Degree of Conversion, Bond Strength and Biocompatibility of the APTES@SiO₂ Strengthened Experimental Dental Adhesive

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The aim of this study was to investigate the effect of incorporation of the nano silica modified with (3-aminopropyl)triethoxysilane (APTES@SiO₂) on the degree of conversion, bond strength and biocompatibility of an experimental dental adhesive. An amphiphilic bonding system was used as the experimental dentin bonding system. The APTES@SiO₂ with diameter of 70 nm was prepared and added into the experimental dentin bonding system as nanofiller, and the effect of the modification on the stability of the nano APTES@SiO₂ dispersed in the experimental adhesive was studied. In addition, the morphology, degree of conversion, bond strength to dental restorative resin and biocompatibility of the experimental adhesives containing nanofiller were tested. After modification, the dispersion stability of the nano particles in the adhesive was improved. With increasing the content of the nano APTES@SiO₂, the rate of light conversion increased significantly. However, the ultimate degree of conversion, bond strength to resin and bulk compressive strength was not significantly affected. Compared with the adhesive without nanofiller, the adhesive with 2 wt% APTES@SiO₂ showed better bond strength to dentin and lower polymerization shrinkage. In addition, the experimental adhesive showed slight cytotoxicity with the incorporation of APTES@SiO₂, but it was attenuated at low concentration.

Keywords experimental dental adhesive; degree of conversion; bond strength; APTES@SiO₂; biocompatibility

Introduction

The success of adhesion to the tooth structure plays an important role in the dental restoration, which can effectively avoid the terrible clinical problems during the bonding process, including the secondary caries, postoperative sensitivity, marginal discoloration, pulpal pathology and microleakage.^[1-3] To ensure the success of the bonding process, various dental adhesives with different adhesion mechanism have been explored according to the surface properties of the restoration resin and tooth structure.^[4-6] The mechanism of the adhesion included physical adsorption, micromechanical interlocking, chemical and ionic bonding and acid-base interactions. Mechanical interlocking on the interface of the enamel was realized after etching. It resulted in a strong fix of the restorative resin onto the enamel. However, dentin is a hydrated biological dynamic composite where hydroxyapatite minerals, organic matrix and water account for 70 wt%, 20 wt% and 10 wt%, respectively. The dental adhesives, mainly composed of

hydrophobic monomers, could hardly penetrate into the dentin. It resulted in the failure of long-term bonding. Therefore, the amphiphilic bonding system is essential to the dental adhesion because it can wet the dentin substrates and tolerate additional moisture.^[7] The hydroxy ethyl methacrylate (HEMA), which is an amphiphilic monomer with both hydrophilic groups and hydrophobic groups in its molecular chains, have been incorporated into the dental adhesives to improve their hydrophilicity and wetting ability to the dentin. As reported, HEMA can promote the diffusion of methyl-based monomers into the dentin after acidic treatment.^[7-9] In addition, HEMA was shown to play an important role in preventing phase-separation reactions in self-etch adhesives and improving the bond strength.^[10] Therefore, the HEMA-containing adhesives have attracted much attention of researchers in the dental adhesives.

In order to further improve the bond strength and reduce the nanoleakage, inorganic nanofillers were introduced into the dental adhesive.^[11-13] The nanofillers diffused into the collagen fibrils in the dentin could

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strengthen the hybrid layer and then increase the bond strength.^[14] However, other studies indicated that the addition of nanofillers did not affect the bond strength significantly.^[15–17] Therefore, there is still some controversy as to whether nanofillers can reinforce the adhesive layer and thereby increase the bond strength. In addition, no adequate information concerning how the nanofillers affect these properties, such as dispersion, bond strength, bulk strength, degree of conversion and biocompatibility. Furthermore, commercial dental adhesive with unknown components was usually used to compare the bond strength before and after filling, whereas it was not easy to illuminate the effect.

