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Biphenyl liquid crystalline epoxy resin as a low-shrinkage resin-based dental restorative nanocomposite

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ABSTRACT

Low-shrinkage resin-based photocurable liquid crystalline epoxy nanocomposite has been investigated with regard to its application as a dental restoration material. The nanocomposite consists of an organic matrix and an inorganic reinforcing filler. The organic matrix is made of liquid crystalline biphenyl epoxy resin (BP), an epoxy resin consisting of cyclohexylmethyl-3,4-epoxycyclohexanecarboxylate (ECH), the photoinitiator 4-octylphenyl phenyliodonium hexafluoroantimonate and the photosensitizer champhorquinone. The inorganic filler is silica nanoparticles (~70-100 nm). The nanoparticles were modified by an epoxy silane of γ -glycidoxypropyltrimethoxysilane to be compatible with the organic matrix and to chemically bond with the organic matrix after photo curing. By incorporating the BP liquid crystalline (LC) epoxy resin into conventional ECH epoxy resin, the nanocomposite has improved hardness, flexural modulus, water absorption and coefficient of thermal expansion. Although the incorporation of silica filler may dilute the reinforcing effect of crystalline BP, a high silica filler content (~42 vol.%) was found to increase the physical and chemical properties of the nanocomposite due to the formation of unique microstructures. The microstructure of nanoparticle embedded layers was observed in the nanocomposite using scanning and transmission electron microscopy. This unique microstructure indicates that the crystalline BP and nanoparticles support each other and result in outstanding mechanical properties. The crystalline BP in the LC epoxy resin-based nanocomposite was partially melted during exothermic photopolymerization, and the resin expanded via an order-to-disorder transition. Thus, the post-gelation shrinkage of the LC epoxy resin-based nanocomposite is greatly reduced, ~50.6% less than in commercialized methacrylate resin-based composites. This LC epoxy nanocomposite demonstrates good physical and chemical properties and good biocompatibility, comparable to commercialized composites. The results indicate that this novel LC nanocomposite is worthy of development and has potential for further applications in clinical dentistry.

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1. Introduction

Methacrylate resin-based dental restorative materials exhibit intrinsic shrinkage problems resulting from the free volume reduction that occurs due to the linking of methacrylate monomer and oligomer during photocuring. This free volume reduction is at the nanometer scale (i.e. reduced entropy) and cannot be compensated for by very small picometer-scale volume increases, such as those that occur when carbon-carbon sigma bonds (154 pm) are formed from carbon-carbon double bonds (147 pm) during polymerization. The contraction stress of shrinkage can lead to tooth structure damage and the formation of internal gaps between restorative materials and the original tooth structure [1-3]. Polymerization shrinkage can be reduced using large molecule resins or other resin types [4]. Special methacrylate monomers and oligomers have been designed to reduce the polymerization shrinkage of restorative resins [5-9]. Recently, liquid crystalline (LC) and crystalline acrylates have been reported to reduce shrinkage. Photopolymerization of methacrylate resins via visible light irradiation is an exothermic reaction. The released heat melts the crystalline acrylate and results in a disordered structure (order-to-disorder transition). The volume expansion from this type of order-to-disorder

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transition compensates the free volume loss that arises from polymerization. Thus, effective shrinkage reduction is achieved using crystalline or LC resin as one component of the organic matrix in resin-based restorative composites [10–13].

LC resins are monomers and oligomers that contain rigid rod segments (mesogens) on the main chain or side chain of the molecule and form highly crystalline domains under certain conditions. For instance, when thermotropic LC resin is heated to its LC state, the ordered crystalline structure can be locked upon cooling. The ordered structure can be further enhanced by electrical or magnetic fields. Moreover, the crosslinking functionality of LC resins can alignment the mesogen in a specific direction. High mechanical strength, bonding strength and thermal conductivity have been achieved by curing the LC resin under these conditions, due to formation of a self-reinforcing crystalline structure [10,14–21].

LC epoxy resins have an additional advantage over LC methacrylate resins in that they reduce volume shrinkage. The ringopening polymerization of the epoxy resin frees its constrained three-member ring structure, which results in a much larger free volume increase compared to that from the curing of methacrylate resin [22]. Thus, the overall volume shrinkage of epoxy resin is much less than that of methacrylate resin after curing. Due to both a high degree of conversion and low polymerization shrinkage, epoxy resins have been investigated for application as dental restorative materials [13,17,23-26]. The LC epoxy resin 4,4'bis(2,3-epoxypropoxy) biphenyl (BP), can be cured with various curing agents, and shows good mechanical and thermal properties after curing [17,18,21,27]. Accelerated curing kinetics are observed when BP resin is reinforced with BP-grafted multi-walled carbon nanotubes (MWCNT) and cured using diaminodiphenylsulfone, due to the extremely high thermal conductivity of the MWCNT. A dendriticcrystalline structure is formed during curing that results in a large improvement in the physical and chemical properties of the composite [28,29].

The purpose of this study is to develop low-shrinkage epoxy resin-based nanocomposite using LC epoxy resin. By incorporating LC epoxy resin into a conventional epoxy resin-based nanocomposite, one could expect to observe extremely low shrinkage due to the occurrence of low-shrinkage ring-opening polymerization and volume expansion from order-to-disorder transition during curing. The physical and chemical properties of the nanocomposite could also be improved by the crystalline character of the LC epoxy resin.

2. Materials and methods

2.1. Materials

The following chemicals were used as received from suppliers without further purification to prepare nanocomposites: Snowtex-ZL colloidal silica (40 wt.% solid content of 70–100 nm SiO₂; Nissan Chemical Industries, Ltd., Tokyo, Japan), 3,4-epoxycyclohexylmeth-yl-(3,4-epoxy)cyclohexanecarboxylate (ECH, 90%; Aldrich Chemicals, Milwaukee, USA), γ -glycidoxypropyltrimethoxysilane (GPS,

Table 1

99%; Acros, Geel, Belgium), 4-octylphenyl phenyliodoniumhexafluoroantimonate (OPIA, 99%; General Electric Co. New York, USA) and champhorquinone (CQ, 97%; Aldrich Chemicals, Milwaukee, USA), anhydrous methanol (99.95%; Mallinckrodt Chemicals, Chesterfield, UK) and anhydrous tetrahydrofuran (THF, 99.9%; Acros, Geel, Belgium).

BP was synthesized according to our previously reported procedure [17]. Four commercial resin-based dental restorative materials, Estelite Sigma (shade A3; Tokuyama Dental, Tokyo, Japan), Permisa (shade A3; Kerr Corporation, Orange, United States), PolofilSupera (shade A3; Voco, Cuxhaven, Germany) and Z350XT (shade A3; 3 M ESPE, St. Paul, USA), are referred to as CMR1, CMR2, CMR3 and CMR4, respectively. They were used to compare resin-based nanocomposites and commercial dental restorative materials. The overall weight percent of commercial resin-based materials and their filler content was measured using a thermogravimetric analyzer (Pyris 1 TGA, Perkin Elmer, USA). The results are summarized in Table 1. Detailed sample preparation and the testing conditions for the thermogravimetric analysis (TGA) are described in Sections 2.4 and 2.5.2.

