.12^{a}	$P < 0.05$

NaB: Sodium pentaborate pentahydrate; Composite: G-Aenial Posterior, GC Corporation, Japan; Polyacid-modified composite: Dyract AP, Dentsply DeTrey, Germany. [a, b, c]: Tukey multiple comparison results, [a]: Significantly different from control ($P < 0.05$), [b]: Significantly different from polyacid-modified composite ($P < 0.05$), [c]: Significantly different from composite + 1% NaB (w/w) ($P < 0.05$). Note: No antimicrobial activity was observed in the control group (composite without NaB additive) and it was accepted as 0%.

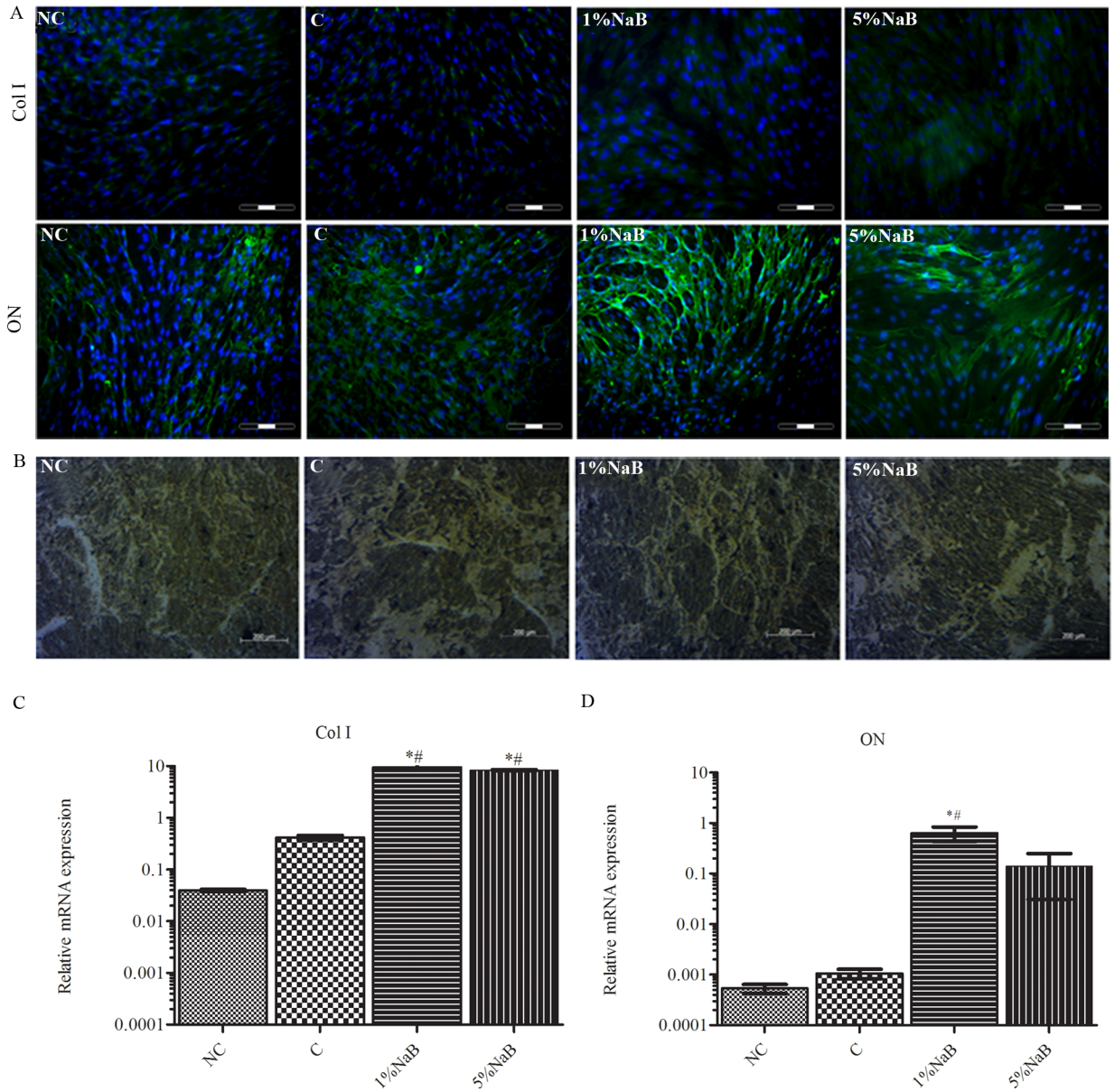

Figure 2. Determination of osteogenic differentiation levels in experimental groups at the end of the 10-day incubation period. **A)** Immunocytochemical staining of Col1 and ON, scale bar: 50 µm. **B)** von Kossa staining, scale bar: 200 µm. Relative mRNA expression levels of **C)** Col1 and **D)** ON. NaB: Sodium pentaborate pentahydrate, NC: Growth medium group, C: Differentiation medium group, Col1: Collagen type I, ON: osteonectin, #: Comparison with C, *: Comparison with NC ($^{\#,*}P < 0.05$).

aqueous agar milieu. In contrast to these findings, 77.81 ± 4.23%, 94.47 ± 2.14%, and 99.38 ± 3.64% bacterial growth inhibitions were obtained in direct-contact assay for 1%, 5%, and 10% boron-impregnated composites, respectively. No inhibition zone was obtained in the agar diffusion assay for Dyract AP, a fluoride-releasing polyacid-modified composite, but low antimicrobial activity was detected with the direct contact method. These results are consistent with the previous studies reporting that no bactericidal activity was noted in agar diffusion tests conducted with Dyract AP due to low fluoride-releasing characteristics (Vermeersch et al., 2005; Marczuk-Kolada et al., 2013). It may be proposed that the amount of fluoride released from Dyract AP was not enough to inhibit bacterial growth in the agar diffusion test, but direct contact between bacteria and the composite provided partial inhibition. These overall results show that while a detailed inhibitory mechanism is yet to be established, polymerized boron-containing dental composites displayed contact antibacterial activity instead of releasing their content into the media, which