Therefore, the aim of this study was to systematically investigate how the nanofillers affected the properties of dental adhesives by using an experimental amphiphilic dental adhesive. The null hypothesis tested was that the properties of an experimental dental adhesive were not influenced by the nanofiller. To verify this hypothesis, SiO₂ was chosen as a model of nanofiller to strengthen the dental adhesive because of its biocompatibility, high strength and abrasive resistance. To facilitate the dispersion of SiO₂ in the dental adhesive, the silane coupling agent, (3-aminopropyl)triethoxysilane (APTES@SiO₂), was used to modify the surface of SiO₂. After that, the modified nano silica was used to strengthen the amphiphilic experimental adhesive to improve its mechanical properties. The properties of the dental adhesives, including dispersion stability of the nanofiller, micro tensile bond strength, compressive strength, light conversion kinetic curves, degree of conversion and biocompatibility, were investigated. As a control, the properties of the dental adhesives filled with unmodified SiO₂ were also studied.

Experimental

Materials

Bisphenol A glycoldimethacrylate (bis-GMA), triethylene glycol dimethacrylate (TEGDMA), 2-(dimethylamino)ethyl methacrylate (DMAEMA) and camphorquinone (CQ) were purchased from Sigma Aldrich. Hydroxy ethyl methacrylate (HEMA) was produced by Tokyo Chemical Industry. Tetraethyl orthosilicate (TEOS), (3-aminopropyl)triethoxysilane (APTES), ammonium hydroxide (25%–28%) and ethanol were purchased from Sinopharm Chemical Reagent. These reagents were all analytical grades and were used without further processing. Phosphate Buffered Saline (PBS) buffer solution (pH=7.4), fetal bovine serum (FBS) and Dulbecco's Modified Eagle Media (DMEM) were produced by Gibco, America. Z350XT resin was produced by 3M ESPE, America. Cell counting kit-8 (CCK-8) was purchased from Fanbo Biochemical, Beijing.

Preparation of the nano SiO₂ spheres and the experimental adhesives

The nano SiO₂ spheres with a narrow size distribution were prepared by the well-known Stöber method.^[18] More specifically, 1.5 mL TEOS was added into the mixture of 50 mL ethanol, 1 mL deionized water and 1.5 mL aqueous ammonia. Then, the mixture was placed in a water bath at 40 °C for 3 h, followed by adding 1 mL more TEOS and being stirred for another 3 h. Then the SiO₂ was modified with APTES and added into the adhesives (Figure 1). In order to prepare the APTES@SiO₂, the mixture of APTES and ethanol was added into the SiO₂ ethanol dispersion while it was stirred at 50 °C for 8 h, then at ambient temperature for 14 h. Then the nano-silica spheres modified with APTES@SiO₂ were

Figure 1 The preparation process of the APTES@SiO₂ filled experimental adhesives.

collected by centrifuging, washing and freeze-drying at $-80\text{ }^{\circ}\text{C}$. Finally, they were added into the experimental adhesive with contents of 1, 2, 5 and 10 wt% according to the formulations in Table 1. The systems were stirred for more than 6 h and ultrasonic dispersed for 30 min to get a homogeneous mixture.

Table 1 The formulations of the experimental adhesives used in this study^a

Materials	(1)	(2)	(3)	(4)	(5)
RESIN	bis-GMA (58.8)+TEGDMA (39.2)+HEMA (2.00)				
MATRIX	+CQ (1.60)+DMAEMA (1.60)				
APTES@ SiO ₂	0.00	1.00	2.00	5.00	10.00

^a Unit, mass fraction percentage.

Characterization of nano SiO₂ spheres

The morphology of the SiO₂ spheres was investigated with JEOL 2100F transmission electron microscopy (TEM) at an accelerator voltage of 200 kV. Before TEM, about 1 wt% SiO₂ spheres were dispersed in ethanol, dropped onto the copper mesh and dried.

The morphology of SiO₂ spheres dispersed in the experimental adhesive was investigated with HITACHI S-4800 field emission scanning electron microscopy (SEM). The specimen with SiO₂ spheres was fully cured by LED light curing unit (400–500 nm, 1000–1200 mW/cm², Woodpecker, Guilin, China) and fractured in liquid nitrogen before SEM.

The structure of the nano SiO₂ and the surface modified nano SiO₂ was analyzed by FTIR on Nicolet 6700 spectrometer. The spectra were measured with an attenuated total reflectance accessory (ATR). Each spectrum was acquired in transmittance mode on a diamond substrate by the accumulation of 32 scans with a resolution of 4 cm⁻¹ and a spectral range of 500–4000 cm⁻¹.

