

# Nanogel-Based Filler-Matrix Interphase for Polymerization Stress Reduction

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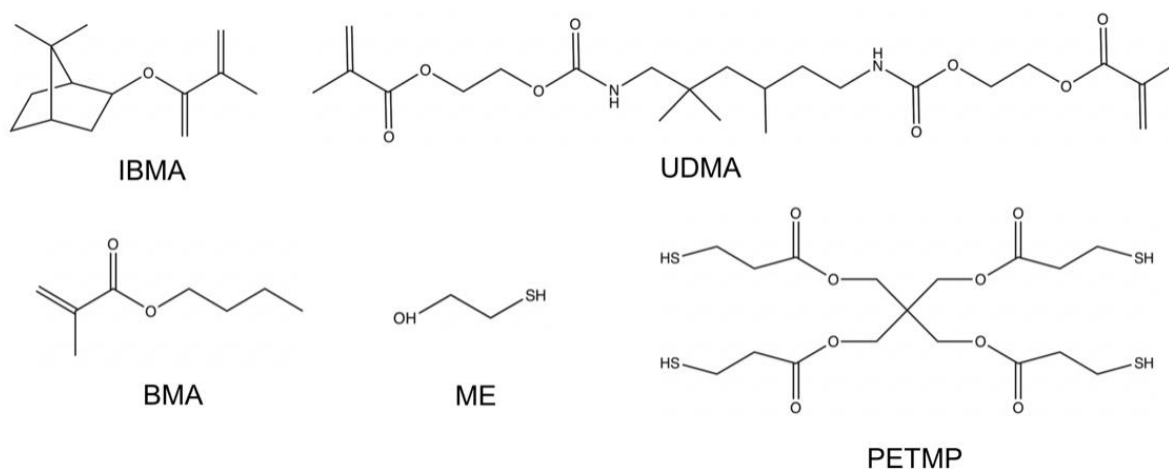
## Appendix

### Materials and Methods

#### *Nanogel characterization*

Appendix Figure 1 shows the chemical structures of the monomers (all from Esstech, USA) used in the nanogel syntheses.

Polymeric nanogels' number average molecular weight ( $M_n$ ), polydispersity index (PDI), and hydrodynamic radius ( $R_h$ ) were characterized using gel permeation chromatography (GPC; Viscotek, Malvern Instruments, UK) with triple detectors (refractive index, right angle light scattering, and differential viscometer) with tetrahydrofuran (EMD Millipore, USA) used as mobile phase. GPC calibration was based on a series of linear polystyrene standards of known molecular weight and dispersity.



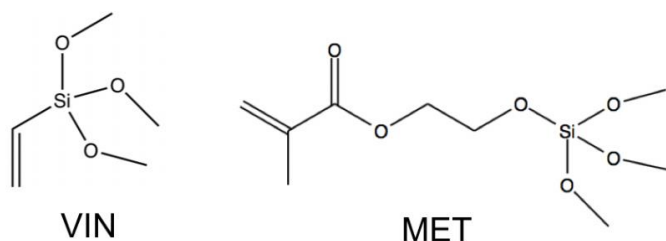
**Appendix Figure 1.** Chemical structures of the monomers used in the nanogel syntheses: mono-functional monomers isobornyl methacrylate (IBMA) and butyl methacrylate (BMA); di-

functional crosslinker urethane dimethacrylate (UDMA); 2-mercaptoethanol (ME), and pentaerythritol tetra(3-mercaptopropionate) (PETMP).

T<sub>g</sub> of nanogel powders (n = 2) was determined by dynamic mechanical analyzer (DMA; Perkin Elmer 8000, USA) by sandwiching 10 mg of nanogel in a thin metallic pocket that was then subjected to single cantilever cyclic displacement of 50 μm at 1 Hz. The nanogel was heated from 0 to 150 °C with tan δ data collected in the second cycle of heating at 2 °C/min in air. Testing of nanogel samples dispersed in tetrahydrofuran with Ellman's reagent (5,5'-dithio-bis-(2-nitrobenzoic acid)) and N,N-diisopropylethylamine as catalyst was used to quantify the free sulfhydryl group concentration. After stirring for 15 min, ultraviolet–visible spectroscopy (Evolution 300; Thermo-Fischer Scientific) was used to measure the absorption at 412 nm and by comparison with a standard curve, thiol functionality concentration of nanogels was determined.

