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RESEARCH AND EDUCATION

Biocompatibility of universal dental adhesives: An in vitro study

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ABSTRACT

Statement of problem. Adhesives play an essential role in restorative dentistry, and Universal Bond Quick (UBQ) has been marketed as having a short application time and long-term resistance to hydrolysis. However, research on its biocompatibility is lacking.

Purpose. The purpose of this in vitro study was to compare the biocompatibility of 3 recently introduced universal dental adhesives: UBQ, Single Bond Universal (SBU), and PrimeBond Universal (PBU), and the popular clinical dental adhesive SE Bond.

Material and methods. The CCK-8 assay, Calcein AM-PI, Real-time qPCR, DNA damage, and Cell cycle were used to evaluate the biocompatibility of each adhesive. Microleakage of the dental adhesives was also tested through immediate and chemical aging dye penetration in vitro experiments. An ANOVA, *t* test, Kruskal-Wallis, and Mann-Whitney tests were used for the statistical analyses (α=.05).

Results. The biocompatibility results showed that the inhibitory effect of UBQ and SBU on the proliferation of L929 cells was lower than that of PBU and SE. The proportion of viable cells in the SBU group decreased when the concentration reached 25%, while the proportion of viable cells in the UBQ group remained unchanged at this concentration. Concentrations of PBU and SE of 6.25% caused DNA damage in L929 cells. After chemical aging, the gingival microleakage of specimens in the UBQ and PBU groups was more obvious than that of occlusal microleakage, and the difference was statistically significant ($P<.05$).

Conclusions. The biocompatibility of UBQ and SBU was similar and better than that of PBU and SE. However, after aging, the microleakage of the UBQ and PBU was slightly poorer in the thin enamel area near the gingival margin. (J Prosthet Dent 2025;133:905.e1-e11)

Dental adhesives play an essential role in restorative dentististy,[1,2] and self-etching adhesives have been widely used clinically.[3] However, their long-term stability needs to be enhanced. The recently introduced adhesive Universal Bond Quick (UBQ) contains an amide monomer, instead of β-hydroxyethyl methacrylate (HEMA), that has been reported to absorb less water. It can be directly light polymerized after being applied to dentin, which has the advantages of short application time and long-term resistance to hydrolysis.[4,5]

Research on UBQ has mainly focused on bond strength and interface morphology.[6,7] The bond strength has been reported to be similar between the wait-free application UBQ and after application for 20 seconds[8] Extending the application time of UBQ has been reported to improve its bond strength immediately but not after aging.[9] Initial shear bond strength and shear fatigue strength were not affected by application time. Acid washout resulted in less microleakage than that from self-etching adhesives. Although the antibacterial effect of UBQ was reported to be similar to that of several universal dental adhesives,[10] studies on the biocompatibility of UBQ are sparse. Demirel et al[11] reported that UBQ diluted 1:10 had no cytotoxic effects on L929 cells after 24 hours, and Wawrzynkiewicz et al[12] showed that UBQ was not

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Clinical Implications

UBQ was found to have good biocompatibility and should also provide good edge sealing performance. While still ensuring dental bonding quality, UBQ can be directly exposed to light after coating because of the special monomers incorporated, thereby shortening the bonding time.

genotoxic and that treatment of human monocyte/macrophage cells for 24 hours did not affect levels of apoptosis or the cell cycle. These studies mainly compared the cytotoxicity of UBQ with other self-etching adhesives, but biocompatibility comparisons with earlier self-etching adhesives are lacking. Cardoso et al[13] compared the interface leakage of UBQ and Tetric N-Bond adhesives in self-etching and full-etching modes. SE Bond has often been compared with recently introduced adhesives, providing a basis for their clinical application.[14–16]

Universal dental adhesives have made progress in both composition and performance, but horizontal comparative research on the biocompatibility and microleakage of UBQ and other recently introduced universal dental adhesives is lacking. The purpose of this study was to compare the differences in biocompatibility and microleakage between UBQ and other recently introduced universal dental adhesives to provide laboratory data for the clinical application of UBQ. The null hypotheses were that no statistical differences would be found in biocompatibility or microleakage between UBQ and other recently introduced universal dental adhesives.

MATERIAL AND METHODS

L929 cells (Pricella) were cultured in Dulbecco modified Eagle medium (DMEM)(Hyclone) containing 10% fetal bovine serum (FBS; Hyclone) and 1% penicillin and streptomycin. The cells were maintained at 37 °C in a 5% CO_2 incubator (Thermo).

Universal Bond Quick (UBQ; Kuraray Co., Ltd), SE Bond (SE; Kuraray Co, Ltd), Single Bond Universal (SBU; 3M Co), and PrimeBond Universal (PBU; Dentsply Sirona) were studied. Unsupplemented culture medium served as the control group. To prepare the leaching solution, 25 µL of each dental adhesive was dispensed in a pipette. During the light-activated polymerization process, the light source was kept 1 mm away from the surface of the dental adhesives. After light polymerization, Ø8×0.5-mm sheets were formed, which were then UV light sterilized (Thermo) for 4 hours. According to the International Organization for Standardization (ISO) 10993–12 standard, serum-containing culture medium was added, and the specimens were soaked at a ratio of extraction of 3.0 cm^2/mL.[15] The specimens were incubated at 37 °C for 24 hours, and the medium was filtered through a sterile 0.22-µm filter (Fig. 1).