Stability of the adhesive

The experimental dental adhesives with 1, 2, 5, 10 wt% of APTES@SiO₂ spheres were stored in darkness at 4 °C, and were photographed at 1 and 7 d to describe the dispersion stability. The experimental dental adhesive with 1 wt% pristine SiO₂ was used as a control group.

Measurement of degree of conversion (DC)

Real time *in situ* monitoring of the photo-curing process of the adhesives was made using a Thermo Scientific iS10 Fourier transform infrared spectrophotometer (FTIR) at a resolution of 4 cm⁻¹. The tested adhesive was dropped on the infrared transparent silicon chips and covered with a mylar film. A 200 s exposure of LED light under a distance of 4 cm was applied for curing the adhesives. Real time IR spectra were continuously recorded for 300 s in total at a rate of one spectrum every 5 s. Then the light conversion kinetic curves

were constructed according to the calculation results of DC.

However, because the LED light unit was not the original accessory of the real time FTIR instrument, the 4 cm distance can not be further reduced. In order to avoid the negative effect of the long curing distance on the DC,^[19,20] the FTIR of the adhesive was also investigated on another FTIR (Nicolet 6700, Thermo Fisher, USA). The adhesive was smeared between two thin KBr disks to form a sandwich testing sample and then cured under a light distance of 4 mm. The spectrum was obtained after every 30 s light-cured. In this way, the FTIR spectrum of the adhesives cured for 0–240 s was measured.

The changes in absorbance ratios of 1637 cm⁻¹ (C=C)/1608 cm⁻¹ (phenyl) were monitored from the FTIR spectrum and DC could be calculated by using the following equation:^[21,22] $DC = [1 - (A_{1637}^{Cure} / A_{1608}^{Cure}) / (A_{1637}^{Monomer} / A_{1608}^{Monomer})] \times 100\%$, where A_{1637}^{Cure} was absorbance of cured adhesive at 1637 cm⁻¹, A_{1608}^{Cure} was absorbance of cured adhesive at 1608 cm⁻¹, $A_{1637}^{Monomer}$ was absorbance of uncured adhesive at 1637 cm⁻¹, $A_{1608}^{Monomer}$ was absorbance of uncured adhesive at 1608 cm⁻¹.

Polymerization shrinkage

The polymerization shrinkage of the experimental adhesives with different contents of nano SiO₂ was determined by measuring the density according to Archimedes method.^[23] Firstly, the mass of uncured adhesive in a 1 mL volumetric flask was determined with an electronic balance (ZTP-114, Denver Instrument, US), which indicated $\rho_{uncured}$ (the density of the uncured adhesive). Then a full filled special vial of 10 mL pure water and a piece of totally cured adhesive was weighed to get m_1 and m_2 , respectively. After that, the adhesive piece was put into the full vial and the mass of the whole assembly m_3 was weighed. Finally, the polymerization shrinkage (PS) could be calculated according to the following equations: (1) $V_{cured} = (m_1 + m_2 - m_3) / \rho_{water}$, (2) $\rho_{cured} = m_2 / V_{cured}$, (3) $PS(\%) = (\rho_{cured} - \rho_{uncured}) / \rho_{cured} \times 100\%$, where V_{cured} was the volume of the cured adhesive specimen, $\rho_{uncured}$ was the density of the uncured specimen, ρ_{cured} was the density of the cured specimen, $\rho_{water} = 1.0\text{ g}\cdot\text{cm}^{-3}$.

Mechanical properties

The bond strength was characterized using the Shenzhen SUNS UTM4104 Universal Testing Machine. A special clamp was designed (Figure 2) and installed on the mechanical testing machine, with its upper slider moving freely while the lower slide static. Firstly, two Z350XT resin sticks (2 mm × 2 mm × 10 mm) were used to test the bond strength of the adhesive between the clinical restorative resin. They were fixed in the rectangular grooves on the clamp. The experimental adhesives were evenly coated on the sides between the two sticks and cured by the LED light curing unit for 2 min.

Figure 2 The specially designed clamp used to fix the tested samples.

