

# Dual-Functionality Evaluation of a Novel Collagen Crosslinking Resin

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## APPENDIX

### Materials and methods

#### General

All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless stated otherwise. Mega Natural Gold grapeseed extract (GSE, contains >90% proanthocyanidin, rich in oligomers of flavonoids with 80% of  $n = 2\sim 7$ ) was donated by the manufacturer (Polyphenolics, Madera, CA, USA). Collagenase (type I, from *Clostridium histolyticum*,  $\geq 125$  U/mg) solution was made at 0.1% (w/v) in TESCA buffer (50-mM N-tris (hydroxymethyl) methyl-2-aminoethanesulfonic acid, 0.36-mM  $\text{CaCl}_2$ , pH = 7.4). Non-carious human molars were collected under a protocol approved by the University of Missouri-Kansas City Adult Health Sciences Institutional Review Board (IRB). Extracted teeth were stored at 4°C in 0.96% (w/v) phosphate-buffered saline containing 0.002% sodium azide.

#### Synthesis and characterization of MAGSE

Grapeseed extract 2.0 g was suspended in N,N-dimethylformamide/triethylamine at 0 °C. Methacryloyl chloride (10 eq) was added dropwise. The reaction mixture was stirred for another 1 h at 0 °C, then stirred at room temperature overnight. The crude product was then rinsed with water and ethyl acetate, purified with silica gel column chromatography. The  $^1\text{H-NMR}$  spectra of methacrylate functionalized grapeseed extract (MAGSE) and GSE were recorded with a Varian Inova 400 MHz NMR spectrometer. Deuterated dimethyl sulfoxide (DMSO- $\text{D}_6$ ) was used as the solvent. Fourier transform infrared (FT-IR) spectra of MAGSE and GSE were performed at 4  $\text{cm}^{-1}$  resolution with a Spectrum One FT-IR Spectrometer (PerkinElmer, Waltham, MA, USA) equipped with an attenuated total reflectance (ATR) attachment.

#### Preparation, demineralization, crosslinking and FTIR analysis of dentin films

Each dentin film was demineralized with 10 % phosphoric acid for 30 min, rinsed in deionized (DI) water for 10 s, and then spread on a plastic cover slip (Fisher Scientific, Pittsburgh, PA, USA) with the aid of a fine paintbrush. After blotting away the excessive water, a small drop of 1% MAGSE (in 50/50 ethanol/water by volume) was immediately applied to cover the entire 5 × 5 mm demineralized dentin film. After 30 s, the film was rinsed with solvent and immersed in copious deionized water for 30 min (change DI water every 10 min) to thoroughly remove residual treatment solution. Additional films received no MAGSE treatment were served as the control. After being air-dried overnight, the films were subjected to FTIR analysis performed at 4  $\text{cm}^{-1}$  resolution with a Spectrum One FT-IR Spectrometer (PerkinElmer, Waltham, MA, USA)

equipped with an attenuated total reflectance (ATR) attachment. The band intensities at 1235 cm<sup>-1</sup> (amide III) and 1450 cm<sup>-1</sup> (CH<sub>2</sub> scissoring) were determined following a two-point baseline correction and band area integration, and the band ratio (A1235/A1450) was calculated.

### **Preparation, acid etching and crosslinking of dentin slabs**

The abraded surfaces of the slabs were etched with 35% phosphoric acid gel (Scotchbond Etchant, 3M-ESPE, St. Paul, MN, USA) for 15 s and rinsed with water for 10 s. The slabs were pooled, randomly selected, and treated with 1% MAGSE for 30 s after being blot-dried. Upon completion of treatment, the slabs were immediately submerged in a copious amount of de-ionized water and then rinsed for an additional 30 min, with water changed every 10 min. Additional slabs received no MAGSE treatment and served as the control. For each group, at least 6 slabs were prepared, 3 of which were not subject to collagenase digestion, whereas the other 3 underwent 1 hour of digestion, respectively, at 37°C in 1 mL of 0.1 % collagenase solution.