According to the instructions provided with the CCK-8 assay (Solarbio), L929 cells were seeded into a 96-well plate at a cell density of 3.0×10^4/mL and placed in a 37 °C, 4% CO_2 incubator (Thermo) for 24 hours. After diluting the leaching solution to obtain concentrations of 6.25%, 12.5%, 25%, 50%, and 100%, 200 µL of each of the diluted dental adhesive leaching solutions was added to appropriate cell cultures. The 100 µL of prepared CCK-8 reagent working solution was added to each well. After incubation at 37 °C for 1 hour, absorbance at 450 nm was measured using a microplate reader (Multiskan SkyHigh; Thermo).

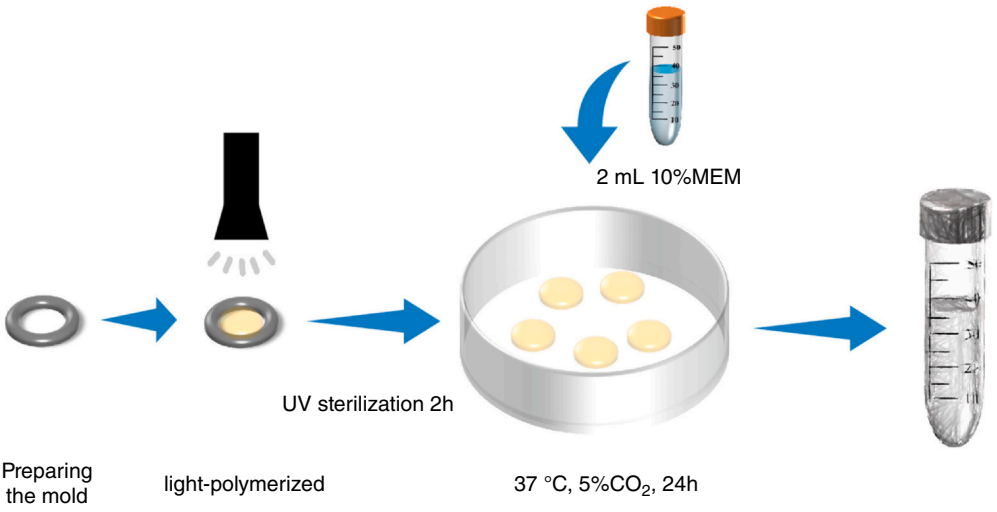

Figure 1. Preparation of leaching solution of dental adhesives.

According to the instructions provided with Cell live and the dead double staining kit (Calcein-AM/PI; Solarbio Science Technology Co, Ltd), extracts of dental adhesives of different concentrations were added to cultures of L929 cells and cultured for a further 48 hours. After that, 1×Assay Buffer (Calcein-AM/PI; Solarbio Science Technology Co, Ltd) was added and incubated for 2 minutes. Next, Calcein-AM (1:1000) was added and incubated for another 20 minutes. Finally, PI (1:400) was added and incubated for 5 minutes in the dark. The staining solution was discarded, the specimens washed twice with phosphate buffered saline (PBS) for 5 minutes, the slides sealed, and the images captured with an inverted fluorescence microscope (Olympus IX71; Olympus).

According to the instructions provided with the total RNA extraction kit (Cell RNA Purification Kit; Adamas life), 1 mL of Trizol (TRIzol; Invitrogen) was added to the cells and mixed by pipetting. Then 200 μL of chloroform (Sangon Biotech) was added, and the mixture was centrifuged at 14 800 rpm for 15 minutes. A volume of 400 μL isopropanol (Sangon Biotech) was added to the mixture and maintained at room temperature for 10 minutes. The mixture was centrifuged at 12 000 rpm for 10 minutes and 3 minutes at 4 °C and then held at room temperature for 2 minutes. A volume of 30 μL of DEPC water was added to dissolve the RNA. The cDNA obtained was then used for quantitative polymerase chain reaction (qPCR) using a kit (SYBR Green Pro Taq HS qPCR; Accurate Biology).

According to the instructions provided with the DNA damage kit (DNA Damage Assay Kit by γ-H2AX Immunofluorescence; Beyotime Biotechnology), the cells were fixed with 4% paraformaldehyde for 10 minutes. The fixed cells were then blocked with staining blocking solution for 15 minutes, and an anti-γ-H2A histone family member X (H2AX) primary antibody was added, followed by the secondary antibody; the cells were incubated at room temperature for 60 minutes.

According to the instructions provided with the assay kit (Cell Cycle Assay Kit; Elabscience), 1.2 mL of absolute ethanol was first added to the cells overnight. The next day, the cells were centrifuged at 300 *g* for 5 minutes and then allowed to stand at room temperature for 15 minutes. They were then centrifuged at 300 *g* for 5 minutes, 100 μL of RNase A Reagent was added, and they were then incubated in a 37 °C water bath for 30 minutes. Finally, 400 μL of PI Reagent (50 μg/mL) was added, the cells were incubated at 4 °C in the dark for 30 minutes.

After culturing the cells for 24 hours, washing with PBS 3 times, fixing with 4% paraformaldehyde for 30 minutes, phalloidin (1:200) was added, and the cells were incubated in the dark at 37 °C for 30 minutes and washed with PBS 3 times. A 4′,6-diamidino-2-phenylindole (DAPI) stain was added, and the cells were incubated at room temperature for 5 minutes, washed with PBS 3 times, and photographed with an inverted fluorescence microscope (Olympus IX71; Olympus).