The samples were then tested with a 100 N capacity load cell at a crosshead speed of 4 mm/min. Secondly, the bond strength between dentin and the clinical restorative resin was tested according to the same method, in which one of the sticks was replaced by a dentin stick (with a cross section area of $1 \times 1 \text{ mm}^2$). The dentin sticks were cut from the third molar using a double face diamond disk of a slow-speed sectioning machine under running water. The teeth were cut perpendicular to the long axis of each tooth to remove the enamel and then along the long axis to get dentin sticks. Finally, after tensile test, the dentin sides of the tested specimens were ultrasonic cleaned, sputter-coated (40 mA for 60 s) with gold and examined through SEM.

The compressive strength of the experimental adhesives was also measured by the mechanical testing machine. The experimental adhesives were molded into columns (radius, 2 mm; height, 6 mm), using a silica rubber mold and cured for 2 min by LED light, and then were stored in distilled water at room temperature for 40 h to ensure that polymerization was completed and the rest of the monomers were dissolved. The compressive strength was determined according to the equation $CS = 4F/(\pi d^2)$, where F was the maximum load measured with the universal testing machine at a crosshead speed of 1 mm/min.

Cytotoxicity experiment

The cytotoxic effect of the adhesives with or without SiO_2 was determined with the CCK-8 assay. The adhesives disks with a diameter of $10 \pm 1 \text{ mm}$ and a thickness of $1 \pm 0.1 \text{ mm}$ were prepared by filling the adhesives into a silica rubber mold and light curing for 1 min. After that, the disks were immersed in 75% alcohol for 30 min, washed with PBS, and then soaked in 5 mL DMEM (containing 10% FBS, 100 U/mL penicillin and 100 $\mu\text{g/mL}$ streptomycin) in a sterile environment at 37°C for 4 d. The extract liquid was collected and filtered using a syringe-driven filter to ensure no bacteria. Then the sterilized extract liquids (diluted with DMEM as 1 : 0, 1 : 5 and 1 : 10) were set as the experimental

groups, and the untreated DMEM as the control group.

Healthy human dental pulp cells (HDPC) were regularly cultured and passed in DMEM medium at 37°C and 5% CO_2 . The cells suspension (5×10^5 cells/mL) was seeded in a 96-well plate (200 μL medium per well) and cultured for 48 h to make the cells well attached and proliferated. Then the medium was substituted with the above experimental groups and control group extract liquid. After 24 h, the cells morphology was observed by an inverted microscopy (Leica DMI 4000B, Germany). Then 100 μL CCK-8 solution (10% in volume) was added into each well and the cells were incubated for another 2 h. Cell viability was measured with the MB-40 ELISA microplate reader by recording the absorbance at 450 nm. The number of the survival cells was calculated as the ratio of the mean absorbance of experimental groups to the mean absorbance of control group.

Result and Discussion

Preparation of APTES@ SiO_2 and their dispersion in the experimental adhesives

The SiO_2 was prepared by the classical Stöber method and characterized by TEM. It had aspherical morphology and well dispersed in ethanol, as shown in Figure 3a. The average particle size of the SiO_2 was about 72 nm, shown in the statistical distribution histogram (Figure 3b), which was calculated according to the TEM photos. APTES was used to modify the surface properties of the SiO_2 . The FTIR spectra of SiO_2 before and after APTES modification were shown in Figure 3c. The characteristic absorption peaks of SiO_2 spectra curve at 786, 1046, and 1629 cm^{-1} and the broad

Figure 3 Modification of SiO_2 and its dispersability. (a) TEM image of SiO_2 dispersed in ethanol; (b) size distribution of the SiO_2 ; (c) FTIR spectra of SiO_2 modified with APTES; (d) SEM image of SiO_2 dispersed in the light-cured experimental dental adhesive.

band around 3262 cm^{-1} were identified as symmetric

vibrations of Si—O—Si, asymmetric vibrations of Si—O—Si, bending vibration of O—H and stretching vibration of OH,^[24-26] respectively. After surface modification by APTES, the broad band at 3262 cm⁻¹ became weak, which indicated the decrease of hydroxyl on SiO₂ surface. Furthermore, new characteristic peaks around 1500 cm⁻¹ belonging to N—H bending vibration were observed.^[25] Several minor bands at around 2800 to 3000 cm⁻¹ were also detected in the spectra of APTES@SiO₂, which were attributed to the N—H stretching vibration.^[25,26] Therefore, this result illustrated that APETS was successfully grafted onto the surface of SiO₂.