2.2. Preparation of the organic matrix: light-curable epoxy resins

In this study, we used "E-BPXX" to denote the formulations of a series of light-curable epoxy resins. The first letter "E" refers to the ECH epoxy resin, while "BP" represents the biphenyl liquid epoxy resin. Finally, "XX" indicates the wt.% of BP in the resin mixture of ECH and BP. For instance: E-BP00 and E-BP07 indicate a resin system without BP and one with 7 wt.% BP as compared to neat resin weight, respectively. All formulations in the series contain the same amount of photoinitiating system (a mixture of 4.00 wt.% of photoinitiator OPIA and 2.00 wt.% of photosensitizer CQ based on the weight of the ECH/BP resin mixture). Five resin formulations with varying amounts of LC epoxy resin were prepared, and their compositions are summarized in Table 2. The actual weight percent of each component was slightly lower than the number indicated on the name of the sample because the formulation was designed and prepared using a two-stage synthesis: the resin composition was prepared first and the photoinitiating system was added later to give the final formulation.

To prepare each formulation, we weighed each component with milligram accuracy, as shown in Table 2. First, BP resin was added to ECH epoxy resin to make neat E-BP resin in an Al-foil = covered flask that was shielded from visible light. The photoinitiator OPIA and the photosensitizer CQ were added to the neat E-BP resin. Finally, the photocurable E-BP resin was obtained by heating the mixtures to 100 °C in an oil bath and stirring for 1 h to ensure homogeneity.

2.3. Preparation of resin-based nanocomposites

E-BPXXZLYY is used to name the formulations prepared for two series of resin-based nanocomposites in this study. The first five letters, "E-BPXX", represent the resin composition. The letters

Composition	of	commercial	recip_based	dontal	restorative materials
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Sample name	Composition				
	Type of resin	Filler	(wt.%)		
CMR1	${\sim}20$ wt.% mixture of bis-GMA and TEGDMA	82 wt.% fillers of silica, zirconia and composite filler	68.33		
CMR2	20–35 wt.% of TEGDMA	Trimodal filler of prepolymerized filler, 0.4 μ m barium glass and 40 nm nanoparticles	72.89		
CMR3	${\sim}25$ wt.% mixture of bis-GMA and UDMA	76.5 wt.% fillers of 0.5–2 μm micron filler and 50 nm nanoparticle	71.64		
CMR4	${\sim}20$ wt.% mixture of bis-GMA, UDMA, PEGDMA, TEGDA, bis-EMA (6)	78.5 wt.% nanocluster comprised of 20 nm silica and 4–11 nm zirconia nanoparticles	72.94		

* According to the data sheet of commercial resins. Bis-GMA: bisphenol-A glycidyldimethacrylate; TEGDMA: triethylene glycol methacrylate; UDMA: urethane dimethacrylate; PEGDMA: polyethylene glycol dimethacrylate; Bis-EMA (6): ethoxylatedbisphenol-A dimethacrylate(bisphenol-A: ethylene glycol = 1: 6).

** As determined by thermogravimetric analysis.

Table 2	
Composition of various nanocomposite resins.	

Sample name	Composition (wt.%)*					
	ECH	BP	OPIA	CQ	G-Si-NP	
E-BP00	94.34	0	3.77	1.89	0	
E-BP05	89.62	4.72	3.77	1.89	0	
E-BP07	87.74	6.60	3.77	1.89	0	
E-BP10	84.91	9.43	3.77	1.89	0	
E-BP15	80.19	14.15	3.77	1.89	0	
E-BP00ZL20	76.33	0	3.05	1.53	19.08	
E-BP00ZL40	57.91	0	2.32	1.16	38.61	
E-BP00ZL60	39.06	0	1.56	0.78	58.59	
E-BP07ZL20	70.99	5.34	3.05	1.53	19.08	
E-BP07ZL40	53.86	4.05	2.32	1.16	38.61	
E-BP07ZL60	36.33	2.73	1.56	0.78	58.59	

^{*} ECH: conventional cyclohexylmethyl-3,4-epoxycyclohexanecarboxylate epoxy resin; BP: LC biphenyl resin; OPIA: 4-octylphenyl phenyliodoniumhexafluoroantimonatephotoinitiator; CQ: champhorquinone photosensitizer; G-Si-NP: γ-glycidoxypropyltrimethoxysilane modifiednanosilica.

"ZL" represent the type of SiO₂ nanoparticle, with 70–100 nm size (Snowtex-ZL colloidal silica) used in the formulation. The last two letters, "YY", indicate the weight percent of SiO₂ in the nanocomposite. For example, E-BP00ZL20 means the nanocomposite was made from ECH epoxy resin without BP liquid epoxy resin and with ~20 wt.% SiO₂ nanoparticles. E-BP07ZL20 means the nanocomposite was made from ECH epoxy resin with ~7 wt.% BP liquid epoxy resin and ~20 wt.% SiO₂ nanoparticles.

Six formulations of resin-based nanocomposite were prepared; their compositions are summarized in Table 2. To prepare each nanocomposite, the surface of SiO₂ nanoparticles was modified with GPS to make epoxy-functionalized silica nanoparticles [25], which we called G-Si-NP. The modified SiO₂ (G-Si-NP) was mixed with the above resin system to make a resin-based nanocomposite. The GPS silane contains two functional groups: silane and an epoxy oxirane ring. After reacting with SiO₂ nanoparticles, the silane group reacts with the hydroxyl group of SiO₂ nanoparticles, but its epoxy group remains unreacted. The GPS-modified SiO₂ can render the SiO₂ compatible with the epoxy resin, and the epoxy group can be linked with epoxy resin with chemical bonds after curing. A detailed experimental procedure follows.

The Snowtex-ZL colloidal silica solution was centrifuged (Eppendorf Centrifuge 5810) at 10,000 rpm for 20 min. After centrifugation, the water-based solvent was decanted and 70–100 nm silica nanoparticles were redispersed in anhydrous methanol. After repeating centrifugation and methanol washing two more times, the pH of the colloidal silica/methanol solution was adjusted to 4.5 using acetic acid to make an ~25 wt.% SiO₂ colloidal methanol solution. The exact concentration of the SiO₂ colloids was determined using TGA by heating the sample from 100 to 650 °C at 10 °C min⁻¹ under nitrogen.

G-Si-NP with a weight ratio of silica/GPS = 1/0.1 were prepared by mixing a SiO₂ colloidal methanol solution and GPS in a flask with stirring at 50 °C for 24 h. The exact amount of G-Si-NP in the solution was determined using TGA. Each E-BP00ZL resinbased nanocomposite was prepared according to the composition listed in Table 2. We first weighed out each component (G-Si-NP solution, ECH, OPIA and CQ) to milligram accuracy in a flask covered with aluminum foil. The mixture was stirred for 1 h at room temperature and then the solvent in the mixture was removed under vacuum (~10⁻³ mm Hg) for 4 h. Each BP epoxy resin that contained E-BP07ZL resin-based nanocomposite was prepared according to the composition listed in Table 2. Again, the G-Si-NP, ECH, OPIA and CQ were weighed and placed in a flask covered with aluminum foil. The mixture was then stirred for 1 h followed by solvent removal under vacuum (~10⁻³ mm Hg) for 1 h to obtain the part I mixture. The 7 wt.% BP epoxy resin, based on the total weight of resin matrix (E-BP07 resin series), was dissolved in THF to make an ~10 wt.% solution. The solution was poured into the part I mixture and stirred for 1 h at room temperature to ensure homogeneity. The solvent in the final mixture was again removed under vacuum ($\sim 10^{-2}$ mm Hg) for 4 h to obtain a solvent-free resin-based nanocomposite. The determination of the viscosity of the nanocomposite is described in the Supplementary information S1.