A total of 80 freshly extracted third molars without obvious caries, cracks, or restorations were collected from patients who visited Dalian Medical University School of Stomatology between October 2021 and June 2022 and immediately placed in chloramine-T. The experiments were approved by the Ethics Committee of the Dalian Medical University (AEE23006). The 80 teeth were divided into 4 groups by simple randomization: UBQ group, SBU group, PBU group, and SE Bond group, with 20 teeth in each group. A 4×3×2-mm cavity was prepared 1 mm occlusal to the enamel–cementum junction of the buccal surface of each tooth with a periodontal probe being used to control the cavity size.

The 20 specimens from each group were selected for dye penetration testing with simple randomization and divided into 2 groups for immediate and chemical aging dye penetration, with 10 teeth in each group. Each tooth was sectioned along the long axis of the tooth, and 400- to 2000-grit abrasive paper was used to polish in sequence. The dye penetration of the bonded interface was evaluated under a stereomicroscope, and the dye penetration depth was graded as Level 0, no dye penetrated into the cavity wall; Level 1, dye penetrated into the cavity wall less than one third; Level 2, dye penetrated into the cavity wall between one-third and two-thirds; Level 3, dye penetrated into the cavity wall more than two-thirds but did not reach the base of the cavity; Level 4, dye penetrated into the axial wall of the cavity base.

A statistical software program (IBM SPSS Statistics for Windows, v25; IBM Corp) was used for statistical analysis. The Shapiro-Wilk test was used to test whether the data followed a normal distribution. The Bartlett homogeneity of variance test was used to test whether the data obeyed the homogeneity of variances. The data of Calcein AM-PI, Real-time qPCR, DNA damage, and Cell cycle followed a normal distribution, and homogeneity of variances and 1-way analysis of variance (ANOVA) were performed on the Calcein AM-PI, Real-time qPCR, DNA damage, and Cell cycle data, Bonferroni test was performed on the pairwise comparison of the Calcein AM-PI, Real-time qPCR, DNA damage, and Cell cycle data. The *t* test was performed on CCK-8 and Cell cycle of data. The Kruskal-Wallis and Mann-Whitney tests were used on the ranked data of microleakage (α=.05).

RESULTS

All 4 dental adhesives promoted cell proliferation at low concentrations but inhibited cell proliferation at high concentrations. When treated with high-concentration

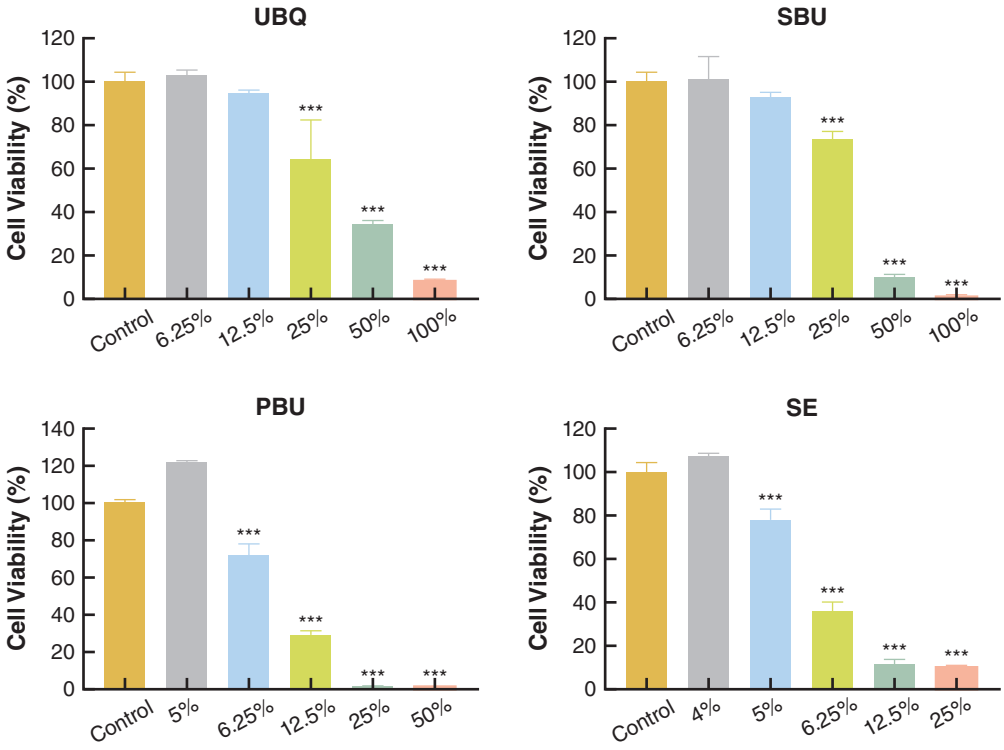

Figure 2. Cell proliferation of L929 cells when treated with different concentrations of dental adhesive extracts. Compared with control group, $^{**}P<.01$.

extracts, cell viability decreased as the concentration of the extracts increased. The 25%, 50%, and 100% concentration extracts of the UBQ and SBU groups inhibited cell proliferation ($P<.001$); in the PBU group, extract concentrations of 6.25% and above inhibited cell proliferation ($P<.001$), while SE extract concentrations of 5% and above inhibited cell proliferation ($P<.001$), as shown in Figure 2. These results showed that the biocompatibility of UBQ and SBU were higher than that of PBU and SE.

