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# Dimeric Proanthocyanidins on the Stability of Dentin and Adhesive Biointerfaces

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**Abstract**

A dentin biomodification strategy with selective proanthocyanidin (PAC)–enriched extracts reinforces dentin and dentin-resin interfaces. Enrichment of the extracts according to the degree of polymerization allows exploration of bioactive principles of PACs and structure-activity relationships. This study investigated the sustained dentin matrix biomodification and dentin-resin bioadhesion of 2 fractions consisting exclusively of B-type PAC dimers with or without a single galloyl motif (specifically, $DIMER_G$ and $DIMER_{NG}$) and their precursor material, enriched grape seed extract (e-GSE; *Vitis vinifera*). The biomodification potential was determined by long-term evaluation of the apparent modulus of elasticity and collagen solubility (hydroxyproline release). Chemical characterization of the dentin matrix was performed by attenuated total reflectance–Fourier-transform infrared spectroscopy. The bioadhesive properties were assessed by a microtensile bond strength test at different time points, and macro-hybrid layers were produced to verify the degree of conversion of the adhesive resin. Fractions consisting of $DIMER_G$, $DIMER_{NG}$, and their precursor, e-GSE, increased the modulus of elasticity at all time points and reduced collagen degradation. Specimens treated with $DIMER_{NG}$ remained stable throughout 12 mo of storage, whereas a significant drop in the modulus of elasticity was observed for the $DIMER_G$ and e-GSE groups at 6 mo. The fractions and precursor did not affect the degree of resin conversion at the hybrid layer. Changes in infrared resonances corresponding to collagen cross-links in the dentin matrix occurred for all treatments. Higher bond strength was observed for dentin treated with e-GSE as compared with $DIMER_G$ and $DIMER_{NG}$; all biointerfaces remained stable after 12 mo. Nongalloylated PACs mediate stable dentin biomodification, which includes protective activity against collagen degradation and reinforcement of the anchoring dentin matrix. Collectively, PACs with a higher degree of oligomerization offer a robust bioadhesion between the hydrophilic dentin matrix and the hydrophobic adhesive.

**Keywords:** extracellular matrix, biomodification, bioadhesion, biostability, grape seed extract, type I collagen

## Introduction

Adhesion protocols in dentistry rely on the formation of micromechanical retention of a dental methacrylate resin infiltrate to enamel and dentin. In the case of dentin, effective infiltration of resin monomers within the dentin extracellular matrix (ECM), biostability of the underlying tissue, and low permeability of the polymerized resin system are key for the success of adhesive restorations (Navarra et al. 2012; Matuda et al. 2016; Silva Sousa et al. 2016; Cadenaro et al. 2019).

Bioinspired approaches to address pitfalls associated with unstable dentin-resin interfaces target the reinforcement of the dentin ECM and dentin-resin adhesion through biomodification by plant-derived proanthocyanidins (PACs; Bedran-Russo et al. 2008; Bedran-Russo et al. 2012; Bedran-Russo et al. 2014). Multiscale enhancement of the mechanical properties, biodegradability of dentin, and extended durability of dentin-resin adhesive interfaces were reported for PAC extracts, specifically from *Vitis vinifera* (Vv) grape seeds (Leme et al. 2015; Leme-Kraus et al. 2017; Aydin et al. 2019). Preliminary studies have indicated that the dentin biomodification potency is influenced by a rich structural diversity of PACs, such as the degree of polymerization (DP; Vidal, Leme, et al. 2014), galloylation (Vidal, Aguiar, et al. 2014), and the stereochemistry or positioning of the hydroxyl groups (Dong et al. 2013; Aydin et al. 2019). Recently, 18-mo analysis found that sources of galloylated PACs exhibited higher initial dentin biomodification; however, these effects were not as sustainable (Aydin et al. 2019).

The development of phytochemical separation protocols enables studies of isolated oligomeric PACs from crude extracts and their enrichment into fractions that contain PACs

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A supplemental appendix to this article is available online.