2.4. Preparation of cured composite samples

A disk sample, 5 mm (diameter) \times 2 mm (thick), was prepared by casting uncured resin-based nanocomposite in a stainless steel mold covered with a glass slide and cured under visible light (halogen lamp, Optilux 501, Kerr Corporation) at 500 mW cm⁻² intensity for 40 s on both sides. A beam sample, 25 mm (length) \times 1 mm $(width) \times 1 \text{ mm}$ (thickness), was prepared by casting resin in a stainless steel mold covered with a glass slide. Because the beam size of the visible light was \sim 6 mm in diameter, the whole length of the sample was cured in five sections every 5 mm from left to right along its length. Each section was cured for 40 s. Because the physical and chemical properties of photocured resin-based dental restorative material can be further improved by dark reactions after curing [32], all samples were post-cured at 100 °C for 24 h. The post-cure procedure was adapted to simulate actual clinical curing of photocured dental restorative material. The details of the determination of curing depth and the post-cure condition are described in the Supplementary information S2 and S3, respectively.

2.5. Characterization of photocurable epoxy resins and resin-based composites

2.5.1. Photocuring of resins

The photocuring of the epoxy resins was monitored by photo differential scanning calorimetry (photo-DSC; Perkin Elmer Diamond DSC equipped with a light source, OmniCure Series 2000). The 3–5 mg epoxy resin sample filled an aluminum pan and the heat of reaction with light irradiation at 25 °C was monitored under an N₂ stream.

2.5.2. Thermal stability of resins and resin-based composites

The thermal stability of visible-light-cured E-BP epoxy resin was probed using a Pyris 1 TGA thermogravimetric analyzer. The thermal stability is expressed by the decomposition temperature at 5 wt.% loss of sample. The powder samples for the TGA test were prepared from cured disk samples by breaking them with a hammer and grinding into a finer powder using an alumina mortar and pestle. A 7–10 mg sample of ground powder was weighed in a platinum pan and the sample was heated from 100 to 650 °C at 10 °C min⁻¹ under nitrogen (n = 1 for each composite).

The dimensional stability of cured samples was monitored by probing the coefficient of thermal expansion (CTE) using a thermomechanical analyzer with a standard expansion quartz probe (TA Instruments Q400, USA) (n = 1 for each composite). A disk sample was tested from -10 to $110 \,^{\circ}$ C at a heating rate of 5 $^{\circ}$ C min⁻¹ under N₂, and the CTE of composites was calculated from 0 to $100 \,^{\circ}$ C using TA Instruments Universal Analysis 2000 software.

2.5.3. Mechanical properties of cured resins and resin-based composites

Hardness tests of cured epoxy resins and composites were conducted on disk samples using a Shimadzu HMV Micro Hardness Tester equipped with a Vickers diamond probe under a loading force of 50 g and a loading time of 10 s (n = 5 for each cured epoxy resin or composite). The Vickers hardness number (VHN) is calculated according to the formula of VHN = $1.85 P/d^2$, where *P* is the applied force and *d* is the size of the diamond probe.

The flexural strength and flexural modulus of resin-based composites were evaluated by a three-point bending test using a PT-1066 tensile strength tester (Perfect International Instruments Co., Taiwan) with a crosshead speed of 1 mm min⁻¹ and a support span width of 20 mm (n = 5 for each composite). Flexural strength and flexural modulus of resin-based composites were calculated according to the equations $\delta = 3F_{max}L/2bh^2$ and $Ef = (F_1L^3)/(4bh^3d)$, where F_{max} is the maximum force during test, L is the support span width, b is the width of sample, h is the thickness of sample and d is the deflection due to the force F_1 applied to the beam.

2.5.4. Morphology and microstructure of cured resins and resin-based composites

The LC and crystalline behavior of samples were studied using polarized optical microscopy equipped with a heating and shearing apparatus (Leica DM 2500M microscope equipped with a cross polarizer, a Leica DFC 420C digital camera and a Linkam CSS450 optical shearing system). For observation of the LC phase of neat BP epoxy resin, samples were heated at a rate of 2 °C until they began to melt at 165 °C. The temperature was held until the BP epoxy resin had completely melted, and the sample was then cooled at a rate of 0.5 °C min⁻¹. When birefringence appeared at 162 °C, the temperature was held constant to observe the LC phase. Other samples were observed at room temperature to obtain polarized optical microscope (POM) images of the crystalline structure.

The microstructure of samples was studied using scanning electron microscopy (SEM; JEOL JSM-6700F field emission scanning electron microscope, accelerating voltage of 10 kV). The fracture surface of the cured composite samples was examined by SEM after the three-point bending test. Before SEM observation, the sample was affixed to the holder using carbon tape and coated with platinum to improve the sample conductivity and the image quality.

The microstructure of cured samples was further studied by transmission electron microscopy (TEM; JEOL JEM 1230 electron microscope, accelerating voltage of 100 kV). The ultrathin TEM samples were prepared using a Leica EM UC6 ultramicrotome machine equipped with a Drukkerultramicrotome knife. The 45° Cutting edge was used to obtain a thin film sample of ~100 nm at room temperature. The sample was loaded on a 200-mesh copper grid to be examined by TEM.

2.5.5. Measurement of water absorption of cured resin-based composites

The water absorption of the cured resin-based composite was determined by the weight difference before and after reflux in boiling water (n = 5 for each composite). The disk sample was first weighed after post-curing and cooled to room temperature in a desiccator overnight. The cured sample was refluxed in deionized water at 100 °C for 24 h. After reflux, each disk sample was weighted after cooling to room temperature and any surface water was wiped off. The weight change represents the water absorption of each disk sample.

2.6. Measurement of post-gelation shrinkage of the composite

Post-gelation shrinkage during photopolymerization of resinbased composites (n = 5 for each sample) was probed using a strain gauge method [30,31]. The strain of resin-based composites was detected and recorded using a single axial strain gauge (EA-06-062AP-120, Vishay Intertechnology Inc., Malvern, USA) linked to a Vishay Stress Analysis Data System 5000. An uncured sample was added to a round poly(methyl methacrylate) mold (5 mm diameter, 1 mm thickness) on the strain gauge with a distance of \sim 1 mm between the upper surface of the mold and the curing light (Optilux 501, Kerr Corporation). The data analysis system was zeroed for the first 5 s and the uncured sample was irradiated with visible light for 40 s. The data points of strain were continuously recorded and visible light irradiation started at 5.00 s. The postgelation shrinkage was calculated from the highest point of each strain curve to the leveled strain value at 185.00 s.