At a concentration of 6.25%, the morphology of cells in the UBQ and SBU groups was no different from the control group. The cell density of the UBQ group was slightly greater than that of the SBU group, while the cell density of the PBU group was reduced, some cells were round or elongated and spindle-shaped, and individual cell nuclei appeared to be divided. The SE group showed the most obvious reduction in cell density, with many round and elongated spindle-shaped cells appearing (Fig. 3A). At a concentration of 12.5%, the cell morphology of the UBQ group and the SBU group was no different from that of the control group; in contrast the cell density of the PBU group further decreased, and many cells were spherical or had a long spindle shape, and, in the SE group, all cells became round (Fig. 3B). Cells in all groups treated with adhesive at a concentration of 25% developed abnormal morphology. Individual cells in the UBQ group and SBU group had irregular morphology; the cells in the PBU group were mostly round and occasionally had a long spindle shape, and all cells in the SE group became round. Lysis was observed in the cytoplasm (Fig. 3C). Nuclei were stained blue with DAPI; the cytoskeleton was stained red with phalloidin.

These results suggested that the cell morphology of the PBU and SE groups changed significantly at 6.25% concentration. In contrast, UBQ and SBU only affected cell morphology when the concentration reached 25%.

At a concentration of 6.25%, live/dead staining of cells in the UBQ and SBU groups showed that cell viability was not significantly different from the control group ($P>.05$). In contrast, the ratio of live cells to dead cells in the PBU and SE groups was reduced ($P=.021$ and.008), as shown in Figure 4A. At 12.5% concentration, the live/dead cell ratio in the UBQ and SBU groups was not significantly different from that in the control group ($P>.05$), while the proportion of live/dead cells in the PBU and SE groups was reduced ($P=.009$ and.003), as shown in Figure 4B. At 25% concentration, the live/dead cell ratio in the UBQ group was not significantly different from that in the control group ($P>.05$), while the proportion of live/dead cells in the SBU, PBU, and SE groups was reduced ($P<.01$), as shown in Figure 4C. Calcein-AM stained live cells green, while PI stained dead cells red.

Combining the results of proliferative activity and live/dead staining analysis, a concentration of 6.25% was