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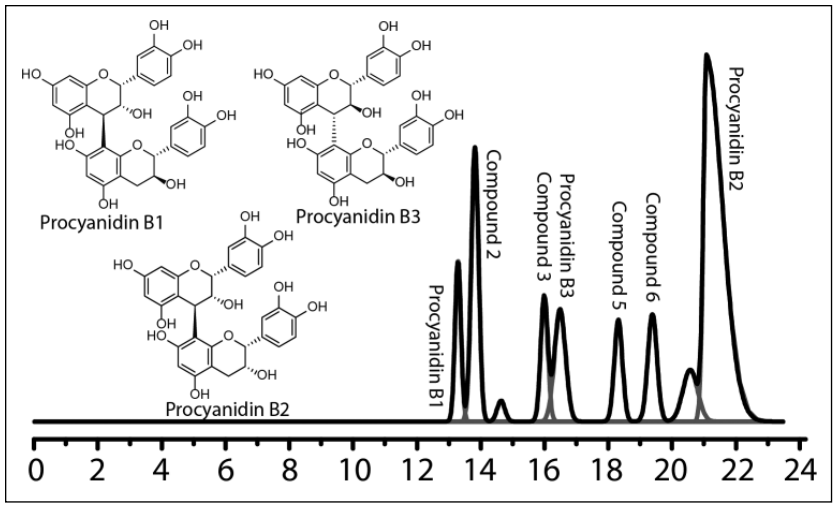


**Figure 1.** Deconvoluted HPLC-UV chromatogram of fraction 4 generated in OriginPro. Peaks are labeled with the constituent dimers (Phansalkar et al. 2019). Structures of major dimeric PACs present in fractions $DIMER_G$ and $DIMER_{NG}$. HPLC-UV, high-performance liquid chromatography–ultraviolet; PAC, proanthocyanidin.

with a desired DP range. The chosen workflow involved the preparation of isolates from Vv with a combination of phytochemical separation methods, such as 2-phase solvent system partitioning and fast centrifugal partitioning chromatography, fraction profiling by HPLC and UHPLC (high- and ultrahigh-performance liquid chromatography, respectively), and structural analysis by NMR (nuclear magnetic resonance). The outcomes show that dimeric PACs from Vv can induce a 5.7-fold increase in the modulus of elasticity of the dentin matrix (Phansalkar et al. 2015). The phytochemical profiles are important fingerprints of the oligomeric PACs and can be used as parameters for the evaluation of their potential for dentin biomodification. Furthermore, it is essential to investigate the separation method and enrichment of the bioactives applied for dentin biomodification for the development of standardized materials, which are a prerequisite for biological and clinical reproducibility.

The primary goal of this study is to conduct an in-depth analysis of PAC structural variations, particularly the role of the galloylation of PACs with an identical DP, targeting their interactivity with the dentin ECM, the stability of the biomodified tissue, and their bioadhesive properties. Therefore, we investigated the effect of 2 exclusively dimeric fractions of B-type PACs—1 galloylated ($DIMER_G$) and 1 nongalloylated ($DIMER_{NG}$), obtained from highly bioactive Vv (grape seed) extract—on the sustained biomodification potential of the dentin ECM and the strength and stability of dentin–hydrophobic resin biointerfaces.

## Materials and Methods

Preparation of dimeric fractions followed a fractionation method previously published (Phansalkar et al. 2015). Briefly, 2 dimeric fractions from an oligomeric enriched grape seed extract (e-GSE) from the crude extract of Vv seeds (MegaNatural Gold Grape Seed Extract, No. 206112508-01/122112505-01; Polyphenolics) were separated by fast centrifugal partitioning chromatography. HPLC-ultraviolet phytochemical profiles confirmed that fraction $DIMER_G$ consisted of monogalloylated dimers and that fraction $DIMER_{NG}$ consisted of nongalloylated dimers. The structures of major dimers present in both fractions are shown in Figure 1.