2.7. Cell culture and cytotoxicity

The cytotoxicity of the resin-based nanocomposite was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay and the lactate dehydrogenase (LDH) assay. The MTT assay is based on the reduction of yellow tetrazolium bromide to a purple formazan product by mitochondrial dehydrogenases in active cells. The LDH assay is based on the presence of LDH inside living cells; when cells are damaged or dead, LDH is released to the medium. The relative amount of dead cells can be obtained by detecting LDH activity. The following chemicals were used for cytotoxicity tests: phosphate-buffered saline (PBS; Biological Industries, BeitHaemek, Israel), MTT Cytotoxicity Detection Kit (Chemicon, Temecula, USA), LDH Cytotoxicity Detection Kit (Takara Bio, Shiga, Japan), Dulbecco's modified Eagle's medium (DMEM; Gibco-BRL, Paisley, UK), fetal bovine serum (FBS, Gibco-BRL), penicillin/streptomycin (P/S; Gibco-BRL), ethylene oxide (99.9%, Aldrich Chemicals, Milwaukee, USA), dimethyl sulfoxide (DMSO; 99.9%, Aldrich Chemicals) and HCl (1 N; Aldrich Chemicals).

MG-63 human osteosarcoma cell lines (American Type Culture Collection, Rockville, MD, USA), were cultured in DMEM supplement with 10% FBS and 1% P/S and maintained at 37 °C in a humidified incubator with 5% CO₂ (SCA-165D, ASTEC, Fukuoka, Japan). For cytotoxicity tests, the cured samples (2 mm thickness \times 5 mm diameter disk) were first sterilized using ethylene oxide (10.5 psi, 130 °F for 12 h), then five sterilized disk samples of each composite were immersed in 12.00 ml DMEM at 37 °C in the incubator for 72 h to extract the soluble or toxic species. The condition medium for cytotoxicity tests contained 100 µl of extract solution and 100 µl of culture medium in each well of the cell culture plate. The MTT and LDH assays were conducted to quantify cytotoxicity after loading for 1, 3 and 7 days, respectively. The culture medium without the extract but under all the same conditions was used as a control. The extracts of CMR1, CMR2, CMR3, CMR4, E-BP00ZL60 and E-BP07ZL60 were named exD-CMR1, exD-CMR2, exD-CMR3, exD-CMR4, exD-E-BP00ZL60 and exD-E-BP07ZL60, respectively, and the blank sample of the 72-h-incubated DMEM was named 72iD. Six culture media were prepared for each test. The results are presented as the mean ± standard deviation (SD).

To conduct the MTT assay, a stock solution of MTT was prepared by dissolving MTT in PBS at a concentration of 5 mg ml⁻¹, sterilized by Millipore filtration and stored protected from ambient light. The upper layer of the conditioning medium was removed from the wells after 1, 3 and 7 days, then 30 μ l of MTT solution was added to each well and was incubated at 37 °C for 3 h. After removing the unreacted MTT solution from the well, 50 μ l of DMSO was added to each well and the plate was agitated for 15 min on a shaker to dissolve any formazan crystals. The optical density (OD) of the formazan solution in each well was measured at 570 nm using an absorbance microplate reader (BioTek ELx800, Winooski, VT, USA).

To conduct the LDH assay, each 10 μ l of upper layer conditioning medium sample was mixed with 100 μ l of LDH kit solution. The mixture was placed in a 96-well plate and incubated at 37 °C for 30 min. After incubation, 50 μ lof 1 N HCl was added to each well to stop the reaction, and OD values of reduced nicotinamide adenine dinucleotide (NADH) at 490 and 630 nm were measured using the microplate reader. The results of the LDH assay were calculated by subtracting the OD at 630 nm from that at 490 nm.

2.8. Statistic analysis

All measurement data for resin-based composites is presented as the mean \pm SD, except for the measurements of curing behaviors by photo-DSC, thermal stability by TGA and thermal expansion coefficient by thermal mechanical analysis (TMA). Statistical significance was calculated using a one-way analysis of variance followed by Student's *t*-test. A *p* value of less than 0.05 indicates an acceptably significant difference between two groups of results.

3. Results

3.1. Compositions of resins and composite

Table 1 summarizes the composition of four commercial methacrylate resin-based composites. Table 2 illustrates the composition of 11 epoxy-resin-based nanocomposites. Fig. 1 shows the chemical structures of the two epoxy resins used in this study.

3.2. Characterizations of photocurable epoxy resins and resin-based composites

Fig. 2 shows the hardness and thermal stability of different compositions of organic matrix (E-BP series cured epoxy resin). Fig. 3 shows the morphology of neat LC epoxy resin and organic matrices containing different amounts of LC BP, as evaluated by polarized microscopy.

Fig. 4 illustrates the hardness, flexural modulus, flexural strength and water absorption of epoxy resin-based nanocomposites with various silica contents. Fig. 5 shows the morphology of epoxy resin-based nanocomposite containing ~60 wt.% silica from SEM studies. Fig. 6 shows TEM images of epoxy resin-based nano-composites with (E-BP07ZL60) and without (E-BP00ZL60) LC epoxy, highlighting the distinct morphologies of the two nanocomposites.

Fig. 7 compares the hardness, flexural modulus, flexural strength and water absorption of the LC epoxy resin-based nanocomposites (E-BP07ZL60) and the four commercial methacrylate-based composites. The thermal expansion coefficients of the composites, as measured by TMA, were 96.56 μ m m⁻¹°C⁻¹ for E-BP00, 67.63 μ m m⁻¹°C⁻¹ for E-BP00ZL20, 47.03 μ m m⁻¹°C⁻¹ for E-BP00ZL40, 39.66 μ m m⁻¹°C⁻¹ for E-BP00ZL60, 75.11 μ m m⁻¹°C⁻¹ for E-BP07ZL40, 39.53 μ m m⁻¹°C⁻¹ for E-BP07ZL40, 33.35 μ m m⁻¹°C⁻¹ for E-BP07ZL60, 41.99 μ m m⁻¹°C⁻¹



4, 4'-bis(2,3-epoxypropoxy) biphenyl epoxy resin (BP)

Fig. 1. Chemical structures of conventional epoxy resin (ECH) and LC biphenyl epoxy resin (BP).

for CMR1, 45.32 μ m m⁻¹°C⁻¹ for CMR2, 32.76 μ m m⁻¹°C⁻¹ for CMR3 and 29.15 μ m m⁻¹°C⁻¹ for CMR4.

3.3. Measurement of post-gelation shrinkage of composites

Fig. 8 presents the post-shrinkage behaviors of composites; differences were observed between the LC epoxy resin-based nanocomposite (E-BP07ZL60), the epoxy resin-based nanocomposite (E-BP00ZL60) and the four commercial methacrylate-based composites.

3.4. Cytotoxicity of cured composites

Fig. 9 shows the cytotoxicity results of composites; differences were observed between the LC epoxy resin-based nanocomposite (E-BP07ZL60), the epoxy resin-based nanocomposite (E-BP00ZL60) and the four commercial methacrylate-based composites.