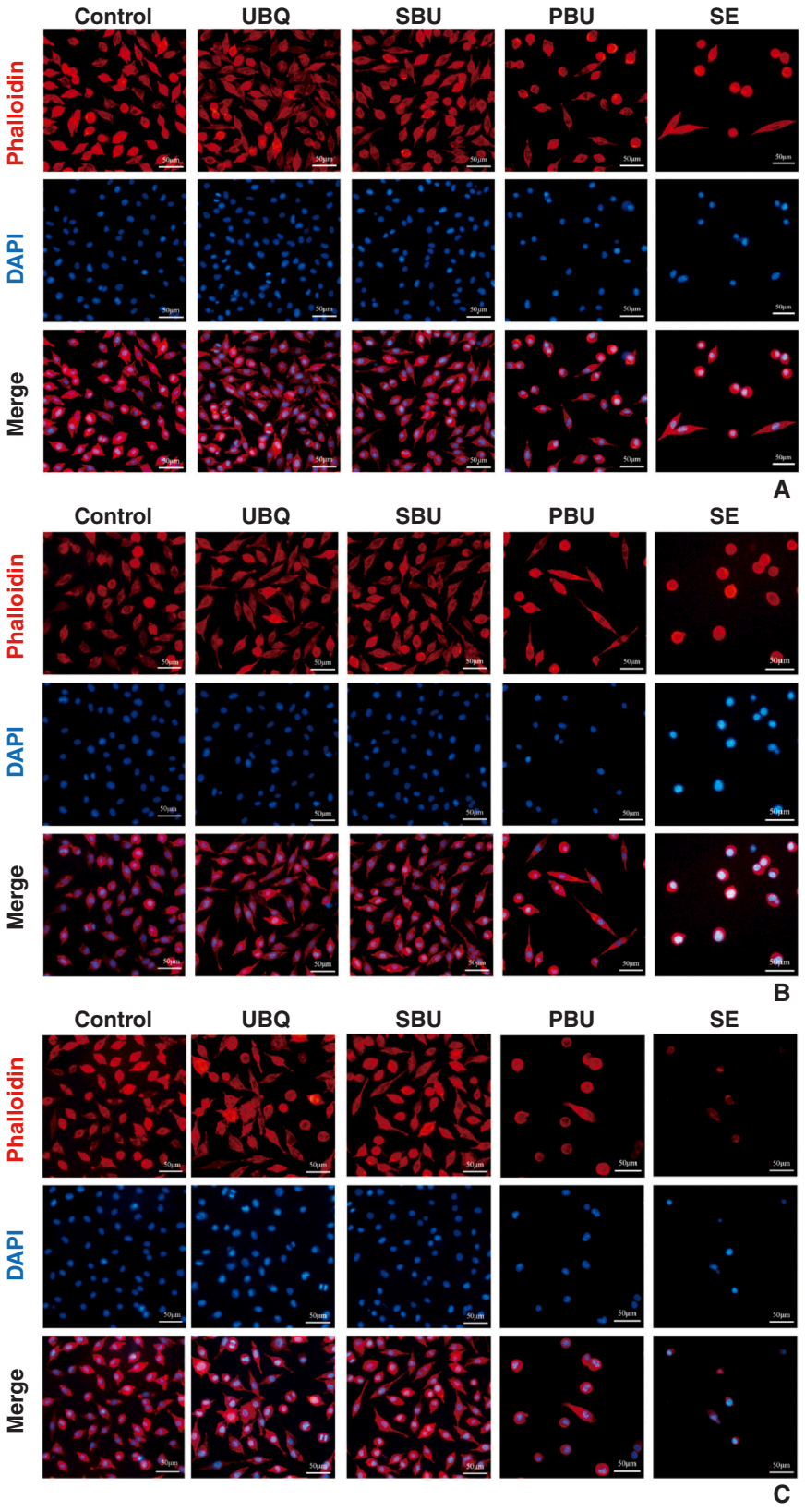

Figure 3. Effects of different concentrations of dental adhesives on cell morphology. A, 6.25%. B, 12.5%. C, 25%. (Scale bar=50 μm).

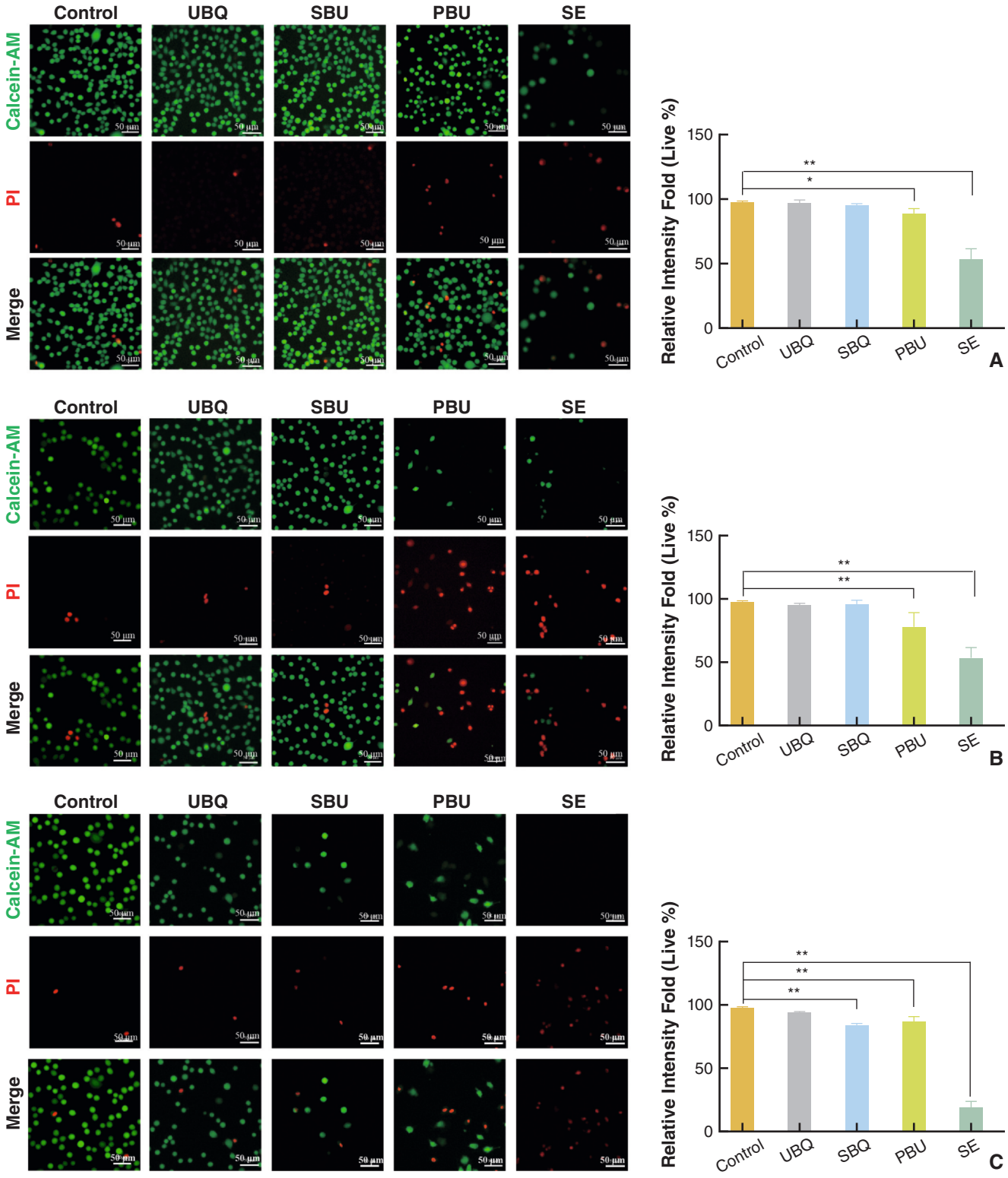

Figure 4. Live/dead staining results of L929 cells treated with dental adhesives. Live/dead staining and statistical results of cells under fluorescence microscopy compared with control group. A, Treated with 6.25% adhesive. B, Treated with 12.5% adhesive. C, Treated with 25% adhesive. **P<.01, *P<.05 (Scale bar=50 μm).