The 5 wt% APTES@SiO₂ was added into the experimental dental adhesive followed by stirring and sonication to make them dispersed evenly. After light curing, the adhesive with APTES@SiO₂ was fractured in liquid nitrogen, and SEM was used to characterize the cross-section of the adhesive, as shown in Figure 3d. Aggregation of the particles was limited,^[27] indicating that the APTES@SiO₂ were well-embedded in the adhesive resin matrix with strong interface effect.

The adhesives with 1, 2, 5, 10 wt% of APTES@SiO₂ were stored in darkness at 4 °C, and were then photographed after 1 d and 7 d to observe the dispersion stability of the APTES@SiO₂ in the experimental adhesives. Either for 1 or 7 d, no significant change for the adhesives with different APTES@SiO₂ contents was observed. However, after 7 d' storage, obvious sedimentation appeared in the adhesive with unmodified SiO₂ (Figure 4a-7). Therefore, surface modification by APTES made the SiO₂ well dispersed in the experimental adhesive. Compared with the PMMA-grafted nanoclay, which showed obvious sedimentation in 12 hours,^[12] the APTES@SiO₂ would be a more appropriate nanofiller for the dental adhesives from the point of view of stability.

Figure 4 The photograph of the dental adhesives with different concentrations of SiO₂ stored for 1 d and 7 d. The character a-e represented the adhesives with 0 wt% SiO₂, 1 wt% APTES@SiO₂, 2 wt% APTES@SiO₂, 5 wt% APTES@SiO₂ and 10 wt% APTES@SiO₂, respectively.

The light conversion kinetic of the experimental adhesive

Degree of conversion is an important property of the dental adhesives, which indicate the degree of polymerization and amount of unreacted monomers. The light conversion kinetic of the experimental dental adhesive was investigated by the real-time FTIR from 0 to 300 s. Representative real-time spectra of the experimental dental adhesive with different APTES@SiO₂ contents during photopolymerization are presented in Figure 5. The absorbance at 1608 cm⁻¹, associated with phenyl groups in Bis-GMA, remained unchanged because the phenyl groups didn't participate in the reaction. However, the intensity of the aliphatic C=C group at 1637 cm⁻¹ decreased with the increasing light curing time, indicating the proceeding of polymerization. Therefore, the polymerization kinetics was studied by tracking the changes of the IR band of C=C at 1637 cm⁻¹ quantitatively. In addition, the phenyl groups at 1608 cm⁻¹ was used as an unchanging and internal standard for the polymerization kinetics. Correspondingly, the changes in absorbance ratios of 1637 to 1608 cm⁻¹ were monitored and DC of the experimental adhesive was calculated.

Figure 5e showed the real-time DC curves of the experimental dental adhesives with different APTES@SiO₂ contents. The DC increased rapidly for the first 1 min curing and then slowed down. With the increasing APTES@SiO₂ content, the final DC increased from 26.6% to 40.8%. CQ/DMAEMA was the most widely used photo-initiator system, where CQ was the hydro-

Figure 5 The real-time FTIR spectra of the experimental dental adhesives: (a) 0 wt% APTES@SiO₂; (b) 1 wt% APTES@SiO₂; (c) 2 wt% APTES@SiO₂; (d) 5 wt% APTES@SiO₂; (e) 10 wt% APTES@SiO₂; and their light conversion kinetic curves (f).

gen transfer type photosensitizer and abstracted hydrogen from tertiary amine groups of DMAEMA^[28] when exposed under the LED light,^[29,30] which generated the active free radicals and initiated the polymerization. As the NH₂ groups modified on the surface of the SiO₂ were relatively active hydrogen donors, hydrogen abstraction occurred on them as well, thus increasing the concentration of radicals, the polymerization rate at beginning and finally the DC of the adhesive.

However, the DC of these experimental dental adhesives exhibited relatively lower than that reported in many other relevant studies. This was due to the large illumination distance of about 4 cm, which was limited by the real-time FTIR instruments and the length of the LED lights. The distance was correlated to the light intensity, which further affected the degree of polymerization.^[19,20] To elucidate this affect, we investigated the FTIR of the adhesives under illumination distance of 0.4 cm, as shown in Figure 6. After curing for 240 s, the DC of the unfilled experimental dental adhesive reached 52.6%. This value was obviously higher than that detected at a distance of 4 cm. Although the illumination distance affected the DC significantly, it followed the same rule that DC of the adhesives increased with increasing the content of APTES@SiO₂ for the first 90 s curing (Figure 6b). However, after curing for 210 and 250 s, the DC gradually reached equilibrium and showed a value of above 50%. Finally, the adhesive with the content of 2%—5% had a relatively higher DC up to 55%.