4. Discussion

4.1. Optimization of compositions of organic matrix

A series of resin compositions with varying amounts of BP LC epoxy resin in ECH epoxy resin (E-BP series of epoxy resins) was prepared to determine the optimal formulation of organic matrix. The synthesized BP resin is an oligomer with 26 mol.% hydroxyl groups. The epoxy resin is photocured through ring-opening polymerization using a cationic photoinitiator made from either aryl iodium or aryl sulfonium salt with a stable anion such as hexa-fluorophosphate (PF₆⁻) or hexafluoroantimonate (SbF₆⁻). A free radical ion is generated upon photoinitiation that further reacts with a



Fig. 2. (a) Hardness and (b) thermal stability of E-BP series cured epoxy resins. E-BP00 resin contains no LC BP epoxy resin; E-BP05, E-BP07, E-BP10 and E-BP15 resins contain 5, 7, 10 and 15 wt.% BP, respectively.



Fig. 3. POM images of (a) neat LC BP epoxy resin, (b) epoxy resin sample with 7 wt.% BP resin (E-BP07ZL00), uncured nanocomposite resin containing silica at (c) ~10 vol.% (E-BP07ZL20), (d) ~24 vol.% (E-BP07ZL40), (e) ~41 vol.% (E-BP07ZL60) and (f) light-cured E-BP07ZL60 nanocomposite resin.

proton to generate a strong Lewis acid such as $H^+PF_6^-$ or $H^+SbF_6^$ and initiate the ring-opening polymerization [32]. Thus, the hydroxyl groups in the BP LC epoxy resin function as co-initiators that accelerate photopolymerization. In this study, we used an iodium salt initiator of OPIA. The crystalline structure of BP that remained after curing reinforces and improves the performance of ECH epoxy resins. The improvements in mechanical properties and thermal stability were evaluated with a hardness test and TGA, respectively. The resin containing 6.6 wt.% of BP epoxy resin (E-BP07) exhibited the highest hardness (Fig. 2(a)) and highest thermal decomposition temperature (T_d , determined by 5 wt.% loss temperature of sample, Fig. 2(b)) among the samples in the series. The BP resin is expected to act as a reinforcing filler and curing accelerator in the organic matrix. It is interesting to note that this reinforcing effect is optimized at 6.6 wt.% of BP; for samples with a higher BP content, the excess BP induces an accelerated polymerization rate that results in early vitrification and a low degree of polymerization, as shown in the TGA analysis. The results indicate that weight loss occurred at as low as \sim 160 °C for resin systems with higher or lower than optimal BP concentrations. Because the weight loss possibly arose from uncured ECH, the E-BP07 resin formulation was selected to fabricate a series of resin-based nanocomposites (E-BP07ZL series) to study the effect of nanoparticle concentration on BP crystalline behavior in the nanocomposite. In addition, the E-BP00 resin formulation (without BP) was used to prepare nanocomposites (E-BP00ZL series) as a control for comparison.

4.2. Characterization of organic matrix and composites

The crystalline behavior of neat LC epoxy resin, organic matrix and composites were evaluated using a POM. The LC phase of the BP resin was observed at 162 °C (Fig. 3(a)). The BP of the organic matrix (E-BP07 resin) exhibited layered crystals in the amorphous ECH epoxy resin host at room temperature (Fig. 3(b)). When the content of surface modified silica nanoparticles was 38.61 wt.% (24.23 vol.%) or higher, the BP crystal size was drastically reduced, from hundreds to less than ten micrometers. This observation can be attributed to the decreasing volume fraction of BP in the nanocomposite when the number of silica nanoparticles serving as nuclei is increased. BP crystals are thus smaller and better dispersed in nanocomposites with a high silica nanoparticle content of 41.85 vol.% (E-BP07ZL60) as compared to resin without any silica (E-BP07ZL00).

Both series of nanocomposites (with and without LC BP) showed large increases in hardness with increasing silica content (G-Si-NP). The increased hardness of both series becomes significant in a nonlinear fashion when the silica content is greater than 24.23 vol.%. This is well-known phenomenon of percolation network formation in composites. A high-performance composite can usually be achieved at a specific filler concentration, called the percolation threshold [33]. In this study, the percolation threshold of nanoparticles in the nanocomposite is close to 24 vol.%, consistent with data reported in the literature [34,35]. With the same silica content, LC epoxy resin containing nanocomposites (E-BP07ZL series) exhibit higher hardness than nanocomposites without LC epoxy resin (E-BP00ZL series). Although significant differences in hardness were observed between the two series (p < 0.05), the difference decreased when the silica content was more than 24 vol.%. The crystalline BP exhibited the expected reinforcing effect on the hardness of the nanocomposite. The organic matrix containing crystalline BP (E-BP07) displayed higher hardness than the matrix without BP (E-BP00) (31.70 ± 1.29 VHN vs. 21.77 ± 0.29 VHN). When silica is incorporated into the nanocomposite, it dilutes the reinforcement effect of the crystalline BP because the BP content is lowered and the crystalline BP size is decreased. It is interesting to note that a 72.29% increase in hardness (from 40.34 ± 1.05 to 69.50 ± 1.24 VHN) is observed in the nanocomposites containing LC epoxy resin (E-BP07ZL series) when the silica content is increased from ${\sim}24$ to ${\sim}42$ vol.% (${\sim}38$ to ${\sim}58$ wt.%). In contrast, the nanocomposites containing no LC epoxy resin (E-BP00ZL series) showed a smaller increase in hardness (38.00%) when the silica volume percent was increased by the same amount, which is rather unusual. We envision a unique microstructure for LC resin composites with a high silica nanoparticle content, and try to deduce the formation mechanism of this microstructure below.

The same phenomenon was also observed in the flexural modulus as we compared the two series of nanocomposites. The average flexural modulus of nanocomposites with LC epoxy (E-BP00ZL series) is always higher than that of nanocomposites without LC epoxy (E-BP07ZL series) with the same silica content (Fig. 4(b)). However, the difference between the two series is insignificant







Fig. 5. SEM images of fracture surface of cured nanocomposite resins containing (a) no LC resin (E-BP00ZL60), (b) 6.6 wt.% LC BP resin (E-BP07ZL60) and (c) higher magnification of detail (red square) from (b).

Fig. 4. Comparison of properties of cured nanocomposite resins without (E-BP00ZL series) and with LC resin (E-BP07ZL series): (a) hardness, (b) flexural modulus, (c) flexural strength and (d) water absorption. * denotes a significant difference (p < 0.05) between the two series.

(p > 0.05) until the modified silica content reaches 24.23 vol.% (38.61 wt.%). This confirms the formation of a percolation network in the nanocomposite. The flexural modulus of LC nanocomposites with 41.85 vol.% (58.59 wt.%) modified silica (E-BP00ZL60) is 9.60 ± 0.26 GPa, which is significantly higher than that of

nanocomposites without LC epoxy resin (E-BP00ZL60) at 8.05 ± 0.53 GPa. When the modified silica content is increased to 41.85 vol.% (58.59 wt.%), similar large increases in flexural modulus are observed. This increasing trend in the flexural modulus of nanocomposites containing LC epoxy resin (E-BP07ZL series) due to increased silica content was similar to that for hardness, as discussed above.