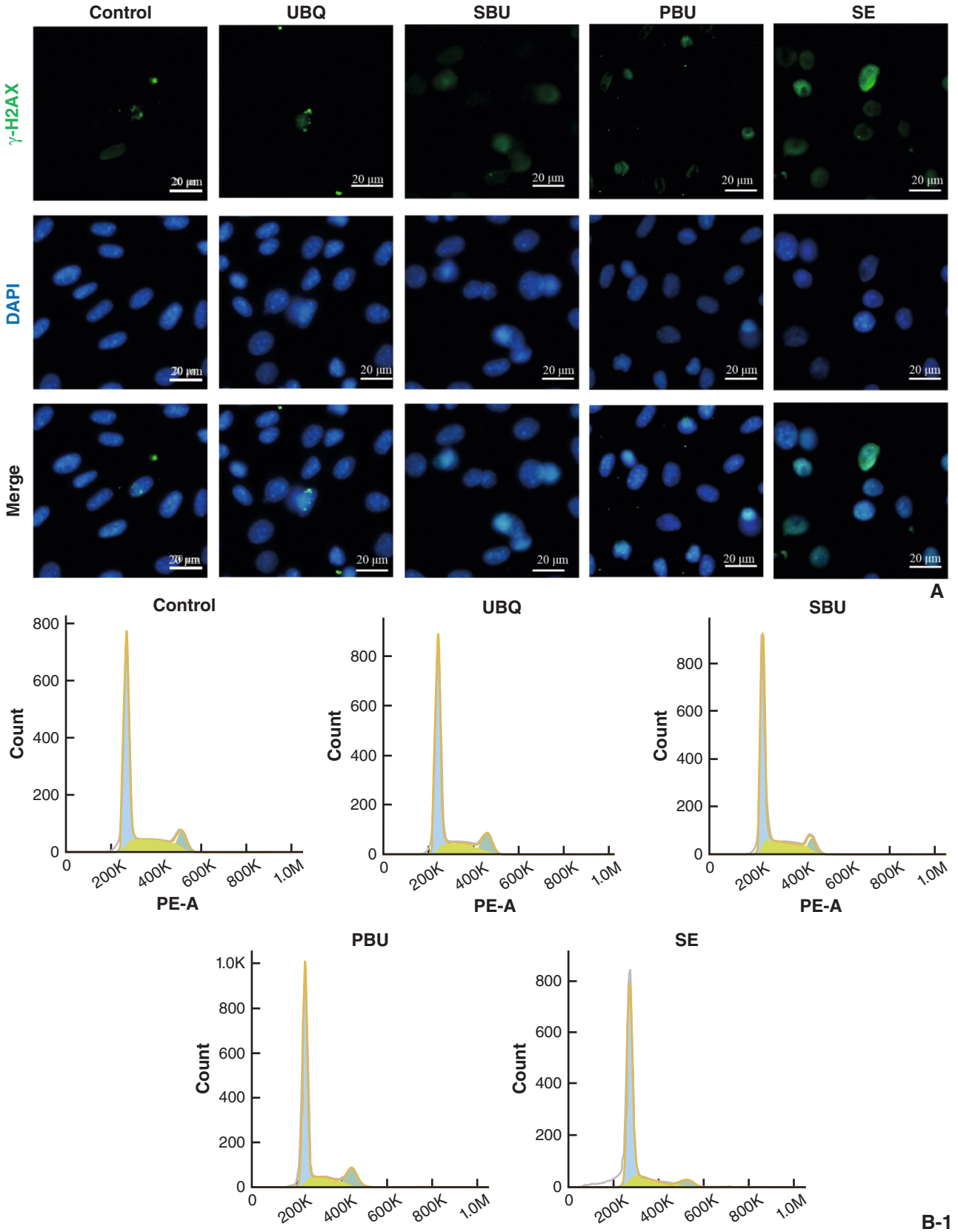

Figure 5. Cytotoxicity of L929 cells caused by dental adhesives compared with control group. A, Effects of dental adhesives on DNA damage (scale bar=50 μm). B, Effects of dental adhesives on cell cycle. C, Effects of dental adhesives on expression of TNF-α and MMP 9., ***P*<.01, **P*<.05.