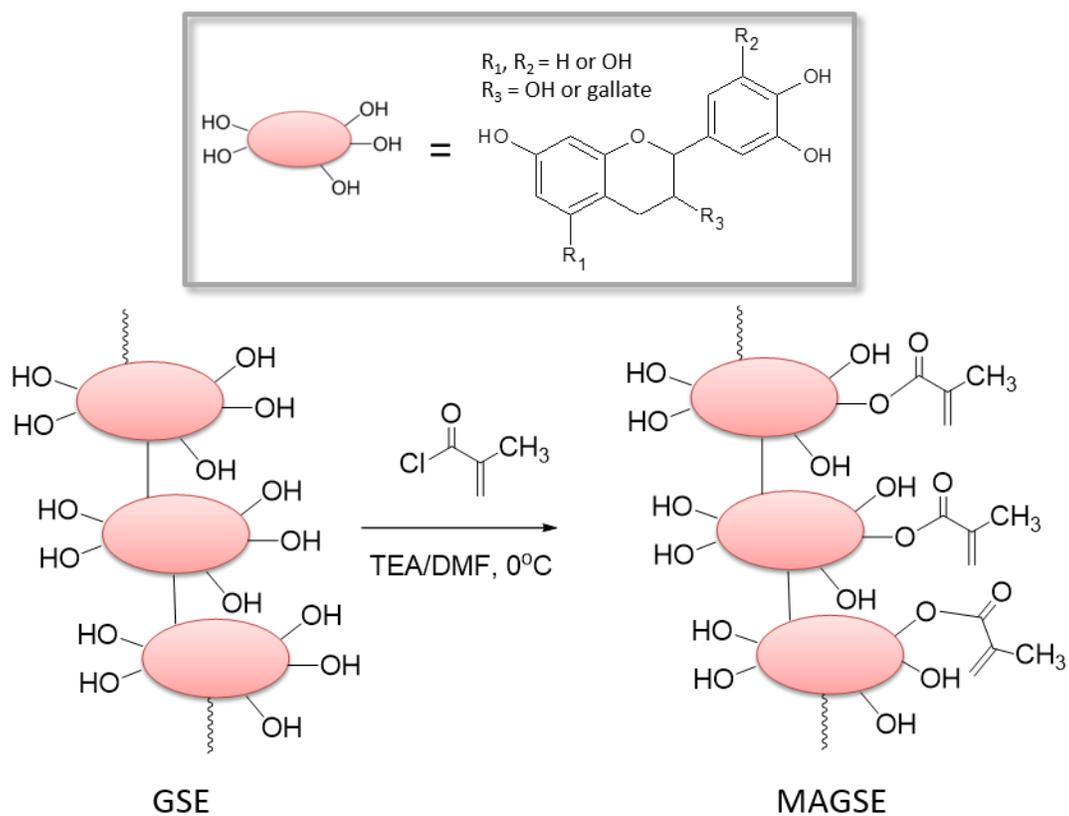
### **Light-curing and real-time FTIR spectral collection for degree of conversion and polymerization rate**

Light curing of each group was monitored real-time by FT-IR (Spectrum One, equipped with an attenuated total reflectance (ATR) attachment, PerkinElmer, Waltham, MA, USA.). The spectra were recorded at a resolution of 4 cm<sup>-1</sup> in the wave-number range between 650 and 4000 cm<sup>-1</sup>. A small volume of adhesive sample was dropped on the horizontal top plate of the ATR crystal, and a transparent plastic cover slip (Fisher Scientific, Pittsburgh, PA, USA) was carefully placed over the adhesive. Time-based spectra collection software (Spectrum TimeBase, Perkin-Elmer, Waltham, MA, USA) was used for continuous and automatic acquisition of spectra at time intervals of 0.4–0.6 s. Each data collection run started with the acquisition of around 50 spectra for the adhesive sample at the uncured state, followed by the photo-curing with a conventional dental light (Spectrum Light, Dentsply, Milford, DE, USA) at the power output of 600 mW/cm<sup>2</sup> for an exposure time of 60 s, and continued for a total of 10 min. When curing, the distance between the curing light and cover slip was kept at approximately 2 mm. Degree of conversion (DC) was calculated by the following equation:

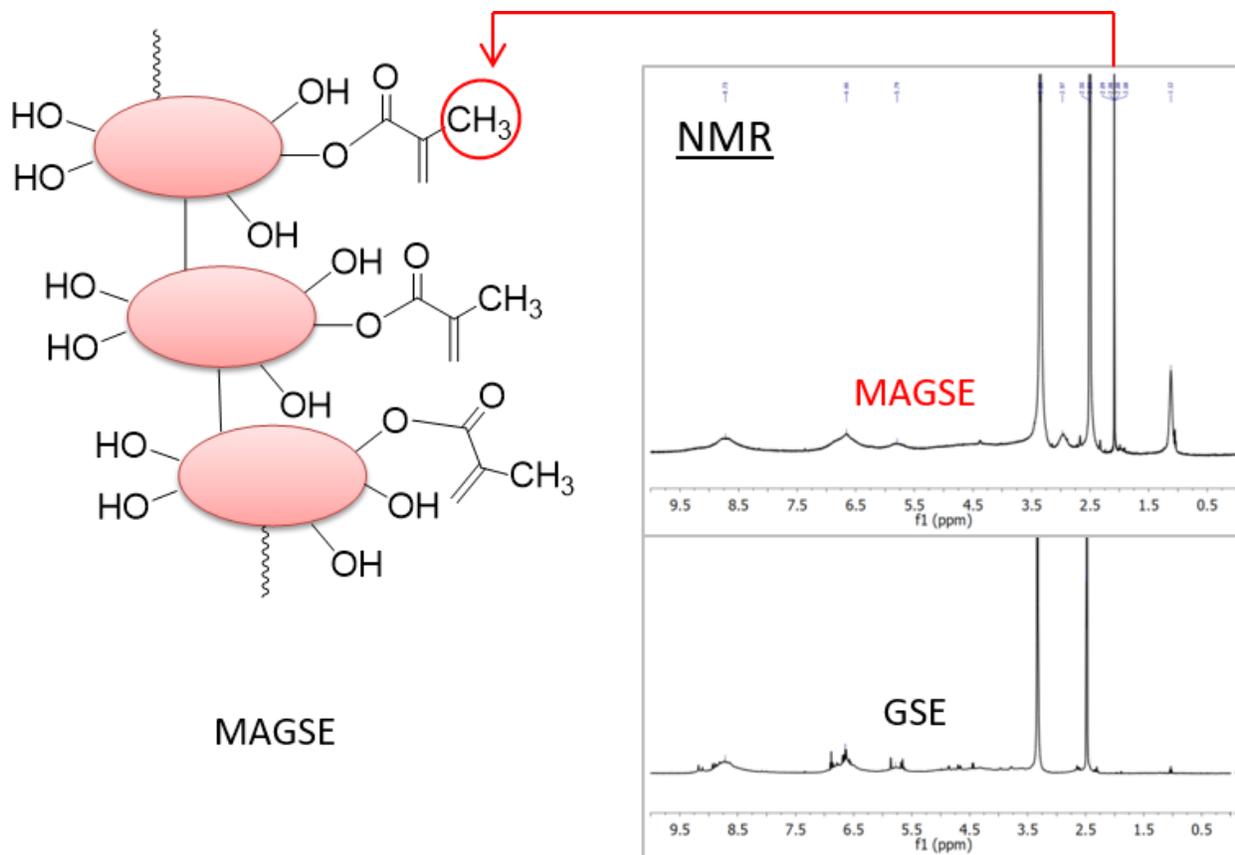
$$DC = \left( 1 - \frac{Absorbance_{1637\text{ cm}^{-1}}^{cured}}{Absorbance_{1454\text{ cm}^{-1}}^{cured}} \right) / \left( \frac{Absorbance_{1637\text{ cm}^{-1}}^{uncured}}{Absorbance_{1454\text{ cm}^{-1}}^{uncured}} \right) \times 100\%$$

Two-point baseline and maximum band height ratio protocol were used to measure the absorption intensity. For each adhesive formulation, the last 20 spectra of time-resolved spectra were employed to generate a single mean DC value, and the five mean values were averaged to obtain the terminal DC. Rate of polymerization (Rp) was calculated with the maximum slope of the linear region of DC versus time and was simulated by least squares linear fitting. The polymerization rate was the average of the five individual slopes for the individual runs for a given group.