### *Studies of the Dentin Matrix*

*Mechanical Properties and Biostability.* The bulk apparent modulus of elasticity of the dentin matrix was assessed by a 3-point bending test with a maximum deformation of 3% (EZ Graph; Shimadzu), as described previously (Bedran-Russo et al. 2008). Briefly, extracted human third molars (Institutional Review Board protocol No. 2011-0312) were sectioned (Isomet 1000; Buehler) into dentin specimens (0.5 × 1.7 × 6.0 mm, thickness × width × length) and demineralized (10% phosphoric acid for 5 h). $DIMER_G$, $DIMER_{NG}$, and e-GSE were assessed at 6.5% w/v (pH 7.2) in 20mM Hepes. Each specimen ($n$ = 15 per group) was immersed in 100 µL of the solution for 1 h under agitation at 25 °C. The apparent modulus of elasticity was assessed immediately after treatment and at 6- and 12-mo storage in simulated body fluid (SBF; 5mM Hepes, 2.5mM $CaCl_2$, 0.05mM $ZnCl_2$, 0.3mM $NaN_3$) at 37 °C. Data were statistically analyzed by 2-way analysis of variance (ANOVA) and Games-Howell test ($\alpha$ = 0.05).

Collagen solubilization by endogenous proteases was estimated by the amount of solubilized hydroxyproline (HYP) in the storage media (SBF; Leme-Kraus et al. 2017). The detailed method is described in the Appendix. Briefly, SBF collected every 2 wk ($n$ = 15) was pooled into 2 time points: 0 to 6 mo and 7 to 12 mo. Aliquots were hydrolyzed and mixed with 0.056M chloramine T reagent (25 min), and 1M Ehrlich's reagent was used to develop color (60 °C for 40 min). Absorbance was measured at a 550-nm wavelength (Spectramax Plus; Molecular Devices). Reference values for HYP concentrations (0.5, 1, 2, 3, 4, and 5 µg/mL; Appendix Fig. 1) were used to produce a standard curve. Specimens were prepared in duplicate and analyzed with 2-way ANOVA and Tukey's post hoc test ($\alpha$ = 0.05).

*Chemical Characterization of the Biomodified Dentin.* Chemical characterization of the biomodified dentin matrix was performed by Fourier-transform infrared spectroscopy (FTIR; Nicolet 6700, Thermo Fisher Scientific), equipped

with an attenuated total reflectance (ATR) apparatus. Spectra were collected between 4,500 $cm^{-1}$ to 600 $cm^{-1}$ with a mean 100 scans per sample and at a resolution of 4.0 $cm^{-1}$ ($n$ = 3). Data were recorded and analyzed with OMNIC Spectra Software (Thermo Fisher Scientific).

The areas under the resonance at 1,630 $cm^{-1}$ (amide I), 1,550 $cm^{-1}$ (amide II), 1,240 $cm^{-1}$ (amide III), and 1,450 $cm^{-1}$ ($CH_2$ scissoring) were obtained following a 2-point baseline correction, normalization, and peak area integration. Structural variations in the collagen triple helix were investigated by the ratio between amide III and $CH_2$ scissoring ($A_{1240}/A_{1450}$) and the degree of cross-linking by the ratio between amide II and $CH_2$ scissoring ($A_{1550}/A_{1450}$; Gordon et al. 1974; Sylvester et al. 1989; Madhan et al. 2005; Liu, Dusevich, and Wang 2014; Liu, Yao, et al. 2014; Liu et al. 2015; Du et al. 2018). Comparisons among $DIMER_G$, $DIMER_{NG}$, e-GSE, and control for each ratio were analyzed by 1-way ANOVA and Tukey's post hoc test ($\alpha = 0.05$).

### *Studies of Dentin-Resin Interface*

*Dentin-Resin Bioadhesion.* The dentin-resin bioadhesive properties of $DIMER_G$ and $DIMER_{NG}$ were compared with their precursor, e-GSE, through a standard microtensile bond strength (TBS) test with an experimental hydrophobic adhesive resin ($H_0$; Silva Sousa et al. 2016; Leme-Kraus et al. 2017). Adhesive composition and detailed method description are available in the Appendix. Briefly, occlusal dentin surfaces ($n$ = 7 per group) were etched (35% phosphoric acid solution), and primer was applied for 1 min ($DIMER_G$, $DIMER_{NG}$, and e-GSE prepared at 15% w/v) and rinsed. $H_0$ adhesive was then applied and light cured for 40 s (Optilux 501; Kerr Dental), followed by incremental placement of resin composite buildup. The same protocol was followed for the control group, except there was no primer. After 24 h at 37 °C in SBF, interfaces were serially sectioned, and dentin-resin specimens were tested in tensile at 24 h and 6 and 12 mo (microtensile tester; Bisco). Data were statistically analyzed with 2-way ANOVA and Tukey's post hoc tests ($\alpha = 0.05$). Pearson's correlation tests with a 1-tailed significance level of 0.05 were performed to investigate correlations between TBS and dentin apparent modulus of elasticity and between TBS and HYP release. Representative fractured specimens (Appendix Fig. 2) were observed under scanning electron microscopy (Hitachi Ltd.).