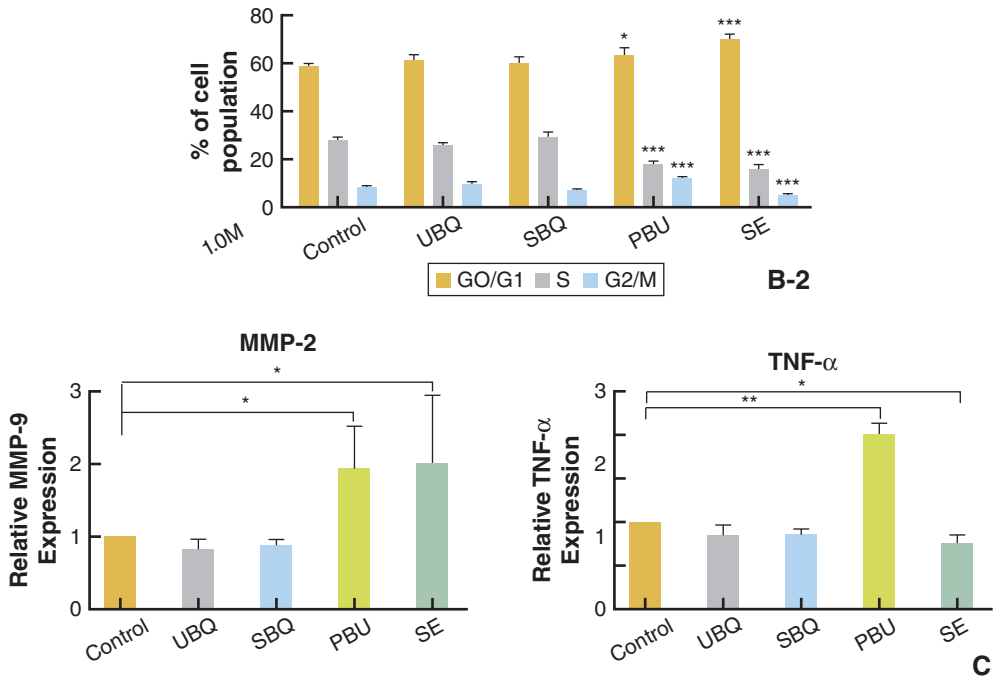

Figure 5. (*continued*)

selected for DNA damage experiments. γ-H2AX is an indicator of DNA damage and displays green fluorescence.[17] The brighter the green fluorescence intensity, the greater the DNA damage. L929 cells were treated with a 6.25% concentration of dental adhesives for 48 hours. In the SE group, focus formation of γ-H2AX was significantly induced ($P<.001$). The results showed that 6.25% SE caused DNA damage in L929 cells, as shown in Figure 5A.

L929 cells were exposed to 6.25% dental adhesives, and the cell cycle was analyzed by flow cytometry. The results showed that cells in the G0/G1 phase increased in the PBU group and the number of S phase cells decreased, while the G2/M phase increased ($P<.001$). In the SE group, cells in the G0/G1 phase increased, those in the S phase decreased, and the G2/M period decreased ($P<.001$). The results showed that both UBQ and SBU also affected the cell cycle. In the PBU and the SE groups, cells were blocked in G0/G1 and S phases, and proliferation was inhibited, while SE had a more obvious inhibitory effect on cell proliferation, as shown in Figure 5B.

The results showed that the gene expression levels of TNF-α and MMP 9 in the UBQ and SBU groups were not significantly different from those in the control group ($P>.05$), while, in the PBU and SE groups, the expression level of MMP 9 increased ($P=.013$). The expression level of TNF-α increased in the PBU group ($P<.001$) but decreased in the SE group ($P=.024$), as shown in Figure 5C.

The UBQ group and SBU group did not show any obvious anti-inflammatory or pro-inflammatory effects. The expression of pro-inflammatory factors in the PBU group increased, which may stimulate inflammatory responses in cells. The SE group may also show anti-inflammatory effects.

Occlusal and gingival microleakage was observed in the immediate group and the chemical aging group using a stereomicroscope, as shown in Figure 6A. The statistical results of occlusal and gingival microleakage in the immediate group and the chemical aging group are shown in Figure 6B, with no statistically significant difference in occlusal and gingival microleakage in the UBQ, SBU, and PBU groups ($P>.05$), as shown by immediate dye penetration experiments with the SE group. After chemical aging, gingival microleakage was more obvious than occlusal microleakage in specimens of the UBQ and PBU groups, and the difference was statistically significant ($P=.031$). TEM showed that cracks appeared in the gingival bonding interface in the UBQ and PBU groups after chemical aging. These results indicated that the new general-purpose adhesives produced good edge sealing performance in the short term, and there was no significant difference in edge sealing performance between them ($P>.05$). However, after chemical aging, the sealing ability of both UBQ and PBU was slightly poorer in the thin enamel area near the gingival margin, as shown in Figure 6C.

DISCUSSION

The biocompatibility of UBQ was compared with the universal dental adhesives SBU and PBU, with the 2-

A

Evaluation of immediate dye penetration experiments (oeclusal microleakage)

	Level 0	Level 1	Level 2	Level 3	Level 4
UBQ	6	4	0	0	0
SBU	8	2	0	0	0
PBU	8	2	0	0	0
SE Bond	8	0	2	0	0

Evaluation of immediate dye penetration experiments (gingival microleakage)

	Level 0	Level 1	Level 2	Level 3	Level 4
UBQ	6	2	0	2	0
SBU	5	3	2	0	0
PBU	5	3	1	1	0
SE Bond	7	3	0	0	0

Evaluation of Chemical aging Dye Penetration experiments (oeclusal microleakage)

	Level 0	Level 1	Level 2	Level 3	Level 4
UBQ	5	4	0	1	0
SBU	6	4	0	0	0
PBU	8	2	0	0	0
SE Bond	6	4	0	0	0

Evaluation of Chemical aging Dye Penetration experiments (gingival microleakage)

	Level 0	Level 1	Level 2	Level 3	Level 4
UBQ	2	3	3	0	2
SBU	4	5	1	0	0
PBU	4	2	1	2	1
SE Bond	5	2	1	2	0

B

Figure 6. Effects of dental adhesives on microleakage. A, Stereomicroscopic observation of microleakage of dental adhesives (scale bar=50 μm). B, Statistical results of marginal microleakage. C, TEM showed that cracks appeared after chemical aging (scale bars=100 and 40 μm).