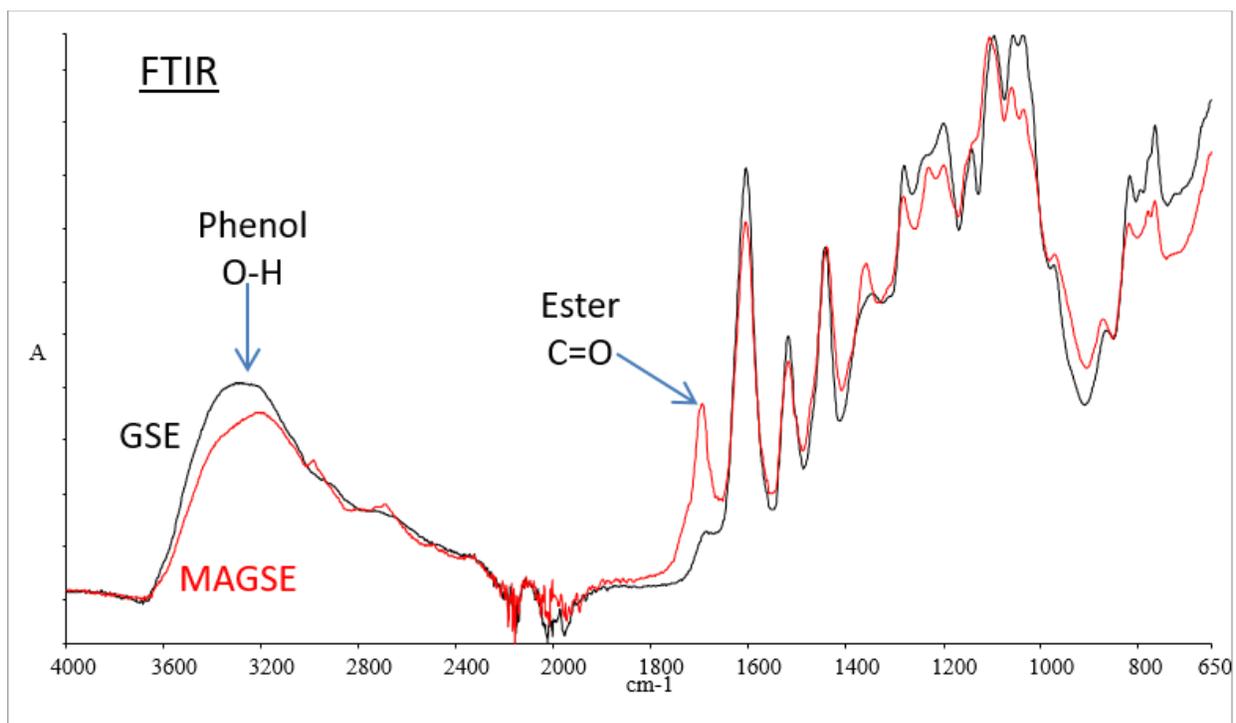
## Schemes & Figures



Appendix Scheme 1. Synthesis of MAGSE via chemical modification of GSE



**Appendix Figure 1.** <sup>1</sup>H-NMR spectra of methacrylate functionalized grapeseed extract (MAGSE) and grapeseed extract (GSE) in DMSO-D<sub>6</sub>. The peaks at chemical shifts of 2.4 and 3.3 indicate the un-deuterated methyl protons in DMSO and the protons in trace water, respectively. An additional peak at the chemical shift of 2.1 is attributed to methyl protons on methacrylate moieties.



**Appendix Figure 2.** FT-IR spectra of methacrylate functionalized grapeseed extract (MAGSE) and grapeseed extract (GSE).