The flexural strength of LC epoxy nanocomposites exhibited different trends from those seen for flexural modulus and hardness. The flexural strength of LC epoxy nanocomposites increases with



Fig. 6. TEM images of cured nanocomposite resins containing (a) no LCBP resin (E-BP00ZL60) and (b) 6.6 wt.% LC BP resin (E-BP07ZL60).

increasing nanoparticle content up to 24.23 vol.% (38.61 wt.%) (Fig. 4(c)). This strength increased slightly from 99.51 ± 8.36 to 103.42 ± 12.56 then further to 103.35 ± 9.46 MPa when the content of modified silica of nanocomposite was increased from 10.33 to 24.23 and then to 41.85 vol.%, respectively. This observation demonstrates that the flexural strength of nanocomposites containing LC BP epoxy resin (E-BP07ZL series) is limited to \sim 100 MPa, which cannot be increased much further by incorporating greater than 10 vol.% of silica. As discussed previously, both the flexural strength and the flexural modulus of nanocomposites without LC epoxy resin BP continuously increase as the silica content increases. Therefore, crystalline BP can increase the flexural modulus of nanocomposites but may limit any increase in flexural strength despite the incorporation of additional silica. This result suggests a unique new microstructure inside nanocomposites containing LC epoxy resin (E-BP07ZL series) when silica is incorporated into the organic matrix containing LC BP resin (E-BP07).

We used SEM and TEM to study the differences in morphology between nanocomposites with and without LC BP resin. The nanocomposite containing 41.85 vol.% silica but no LC BP resin (E-BP00ZL60) has a rough fracture surface that follows the contour of silica (Fig. 5(a)), whereas the layer structure of crystalline BP is observed on the fracture surface of the nanocomposite (E-BP07ZL60) with the same amount of silica and LC BP resin (Fig. 5(b)). We refer to this unique microstructure as "nanoparticle-embedded layers" (NPEL). The nanoparticles and layers support each other, leading to abnormally high hardness and flexural modulus for the LC resin composites with nanoparticle contents above the percolation threshold (>24 vol.%). The same reinforcement was not observed in the flexural strength of nanocomposites containing LC BP epoxy resin (E-BP07ZL series). We speculate that a heteroge-



Fig. 7. Comparison of properties of cured LC nanocomposite resin (E-BP07ZL60) and cured commercial restorative composite resins (CMR1, CMR2, CMR3, CMR4): (a) hardness, (b) flexural modulus, (c) flexural strength and (d) water absorption. * denotes that a significant difference (p < 0.05) is achieved compared with the nanocomposite epoxy resin containing LC resin (E-BP07ZL60).

neous microstructure is formed in the LC epoxy nanocomposite of the E-BP07ZL60 sample (Fig. 5(c)). This unique microstructure resulted from the large amount of silica, which induces the formation of NPEL in BP LC epoxy resin containing nanocomposite; the layer



Fig. 8. Comparison of post-gelation shrinkage behavior of composite resins: (a) amount of post-gelation shrinkage and (b) shrinkage strain curves. (c) Photo-DSC curves of epoxy resins with (E-BP07) and without (E-BP00) LC resin. * denotes that a significant difference (p < 0.05) is achieved compared to the nanocomposite resin containing no LC resin (E-BP00ZL60).

structure of neat BP crystalline domains is distinct from the nanoparticle domains. The failure of flexural strength apparently occurs at these NPEL; this heterogeneous structure may limit flexural strength improvements in nanocomposites with even higher silica nanoparticle contents. This hypothesis was further confirmed by the TEM images of LC epoxy nanocomposite sample (E-BP00ZL60) (Fig. 6(a)), which show that epoxy modified silica nanoparticles are well dispersed in the resin matrix. The NPEL microstructure is clearly visible (Fig. 6(b)), as the silica was embedded in the LC epoxy resin to create the layer structure. Therefore, when the BP crystalline layer undergoes large-scale deformations, such as bending, it will peel off, leading to fracture (Fig. 5(c)). Despite its limitation of flexural strength, crystals obtained from LC BP resin not only contribute higher flexural modulus but also improve the physical and chemical properties of nanocomposites compared to nanocomposites without LC BP.

The water absorption of nanocomposites with and without LC epoxy resin decreased with increasing silica content because the solid silica nanoparticles incorporated into the organic matrix are dense and nonabsorbent. Nanocomposites with LC epoxy resin (E-BP07ZL series) exhibit lower water absorption than nanocomposites without LC epoxy resin (E-BP00ZL series). A hydrophobic phenyl structure and dense crystalline BP were incorporated into the organic matrix, resulting in lower water absorption. The difference between the two series of nanocomposites was not significant (p > 0.05) because the BP content was relatively low (6.60 wt.%). When the silica content increased, the difference in water absorption between the two series of nanocomposites was larger and significant (p < 0.05), due to the presence of low-water-absorption BP resin.

The CTE of the nanocomposites was evaluated using a thermal mechanical analyzer. The CTE of the organic matrix (E-BP00) decreased by 22.21% by adding 6.60 wt.% LC BP epoxy resin to the conventional epoxy resin ECH (E-BP07). The decrease in CTE is due to the restriction of thermal motion resulting from the incorporation of layered BP crystalline. However, the difference in CTE between the two series of nanocomposites with and without LC epoxy resin decreases after the incorporation of silica. The decreased CTE was reduced to 15.91% when the silica content was increased to 41.85 vol.% because the size of crystalline BP in nanocomposites decreases after the addition of silica nanoparticles.

4.3. Performance comparison of LC resin-based nanocomposites and commercial resin-based composites

To evaluate the potential application of LC resin-based nanocomposites (E-BP07ZL60 formulation) for dental restoration, the properties of this LC epoxy nanocomposite were compared to those of commercial methacrylate resin-based dental restorative materials (CMRs). CMR4 performed best in hardness, flexural strength, flexural modulus (Fig. 7) and CTE, but displayed the highest water absorption among the CMRs. Compared with the CMRs, the LC epoxy nanocomposite exhibited a hardness (69.50 ± 1.24 VHN)



Fig. 9. Results of (a) MTT and (b) LDH assays of MG-63 cells on extracts of cured dental restorative composite resins for 7 days. * denotes a significant difference (*p* < 0.05) compared to extracts of cured nanocomposite resin without LC resin (E-BP00ZL60).

significantly higher than for CMR1 and CMR2 but similar to CMR3 and significantly lower than CMR4 (100.58 ± 3.49 VHN). In general, the mechanical properties of resin-based composites are greatly influenced by their inorganic content. According to the TGA results of the commercial dental restorative resins (Table 1), CMR2 and CMR4 have almost the same inorganic filler content, but CMR4 exhibits much higher hardness compared to CMR2 (54.96 ± 1.75 VHN). This indicates that other factors, such as the type and composition of the fillers and the chemical structures of the monomers and oligomer, may dominate the performance of resin-based composites. The flexural strength of LC epoxy nanocomposite is limited to ~ 100 MPa, which is slightly lower than that of CMRs due to the formation of the heterogeneous NPEL microstructure, which cannot tolerate large-scale deformation and leads to lower flexural strength than for the homogeneous structure. Nevertheless, this value (103.35 ± 9.46 MPa) was not statistically significantly diffeent from those of CMR1 (114.10 ± 6.50 MPa) and CMR2 (114.81 ± 10.14 MPa). The flexural modulus of the LC epoxy nanocomposite was 9.60 ± 0.26 GPa, which is higher than those of CMR1 and CMR3 but lower than those of CMR2 and CMR4. The CTE of the LC epoxy nanocomposite was lower than those of CMR1 and CMR2 but higher than those of CMR3 and CMR4. As discussed previously, the water absorption of the LC epoxy nanocomposite was reduced by 21.37% compared to the nanocomposite without LC BP epoxy resin, due to the presence of hydrophobic BP resin. The water absorption of the LC epoxy nanocomposite is significantly lower than that of CMR4 (p < 0.05) but higher than all the other CMRs. The reason why this epoxy nanocomposite has such high water adsorption is not clear, but we speculate that it is caused by two main factors. First, it contains relatively low loading of silica nanoparticles (~58 wt.%) and relatively polar epoxy resin. For light-cured epoxy resins, unreacted epoxy functional groups can increase the uptake of water in a water absorption test [23]. Furthermore, both the ether-linked main chain of photopolymerized epoxy resins and the polar carbonyl group of ECH contribute high water absorption to ECH-based nanocomposites. By the same token, the high water absorption of CMR4 may be due to the ether-linked main chain oligomer of polyethylene glycol dimethacrylate (PEGDMA) in the resin composition (Table 1).