#### *Filler surface treatment*

For both silanes (Appendix Figure 2), i.e. trimethoxyvinylsilane (VIN; Sigma Aldrich, USA) and methacrylate γ-methacryloxypropyltrimethoxy silane (MPS; Sigma Aldrich), fillers were silanized with 5 wt% silane (relative to fillers) in cyclohexane using η-propylamine at 2 wt% as catalyst. The mixture was stirred at room temperature for 30 min and then at 60±5 °C for additional 30 min at atmospheric pressure and then placed in a rotary evaporator at 60 °C for the removing of the solvent and the volatile byproducts. The powder was then heated at 95±5 °C for 1 h on the rotary evaporator and finally was dried at 80 °C in a vacuum oven for 23 h. Extensive solvent washing with acetone was performed to remove physically adsorbed silane with TGA used to verify complete removal of unbound silane.



**Appendix Figure 2.** Chemical structures of the silanes: trimethoxyvinylsilane (VIN) and  $\gamma$ -methacryloxypropyltrimethoxysilane (MPS).

### *Filler surface characterization*

Filler treatments were identified by diffuse reflectance Fourier transform infrared spectroscopy (DR-IR; Nicolet 6700, Thermo Scientific, USA) spectroscopy. Spectroscopic grade KBr and filler powders were ground together and placed in the DR-IR accessory sample slide. Spectra were taken at  $8\text{ cm}^{-1}$  resolution, 64 scans, from  $4000$  to  $1350\text{ cm}^{-1}$  range, using KBr as background.

Thermogravimetric analysis (TGA; Pyris 7 TGA, Perkin Elmer, USA) was used to determine the amount of nanogel deposited on the surface of treated fillers. Samples ( $5\pm 1\text{ mg}$ ) were placed in platinum pans in nitrogen atmosphere flow of  $20\text{ ml/min}$  and heated from  $50\text{ }^{\circ}\text{C}$  to  $850\text{ }^{\circ}\text{C}$  with a heating rate of  $10\text{ }^{\circ}\text{C min}^{-1}$ . The mass loss as a function of temperature was recorded.

Energy dispersive X-ray spectrometry (EDX; Oxford Instruments SDD X-MaxN) analysis coupled to a scanning electron microscope (JSM IT 300, JEOL, Japan) was used to identify the elemental composition of filler surface treatment. Fillers were placed on stubs and sputter-coated with carbon. Each spectrum was acquired for  $300\text{ s}$  (voltage  $15\text{ kV}$ , working distance  $10\text{ mm}$ ). Images showing the identified chemical elements and their relative concentration were obtained from five different spectra of each material at different locations on the stub.

Filler surface images were obtained by transmission electron microscopy (TEM; JEM 2100, JEOL, Japan). Fillers were dispersed in acetone, then few mL of the solution were dropped in formvar grids and TEM images were then obtained. Images were obtained at magnification of 300K and 500K $\times$ .

### *Resin and composites testing*

#### *Polymerization kinetics*

Real-time polymerization kinetics was monitored by Fourier transform near-infrared spectroscopy (FT-NIR, Nicolet 6700) in specimens (n=3) of 6 mm in diameter and 0.8 mm thick laminated between two glass slides. Specimens were light-activated for 20 s at an incident irradiance of 1470 mW/cm<sup>2</sup> at 430-480 nm wavelength (Elipar DeepCure-S LED, 3M ESPE, USA). The area of the methacrylate vinyl absorbance band centered at 6165 cm<sup>-1</sup> (Stansbury and Dickens 2001) was used to follow the polymerization reaction. Measurements were taken at a wavenumber resolution of 4 cm<sup>-1</sup> with 32 scans per spectrum acquired for static scans before and after polymerization to measure degree of conversion (DC) and 2 scans per spectrum for dynamic measurements of polymerization kinetics, which provides a 2 Hz acquisition rate. Data was collected for 10 min during and continuing after curing light exposure. Polymerization rate ( $R_{Pmax}$ ) was calculated as the first derivative of the conversion vs. time curve.