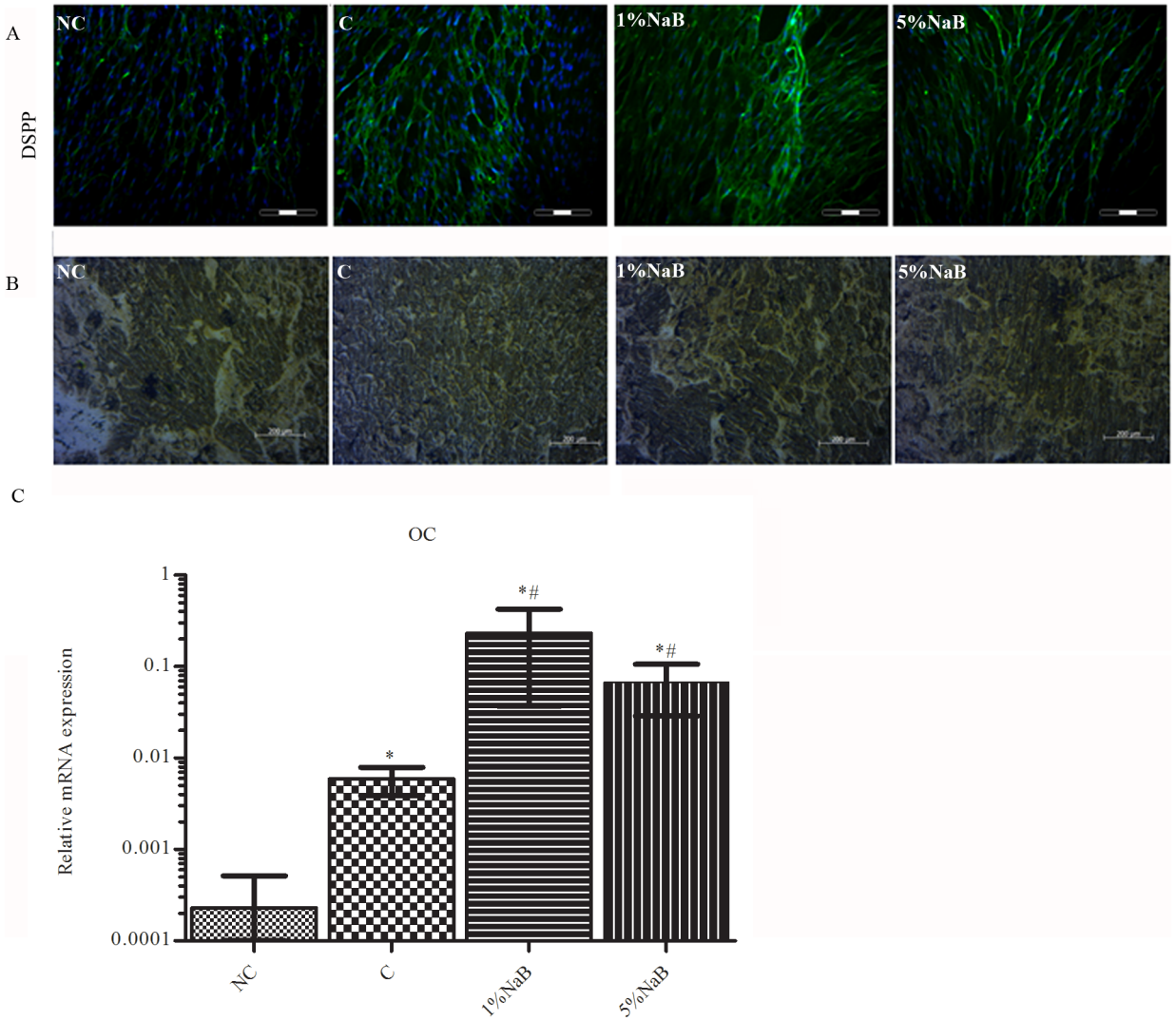

Figure 3. Determination of odontogenic differentiation levels in experimental groups at the end of the 10-day incubation period. **A)** Immunocytochemical staining of DSPP, scale bar: 50 µm. **B)** von Kossa staining, scale bar: 200 µm. **C)** relative mRNA expression levels of DMP1 and OC. NaB: Sodium pentaborate pentahydrate, NC: Growth medium group, C: Differentiation medium group, DSPP: dentin sialophosphoprotein, OC: osteocalcin, #: Comparison with C, *: Comparison with NC (#,*$P < 0.05$).

may provide durable antibacterial effect and safety due to low-release kinetics.

When considering the use of boron in dental composites for antibacterial purposes in medical applications, its biocompatibility must be understood. Materials released from dental composites could diffuse into dentinal tubules in a moist environment and arrive at the dental pulp, resulting in pulpal inflammation (Beyth et al., 2008). To test the biocompatibility of modified materials, hDPSCs were incubated in the presence of boron-containing dental composites in an indirect contact system to mimic clinical conditions. Boron has previously been shown to have a role in healing the alveolar bone after tooth extraction (Gorustovich et al., 2008a, 2008b). Moreover, we have previously reported that boron increases the osteogenic and odontogenic differentiation of dental stem cells (Taşlı et al., 2013; Demirci et al., 2014). Consistent with these findings, the results revealed that the osteogenic and odontogenic potential of hDPSCs was found to be higher in the presence of boron-containing dental composites. However, other dental resident cells, such as periodontal ligaments, dental follicle cells, and gingival fibroblasts, should also be tested for biocompatibility with boron-incorporated dental composites.

The approaches to create an antibacterial and biocompatible dental composite described in the present study have the potential to reduce secondary caries that result in restorative failures. It is noteworthy that boron-

containing composites not only displayed a remarkable antibacterial effect against *S. mutans* but also increased the osteogenic and odontogenic differentiation capacity of hDPSCs. However, several questions remained to be clarified, such as whether boron with its diffusion rate will provide a real benefit under in vivo conditions. Prospective studies are needed to provide additional insights and substantiate the clinical relevance and applicability of boron-containing composites in dental practice.

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