4.4. Post-gelation shrinkage of resin-based composites

The total volume shrinkage of epoxy resins was expected to be lower than that of methacrylate resins, as discussed in the introduction [23,24]. The total volume shrinkage results from two stages of polymerization: pre-and post-gelation. Usually, a violent contraction force between the resin and the tooth cavity occurs after gelation due to a substantial increase in the elastic modulus of resin [30,36]. To avoid gap formation between the resin and the cavity, post-gelation shrinkage should be minimized. Using a strain gauge, post-gelation shrinkage is monitored by finding the peak of the strain curve, which indicates the starting point of physical contraction and chemical gelation. The post-gelation shrinkage of our resins can be calculated from the peak of the strain curve to its leveling-off point at \sim 185.00 s. Fig. 8(a) shows epoxy resinbased nanocomposites without LC epoxy resin (E-BP00ZL60) and with LC epoxy resin (E-BP07ZL60), which exhibited significantly lower (p < 0.05) post-gelation shrinkage than all commercial resins. The free volume reduction was reduced during polymerization by replacing the methacrylate linear structure with the epoxy ring structure. The LC epoxy resin BP-containing nanocomposite (E-BP07ZL60) further lowered volume shrinkage, by 18.97% compared with nanocomposite containing no BP (E-BP00ZL60). A suppressed strain curve for E-BP07ZL60 was observed compared with that of E-BP00ZL60 during visible light irradiation (from 5.00 to 45.00 s; Fig. 8(b)). This observation demonstrates that the nanocomposite

containing LC BP resin (E-BP07ZL60) has a lower contraction rate after gelation than the nanocomposite containing no BP (E-BP00ZL00). This result may be caused by another mechanism that occurs during the curing of E-BP07ZL60. Fig. 3(e) and (f) shows that both the number and size of crystalline BP decreased after light curing. These results indicate that partial melting of crystalline BP may occur due to heat generation during polymerization. Melting is an order-to-disorder transition that induces volume expansion, and the total volume shrinkage of composites is significantly reduced by it. Moreover, the gel point of E-BP07ZL60 curing takes place before that of E-BP00ZL60 (Fig. 8(b)) under the same curing process, which means that E-BP07ZL60 was cured faster than E-BP00ZL60. The cure behaviors of these two resin systems were further investigated by photo DSC, as shown in Fig. 8(c). The photopolymerization of epoxy resin is exothermic. The onset and the exothermic peak of organic matrix containing liquid crystal epoxy resin (E-BP07) preceded those of matrix without LC epoxy resin (E-BP00). These curves confirm that the LC epoxy resin BP accelerated the curing of LC epoxy nanocomposite (E-BP07ZL60), as discussed in the first paragraph of the Discussion.

4.5. Cytotoxicity of restorative composites

The cytotoxicity of composites was used to determine their biocompatibility. Both MTT and LDH assays were used to evaluate their cytotoxicity by incubation of the composite extracts with a human osteosarcoma cell line (MG-63) for 7 days. After 1 day, the MG-63 cells cultured with the extract of commercial resin 1 (exD-CMR1) and blank (72iD) converted more MTT into violet formazan than the other samples. The absorbance of MTT gradually increased as the MG-63 cells proliferated with the exD-CMR1 and 72iD with time. Compared with the extracts of the LC epoxy nanocomposite (exD-E-BP00ZL60) and the extracts of all the other CMRs, exD-E-BP07ZL60 shows almost no difference in MTT absorbance. This result indicates that the cytotoxicity of the LC epoxy nanocomposite is comparable to that of commercial resin-based dental restorative materials.

The cytotoxicity of resin-based composites was further evaluated using an LDH assay. MG-63 cell death in the conditioning medium containing the extract of the cured composite was quantitatively determined by measuring the OD of NADH in the condition medium after 1, 3 and 7 days of incubation. An increase in the OD value indicates an increase in cell death (Fig. 9(b)). There was no significant difference in LDH assay observed among the extracts of the LC epoxy nanocomposite (exD-E-BP07ZL60) and the commercial resins (except CMR1) after culturing for 1, 3 or 7 days. The NADH OD value of the extract of the epoxy resin nanocomposite (exD-E-BP00ZL60) was significantly higher than that of the extract of the LC epoxy nanocomposite (exD-E-BP07ZL60) at day 7; this demonstrates that the incorporation of LC BP inhibits cell death caused by unreacted ECH epoxy resin. The low water absorption and low CTE characteristics of the LC epoxy nanocomposite (E-BP07ZL60) suggest that the dense arrangement of molecules obtained from the NPEL microstructure are tied to low cytotoxicity.

5. Conclusions

In this study, we have demonstrate that the physical and chemical properties of epoxy resin-based dental restorative composite can be greatly improved by incorporation of the LC epoxy resin BP into the nanocomposite. The crystalline BP resin of LC epoxy nanocomposite resin (E-BP07ZL60) provides a favorable mechanism of order-to-disorder transition that reduces its post-gelation shrinkage. A unique microstructure of NPEL is formed at a silica content of 42 vol.%, which results in an unusually large increase

in performance of the nanocomposite. The addition of 6.60 wt.% hydrophobic and dense BP in the organic matrix of the nanocomposites (E-BP07ZL series) improves performance with regard to hardness, flexural modulus, water absorption and CTE compared with those of the nanocomposite containing no BP (E-BP00ZL series). The flexural strength of LC epoxy nanocomposites is limited to \sim 100 MPa due to the formation of neat BP layered crystalline without reinforcing nanoparticles. Both the ring-opening reaction and the order-to-disorder transition of LC epoxy nanocomposite result in a large reduction in post-gelation shrinkage by 50.57% compared with the methacrylate resin-based materials. Overall, LC epoxy nanocomposite (E-BP07ZL60) performs comparably with regard to physical and chemical properties, as well as cytotoxicity, to those of commercial methacrylate resin-based materials, which demonstrates the potential usage of LC epoxy resins for low shrinkage and high performance dental restorative materials.

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Appendix A. Figures with essential color discrimination

Certain figures in this article, particularly Figs. 2–5 and 7–9, are difficult to interpret in black and white. The full color images can be found in the on-line version, at http://dx.doi.org/10.1016/j.actbio.2012.07.030.