#### *Polymerization stress*

Dynamic polymerization stress was evaluated with a tensometer (Volpe Research Center, American Dental Association). Glass rods (6 mm diameter) were sectioned into lengths of 28 and 5 mm. One surface of the 28-mm long rods was polished with a sequence of silicon carbide

abrasive paper felt disks in order to optimize the transmission of light through the rod end into the specimen during photoactivation. The opposite surfaces of the 28 and 5 mm rods were polished with 600 grit using abrasive papers to create a rough surface. Silane was applied to these surfaces. The rods were subsequently attached to the tensometer. The 28 mm rod were attached to the lower clamp and the 5 mm rods to the upper clamp. Composite was placed (6 mm x 1 mm) between the rods with a Centrix syringe. The tip of the light-curing unit was positioned in contact with the polished lower rod. Force development was monitored for 10 min from the beginning of light-activation, and the maximum nominal stress (MPa) was calculated by dividing the maximum force value recorded by the cross-sectional area of the rods. FT-NIR spectroscopy was simultaneously incorporated in direct transmission mode via fiber optic cables (1 mm diameter single fiber) to obtain the concomitant reaction kinetics profile correlated with stress evolution (Lu et al. 2004).

### *Volumetric shrinkage*

A constant volume of each material was placed onto an aluminum disc in a non-contact linear variable differential transducer-based linometer (Academic Center for Dentistry Amsterdam, The Netherlands). The material was covered with a glass slide, which was adjusted to produce a specimen disc (approximately 1 mm × 6 mm). Both resin and composite specimens were irradiated for 20 s through the glass slide. The displacement of the aluminum disk, caused by the lifting action of the material's shrinkage, was registered during and extending beyond the irradiation interval for a total period of 10 minutes. The linear shrinkage during polymerization was measured and converted to the corresponding volumetric shrinkage (de Gee et al. 1993), as follows:

$$lin\% = \frac{\Delta L}{L + \Delta L} \times 100\%$$

where  $\Delta L$  is the recorded displacement and  $L$  the thickness of the sample after polymerization. A thin film of silicon grease applied to the substrate surfaces promotes free shrinkage in the x/y directions as well as along the z axis, which allows the volumetric shrinkage to be derived from the linear shrinkage by:

$$vol\% = 3lin\% - 0.03(lin\%)^2 + 0.0001(lin\%)^3$$

### *Rheology*

Viscosity measurements of the nanogel resin blends (n=5) were performed using a cone-plate digital viscometer (CAP 2000, Brookfield, USA). A defined volume of the materials was tested at room temperature (23 °C) using a 14 mm diameter spindle 200 rpm and 1 Hz (hold time: 15 s; run time: 30 s). Rheology of the composites (n=2) was assessed in a photorheometer (ARES, TA Instruments, USA), with materials placed between two 20 mm parallel quartz disc plates and tested in shear at a frequency of 100 rad/s with 10 % strain (ensuring that the test was carried out within the linear viscoelastic regime), while being photopolymerized at 50 mW/cm<sup>2</sup> (mercury arc lamp 460 nm, Acticure 4000, EXFO, USA) for 10 min. An optical apparatus (Pfeifer et al. 2011) allowed both curing light and FT-NIR direct transmission access to the specimen within the photorheometer, in this way methacrylate conversion was followed concomitantly with modulus development.

### *Mechanical properties*

The three-point bending test was used to assess the flexural strength and flexural modulus. The bar specimens were prepared in dimensions of 2 mm x 2 mm x 25 mm according to ISO 4049 (Iso 4049. Dentistry - polymer-based restorative materials 2009). Specimens (n = 8) were fabricated between glass slides and polymerized with three overlapping 20 s light exposures each

glass side. Specimens were stored dry for 24 h in dark containers at room temperature. The three-point bending test was performed on the MTS testing machine using a span of 20 mm and a cross-head speed of 1 mm/min (MTS Mini Bionix II, MTS, Eden Prairie, MN, USA). The flexural strength (FS) in MPa was then calculated as:

$$FS (\sigma) = \frac{3F\iota}{2bh^2}$$

where F stands for load at fracture (N),  $\iota$  is the span length (20 mm), and b and h are the width and thickness of the specimens in mm, respectively.

The flexural modulus was determined from the slope of the initial linear part of stress–strain curve.