Figure 6 The FTIR spectra of the experimental dental adhesives: (a) the pristine experimental adhesive (without SiO₂) at different curing time point; (b) DC of the experimental dental adhesives with different APTES@SiO₂ contents at different time.

The mechanical properties of the experimental adhesive

In a dental restoration, when the occlusal loading exceed the inherent strength of the adhesive or the adhesive strength at the interface between the dentin and resin, defects or cracks occurred at the adhesive layer leading to the failure of the restoration. Therefore, bond strength and bulk strength are crucial properties of the dental adhesives.

Microtensile bond test has now been accepted as a reliable and facile method measuring the bond strength of dental adhesives to the tooth structure. Figure 7a showed the means and standard deviations of the bond

Figure 7 Mechanical behavior of the dental adhesive with various APTES@SiO₂ contents. (a) Bond strength between two restorative resin sticks; (b) bulk compressive strength.

strength between resin sticks. All the adhesives with different APTES@SiO₂ content had bond strength higher than 38 MPa. Only a slightly increase was observed with increasing the APTES@SiO₂ content. However, there was no statistically difference among the bond strengths of these adhesives. As reported, at higher nanofiller content, the viscosity of the adhesive increased and the fluidity decreased, which also resulted in the coagulation of nanofiller and the decrease of bond strength.^[12,13,31-33] However, the well dispersed nanofillers did little negative influence^[15-17] or even an enhancement^[34-37] on the mechanical properties of the resin composites.

The compressive strength of the adhesives was also investigated to elucidate their bulk strength, as shown in Figure 7b. The adhesives without nanofiller had the lowest compressive strength of 183.8 MPa. With the addition of 2 wt% APTES@SiO₂, the compressive strength reached 291.5 MPa. A slight decrease was observed in the compressive strength of the adhesives containing 5 wt% and 10 wt% APTES@SiO₂. At higher filler contents, nano particles aggregate more easily. The aggregation of nano particles would result in the unevenly distribution of stress and further the mechanical defect sites. However, the decrease was only slight indicating that no obvious aggregation of the nano particles existed in the adhesives. Therefore, the adhesive containing 2 wt% APTES@SiO₂ with the best bond strength and compressive strength was used for further study.

Polymerization shrinkage of the adhesives appeared due to the conversion of intermolecular van-der-Waals forces to the covalent single bonds during the polymerization. The nanofiller in the resin composites played an important role in reducing the polymerization shrinkage.^[38-40] The increase of filler content reduced the concentration of carbon double bonds in resin matrix, therefore reducing the shrinkage.^[40] With the addition of APTES@SiO₂, the polymerization shrinkage decreased from 10.14% to 9.04% (Table 2). Generally, the higher viscosity of the adhesives with higher nanofiller content would affect the penetration of adhesive into the dentin tubules and decrease the micromechanical retention. However, incorporation of a small quantity of APTES@SiO₂ into the adhesive showed opposite result in this study. As shown in Figure 8a, the dentin tubules were

Figure 8 The SEM of adhesive interface on the dentin. (a) Polished dentin; (b) the fracture surface of the dentin after bonded by the adhesive without APTES@SiO₂; (c) the fracture surface of the dentin after bonded by the adhesive with 2 wt% APTES@SiO₂; the orange, blue and red arrow indicated dentin surface, the tubule lumens and adhesive resin, respectively.

clearly exposed after removing the enamel. The experimental adhesives with or without nanofiller were then applied to bond the resin and dentin. After tensile test, the fracture surface of dentin-resin samples was observed by SEM. The resin tags were observed on the fracture surface bonded by the adhesive filled with 2 wt% APTES@SiO₂. Comparatively, there left several dentin tubules empty on the fracture surface when using the adhesive without APTES@SiO₂. This indicated that incorporation of 2 wt% APTES@SiO₂ into the experimental adhesive resulted in complete penetration into the dentin tubules and extra micromechanical interlocking on account of the lower shrinkage (Table 2). As it was recognized that shrinkage caused the debonding of the dentin-resin interface and further resulted in increased microleakage and ultimately secondary caries^[41, 42], incorporation of the nano silica filler provided some implications to solve this problem.