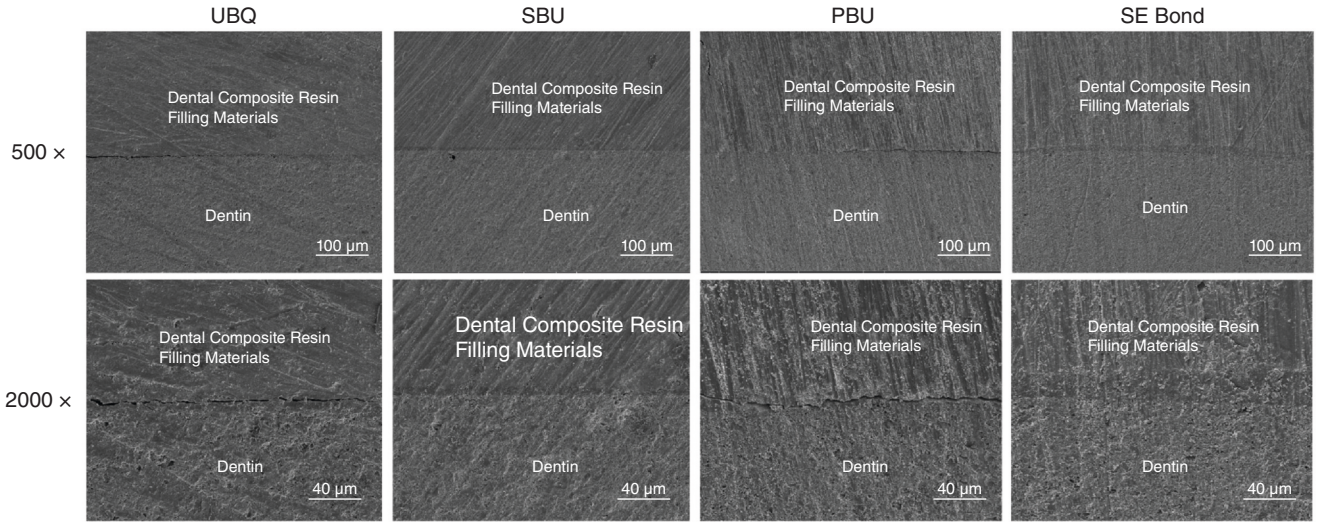

Figure 6. (*continued*)

step self-etching adhesive SE. The biocompatibility results showed that the inhibitory effect of UBQ and SBU on the proliferation of L929 cells was lower than that of PBU and SE. The proportion of viable cells in the SBU group decreased when the concentration reached 25%, while the proportion of viable cells in the UBQ group remained unchanged at this concentration. Concentrations of PBU and SE of 6.25% caused DNA damage in L929 cells. Therefore, the null hypotheses that no difference in biocompatibility would be found between UBQ and other adhesives was rejected. The results showed that the biocompatibility of UBQ and SBU was similar and better than that of PBU and SE. SBU was the first commercial universal dental adhesive and was launched in 2012. Its good biocompatibility has been established previously.[18] The cytotoxicity of PBU as reported previously[19] required verification. The self-etching adhesive SE has been an important reference for comparative studies on the bond strength of self-etching adhesives or universal dental adhesives.[20–23]

The self-etching bonding mode was used to treat the dental tissue, and no separate acid etching treatment was performed on the enamel, as previous studies have confirmed that a universal dental adhesive can provide the same bond strength when bonding enamel without phosphoric acid etching as self-etching adhesives with phosphoric acid etching. After chemical aging, microleakage in the UBQ and PBU groups was found to be greater gingivally than occlusally, with the dye penetrating deeper near the cervical margin where the enamel was thin with a high inorganic content of up to 96% to 97%.[24] The bonding to occlusal enamel was typically better than to dentin, but the weakness of the cervical enamel meant that bonding mainly relied on dentin. Therefore, the sealing was slightly worse, consistent with Jordehi et al.[25]

UBQ has not yet been widely used clinically. The difference in biocompatibility between UBQ and other universal dental adhesives has been researched in terms of cytotoxicity, genotoxicity, and cell cycle.[12–14] Extracts of each dental adhesive were prepared for biocompatibility evaluation. The present study used the ISO 10093–12 standard based on previous experiments to prepare a dental adhesive leaching solution with higher repeatability. In some situations, when a bonding agent is applied to a carious lesion on the cervical portion of a tooth, unpolymerized components from the bonding agent may directly penetrate the periodontal ligament or enter the circulation. Therefore, we used complete DMEM containing serum as the extraction carrier to approximate clinical application. The present study examined the effect of dental adhesives on the expression of inflammatory factors in cells. UBQ extract did not affect the expression of inflammatory factors in L929 cells when the dilution concentration was 6.25%.

Limitations of this study included the in vitro design which cannot completely simulate the in vivo microenvironment. If the dental adhesive spreads deeper into the dentinal tubules and fails to fully polymerize, cytotoxicity will occur. Therefore, when selecting a cementing system and solution, it is recommended to consider influencing factors such as the remaining tooth tissue, the depth of the pulp chamber, and the proximity of the material to the pulp. In addition, the application of bonding systems in deep carious lesions must be carried out with caution. Therefore, evaluating the biological activity of dental adhesives is crucial for their clinical application.

CONCLUSIONS

Based on the findings of this in vitro study, the following conclusions were drawn:

1. The biocompatibility of UBQ was similar to that of the universal dental adhesive SBU and better than that of the universal dental adhesive PBU or the dental adhesive SE bond.
2. After aging, the microleakage of the UBQ and PBU was slightly poorer in the thin cervical enamel area near the gingival margin.

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