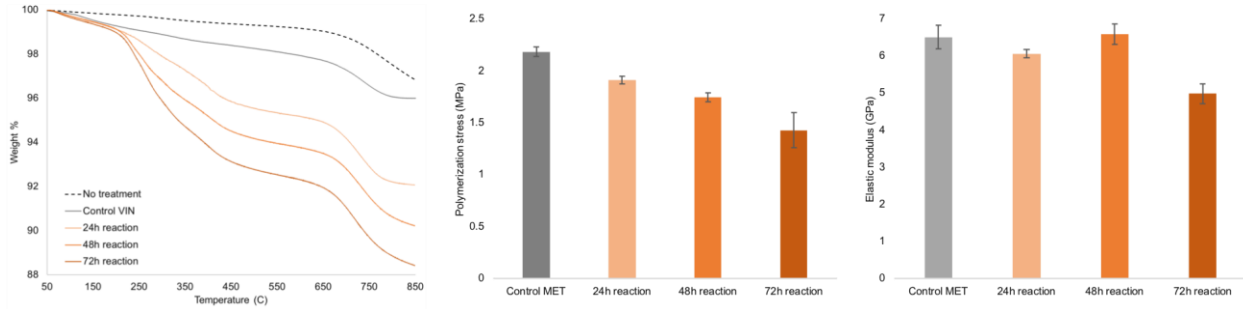
$$FM = \frac{F\iota^3}{4bh^3d}$$

where F is the load at some point on the linear region of the stress–strain curve, d the slack compensated deflection at load F, and  $\iota$ , b, and h are as defined above.

## Results

In a pilot study, fillers were reacted with nanogel (Ng 2) at the times of 24, 48 and 72 h in order to create different concentrations of nanogel on the surface (Appendix Figure 3). It is noticeable that polymerization stress progressively decreases with higher amount of nanogel attached to the surface, i.e. longer nanogel addition reaction times, providing a more complete nanogel layer on the surface as well as a higher thiol concentration (Appendix Table 2 and Appendix Figure 4). However, there may be a limit with respect to the final polymeric flexural modulus, as is presented in the plot that indicates at 72 h reaction, the modulus may be decreasing

as well. The coverage for 48 h is estimated by TGA to be 3 wt%, which would offer reasonable stress reduction with no compromise in the mechanical properties of the material.



**Appendix Figure 3.** TGA analysis of fillers reacted with nanogels for 24, 48 and 72 h, and its respectively composites results for polymerization stress and flexural modulus.

**Appendix Table 1.** Apparent element concentration for fillers with different surface treatments.

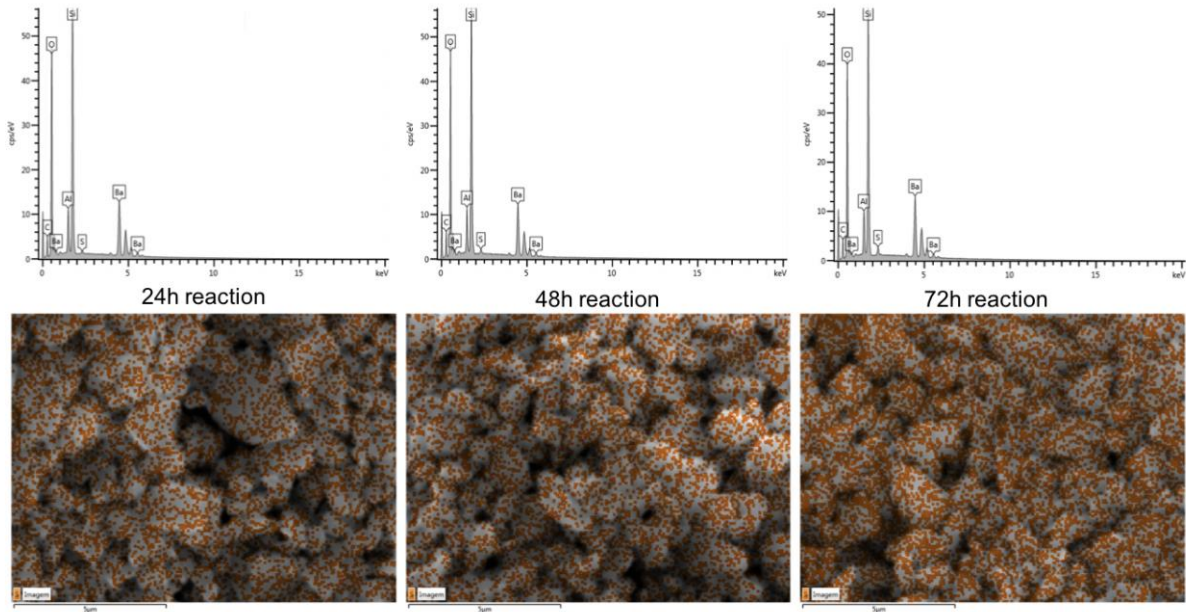
	No treatment	VIN silane	VIN Ng 1	VIN Ng 2	VIN Ng 3
C	4.6 (0.5)	6.3 (0.2)	7.0 (1.2)	4.4 (0.2)	7.7 (0.5)
O	96.6 (8.2)	128.8 (11.2)	114.2 (2.3)	64.5 (1.5)	103.0 (5.1)
Al	5.8 (5.8)	7.3 (0.4)	6.6 (0.3)	4.3 (0.1)	6.1 (0.2)
Si	33.4 (2.3)	40.8 (1.8)	36.5 (0.3)	25.3 (0.2)	34.0 (1.2)
Ba	56.0 (1.6)	55.8 (0.8)	51.1 (2.0)	47.5 (0.8)	47.7 (0.7)
S	-	-	0.4 (0.0)	0.6 (0.1)	0.7 (0.3)