Table 2 Polymerization shrinkage and bond strength between resin and dentin of the dental adhesives with and without APTES@SiO₂

Content of APTES@SiO ₂ /(wt%)	Polymerization shrinkage/(%)	Bond strength/(MPa)
Without APTES@SiO ₂	10.14 ± 0.89	8.79 ± 0.78
With 2% APTES@SiO ₂	9.04 ± 0.33	8.89 ± 1.00

The cytotoxicity of the dental adhesives

The cytotoxicity to the human dental pulp cells (HDPCs) of the dental adhesives was evaluated by CCK-8 test, as shown in Figure 9. The results demonstrated that the cells proliferated better with increasing the dilution ratio of the extract liquid. The morphology of the HDPCs cultured in the extract liquid of the experimental adhesives was observed by the inverted microscopy. In the control group without any components of the adhesive, the HDPCs had obvious spindle morphology and proliferated well (Figure 10a). With decreasing the dilution ratio of the extract liquid of the adhesive without APTES@SiO₂, the cells retracted slightly but most of the cell maintained prototype (Figure 10b1—b3). Comparatively, for the adhesive with 2 wt% APTES@SiO₂, the cell retracted obviously at a

Figure 9 The cell viability of the dental adhesive without APTES@SiO₂ and adhesive with 2 wt% APTES@SiO₂.

dilution ratio of 5 : 1 (Figure 10c2), and the cells lost their activity at a dilution ratio of 1 : 1 (Figure 10c3). The cytotoxicity of the adhesive could be attributed to the ingredients, the interactions between them and the residual monomers during polymerization.^[43] While the DC of the adhesive with or without APTES@SiO₂ was similar to each other, the cytotoxicity was mainly contributed to the incorporation of nano APTES@SiO₂. The compatibility of APTES@SiO₂ was dependent on the surface characteristics, geometry, particle size and porosity. After modification with primary amine functionalities, the toxicity of the APTES@SiO₂ was relatively weak.^[44] The adhesive with APTES@SiO₂ still showed obvious cytotoxicity when the dilution ratio was 1 : 1. Nonetheless, the cytotoxicity can be attenuated by decreasing the content of APTES@SiO₂.

Conclusions

In summary, it was proved that the null hypothesis at the beginning was wrong and that the nano silica as a filler had an effect on the properties of an experimental dental adhesive. We investigated the benefits of incorporation of APTES@SiO₂ on the degree of conversion, bond strength and biocompatibility of an experi-

Figure 10 The micrograph of HDPCs after culture in the extract liquid of the experimental adhesives (a) control group; (b1—b3) represented the adhesives without 2 wt% APTES@SiO₂ at the dilution ratio of 1 : 10, 1 : 5 and 1 : 0; (c1—c3) represented the adhesives with APTES@SiO₂ at the dilution ratio of 1 : 10, 1 : 5 and 1 : 0.

mental dental adhesive. The APTES@SiO₂ was well dispersed in the amphiphilic experimental dental adhesive even after 7 d storage. Incorporation of 2 wt% APTES@SiO₂ into the experimental adhesive resulted in the highest bond strength to dentin, lowest polymerization shrinkage and best penetration into the dentin tubules. The real-time FTIR results indicated that the adhesive showed an obvious increase in the polymerization rate with increasing the content of APTES@SiO₂. However, the final DC of the adhesive filled with different content of APTES@SiO₂ all reached about 50%, which led to the small difference on the bond strength. The compressive strength was significantly improved by filling the APTES@SiO₂ into the adhesive. Slight cytotoxicity appeared due to the incorporation of APTES@SiO₂, which was attenuated at low concentration. Therefore, incorporation of the APTES@SiO₂ into the adhesive increased the polymerization rate and improved the properties of the adhesive, such as bond strength and penetration into the dentin tubules. These systematic studies may open up opportunities to develop

various high-performance dental adhesives filled with the nano silica particles with good potential in industrial applications.

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