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.actbio.2012.07. 030.

References

- Feilzer AJ, Degee AJ, Davidson CL. Curing contraction of composites and glassionomer cements. J Prosthet Dent 1988;59:297–300.
- Walls AWG, McCabe JF, Murray JJ. The polymerization contraction of visiblelight activated composite resins. J Dent 1988;16:177–81.
 Ciucchi B, Bouillaguet S, Delaloye M, Holz J. Volume of the internal gap formed
- [3] Ciucchi B, Bouillaguet S, Delaloye M, Holz J. Volume of the internal gap formed under composite restorations in vitro. J Dent 1997;25:305–12.
- [4] Ge JH, Trujillo M, Stansbury J. Synthesis and photopolymerization of low shrinkage methacrylate monomers containing bulky substituent groups. Dent Mater 2005;21:1163–9.
- [5] Kim Y, Kim CK, Cho BH, Son HH, Um CM, Kim OY. A new resin matrix for dental composite having low volumetric shrinkage. J Biomed Mater Res B Appl Biomater 2004;70B:82–90.
- [6] Atai M, Ahmadi M, Babanzadeh S, Watts DC. Synthesis, characterization, shrinkage and curing kinetics of a new low-shrinkage urethane dimethacrylate monomer for dental applications. Dent Mater 2007;23:1030–41.
- [7] Chen CY, Huang CK, Lin SP, Han JL, Hsieh KH, Lin CP. Low-shrinkage visiblelight-curable urethane-modified epoxy acrylate/SiO₂ composites as dental restorative materials. Compos Sci Technol 2008;68:2811–7.
- [8] He JW, Luo YF, Liu F, Jia DM. Synthesis and characterization of a new trimethacrylate monomer with low polymerization shrinkage and its application in dental restoration materials. J Biomater Appl 2010;25:235–49.

- [9] Kim LU, Kim JW, Kim CK. Effects of molecular structure of the resins on the volumetric shrinkage and the mechanical strength of dental restorative composites. Biomacromolecules 2006;7:2680–7.
- [10] Satsangi N, Rawls HR, Norling BK. Synthesis of low-shrinkage polymerizable liquid-crystal monomers. J Biomed Mater Res B Appl Biomater 2004;71B:153-8.
- [11] Buruiana T, Melinte V, Costin G, Buruiana EC. Synthesis and properties of liquid crystalline urethane methacrylates for dental composite applications. J Polym Sci Pol Chem 2011;49:2615–26.
- [12] Liu WW, He XP, Mo AC, Yao QQ, Ye J, Jing N. Investigation of the mechanical properties of a low-shrinkage liquid crystalline matrix combined with nanohydroxyapatite. Int J Nanomed 2011;6:1787–91.
- [13] Moszner N, Salz U. New developments of polymeric dental composites. Prog Polym Sci 2001;26:535–76.
- [14] Harada M, Watanabe Y, Tanaka Y, Ochi M. Thermal properties and fracture toughness of a liquid-crystalline epoxy resin cured with an aromatic diamine crosslinker having a mesogenic group. J Polym Sci Pol Phys 2006;44: 2486–94.
- [15] Harada M, Sumitomo K, Nishimoto Y, Ochi M. Relationship between fracture toughness and domain size of liquid-crystalline epoxy resins having polydomain structure. J Polym Sci Pol Phys 2009;47:156–65.
- [16] Barclay GG, Ober CK, Papathomas KI, Wang DW. Liquid-crystalline epoxy thermosets based on dihydroxymethylstilbene – synthesis and characterization. J Polym Sci Pol Chem 1992;30:1831–43.
- [17] Su WFA. Thermoplastic and thermaoset main-chain liquid-crystal polymer prepared from biphenyl mesogen. J Polym Sci Pol Chem 1993;31:3251–6.
- [18] Su WFA, Chen KC, Tseng SY. Effects of chemical structure changes on thermal, mechanical, and crystalline properties of rigid rod epoxy resins. J Appl Polym Sci 2000;78:446–51.
- [19] Mititelu-Mija A, Cascaval CN. Liquid crystalline epoxy azomethine thermoset. High Perform Polym 2007;19:135–46.
- [20] Harada M, Ochi M, Tobita M, Kimura T, Ishigaki T, Shimoyama N, et al. Thermal-conductivity properties of liquid-crystalline epoxy resin cured under a magnetic field. J Polym Pol Phys 2003;41:1739–43.
- [21] Kato T, Nakamura T, Agari Y, Ochi M. Relation between thermal conductivity and network formation with polymerizable liquid crystals. J Appl Polym Sci 2007;103:3169–74.
- [22] Odian G. Principles of polymerization. 4th ed. New York: Wiley-Interscience; 2004.
- [23] Tilbrook DA, Clarke RL, Howle NE, Braden M. Photocurable epoxy-polyol matrices for use in dental composites I. Biomaterials 2000;21:1743–53.
- [24] Weinmann W, Thalacker C, Guggenberger R. Siloranes in dental composites. Dent Mater 2005;21:68–74.
- [25] Chen MH, Chen CR, Hsu SH, Sun SP, Su WF. Low shrinkage light curable nanocomposite for dental restorative material. Dent Mater 2006;22:138–45.
 [26] Ilie N, Jelen E, Clementino-Luedemann T, Hickel R. Low-shrinkage composite
- for dental application. Dent Mater J 2007;26:149–55.
- [27] Su WF, Huang HW, Pan WP. Thermal properties of rigid rod epoxies cured with diaminodiphenylsulfone and dicyandiamide. Thermochim Acta 2002;392:391–4.
- [28] Chen S, Hsu SH, Wu MC, Su WF. Kinetics studies on the accelerated curing of liquid crystalline epoxy resin/multi-walled carbon nanotube nanocomposites. J Polym Pol Phys 2011;49:301–9.
- [29] Hsu SH, Wu MC, Chen S, Chuang CM, Lin SH, Su WF. Synthesis, morphology and physical properties of multi-walled carbon nanotube/biphenyl liquid crystalline epoxy composites. Carbon 2012;50:896–905.
- [30] Sakaguchi RL, Versluis A, Douglas WH. Analysis of strain gage method for measurement of post-gel shrinkage in resin composites. Dent Mater 1997;13:233-9.
- [31] Atai M, Watts DC, Atai Z. Shrinkage strain-rates of dental resin-monomer and composite systems. Biomaterials 2005;26:5015–20.
- [32] Pappas SP. UV curing: science and technology. Norwalk, CT: Technology Marketing Corporation; 1978.
- [33] Vaia RA, Giannelis EP. Polymer nanocomposites: status and opportunities. MRS Bull 2001;26:394–401.
- [34] Lin C-C, Chang K-H, Lin K-C, Su W-F. In situ probe nanophase transition in nanocomposite using thermal AFM. Compo Sci Technol 2009;69:1180–6.
- [35] Lin C-C, Hsu S-H, Chang Y-L, Su W-F. Transparent hydrophobic durable low moisture permeation poly(fluoroimide acrylate)/SiO(2) nanocomposite from solventless photocurable resin system. J Mater Chem 2010;20:3084–91.
- [36] Berge HX, Sakaguchi RL. Strain-rate, temperature and hardness measurements of an autocuirng composite. J Den Res 1995;74:183.