**Appendix Table 2.** Apparent element concentration for fillers treated with nanogels for different reaction times.

	VIN Ng 2 24 h	VIN Ng 2 48 h	VIN Ng 2 72 h
C	5.4 (0.9)	4.4 (0.2)	5.8 (1.0)
O	93.0 (17.2)	64.5 (1.5)	109.5 (24.1)
Al	5.6 (0.8)	4.3 (0.1)	6.3 (0.9)
Si	32.2 (4.0)	25.3 (0.2)	35.4 (4.5)



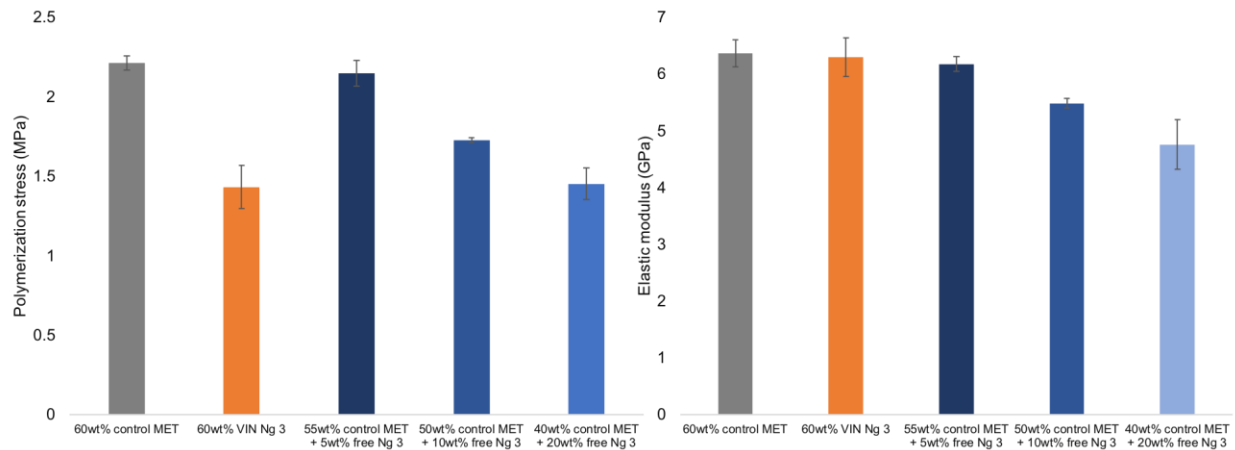
Ba	49.3 (1.4)	47.5 (0.8)	50.0 (0.2)
S	0.2 (0.0)	0.6 (0.1)	1.2 (0.3)



**Appendix Figure 4.** Elemental composition through EDX and S mapping for 24, 48 and 72 h nanogel addition reaction to the fillers.

## Discussion

Different loading of fillers and free nanogel (Ng 2) were evaluated, but always keeping the total loading of 60 wt% (Appendix Figure 5). When an equivalent portion (in terms of weight fraction not volume fraction) of the glass filler is replaced by nanogel at 5 wt%, it still presents similar PS and FM as the MPS control. At free nanogel loading levels of 10 and 20 wt%, the PS is decreased while the FM is also reduced as expected with the lower inorganic filler content. This demonstrates that the interphase created at the filler surface with minimum amount of nanogel (3 wt%) has the potential to significantly reduce the PS without compromising the FM, and this effect is not observed when similar amount of free nanogel (5 wt%) is added to the resin.



**Appendix Figure 5.** Polymerization stress and flexural modulus of composites with different filler and free nanogel loadings.

## Appendix References

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