*Adhesive Resin Degree of Conversion in a Macro-Hybrid Layer Model.* Macro-hybrid layers (Chiaraputt et al. 2008; Matuda et al. 2016) were prepared, and the degree of conversion (Cadenaro et al. 2009; Malacarne-Zanon et al. 2009; Parthasarathy et al. 2012) of the adhesive system at the hybrid layer was assessed, as described in the Appendix. Briefly, dentin specimens were demineralized and treated for 1 h with biomodification primers ($DIMER_G$, $DIMER_{NG}$, and e-GSE) and control (Hepes). Each specimen ($n$ = 5 per group) was dehydrated in ascending ethanol/water concentrations and infiltrated with $H_0$ resin (Matuda et al. 2016). Specimens were light cured (60 s) on the top and bottom surfaces (Optilux 501) and polished with SiC paper grits 600, 800, and 1,200 with a water-free silicon (Silicon Oil; Aldrich). Infrared spectra of the uncured and cured macro-hybrid specimens were obtained with a FTIR-ATR (Nicolet 6700; Thermo Fisher Scientific), and the degree of conversion was calculated with the following formula: degree of conversion (%) = [1 – (R cured / R uncured)] × 100, where R is the ratio between the aliphatic C=C and aromatic C=C (constant) peaks, respectively at 1,638 $cm^{-1}$ and 1,608 $cm^{-1}$ of the cured and uncured resin. Data were statistically analyzed by 1-way ANOVA and Tukey's post hoc ($\alpha = 0.05$).

## Results

### *Studies of Dentin Matrix*

*Mechanical Properties and Biodegradation.* Figure 2A shows the results of the apparent modulus of elasticity. There were significant interactions between the studied factors (time and dentin treatment, $P < 0.001$). Statistically significant differences were found among treatments, with a higher modulus of elasticity for the e-GSE, $DIMER_G$, and $DIMER_{NG}$ groups when compared with control at all time points ($P < 0.001$). A significant decrease in modulus of elasticity occurred at 6 mo for dentin treated with $DIMER_G$ ($P < 0.001$) and e-GSE ($P < 0.001$). The $DIMER_{NG}$ group remained stable after 6 mo in SBF ($P = 0.056$). There were no significant differences in the modulus of elasticity of specimens treated with $DIMER_G$ ($P = 0.0894$), $DIMER_{NG}$ ($P = 0.973$), and e-GSE ($P = 0.630$) at 6 and 12 mo.

Results of cumulative HYP release over the 12-mo aging period (Fig. 2B) showed significantly lower HYP (1.7- to 2.6-fold lower) released from dentin matrices treated with e-GSE, $DIMER_G$, and $DIMER_{NG}$ when compared with the control group ($P < 0.001$). There were no significant differences among e-GSE, $DIMER_G$, and $DIMER_{NG}$ ($P > 0.05$).

*Chemical Characterization of the Biomodified Dentin.* The FTIR spectra of the dentin matrix biomodified with $DIMER_G$, $DIMER_{NG}$, e-GSE, and control are shown in Figure 3A. All spectra showed resonances that are characteristic of type I collagen, such as those of the stretching vibrations of the C=O groups associated with amide I (~1,630 $cm^{-1}$), the presence of mixed C-N stretch and in-plane bend of N-H vibrations and $CH_2$ bending as amide II (~1,550 $cm^{-1}$), N-H bending and C-N stretching vibrations with contributions from the C=O in-plane bending, the C-Cα stretching vibration for amide III (~1,240 $cm^{-1}$), and scissoring mode vibrations of $CH_2$ bonds (~1,450 $cm^{-1}$). The lowest $A_{1240}/A_{1450}$ ratio was observed for $DIMER_G$ and

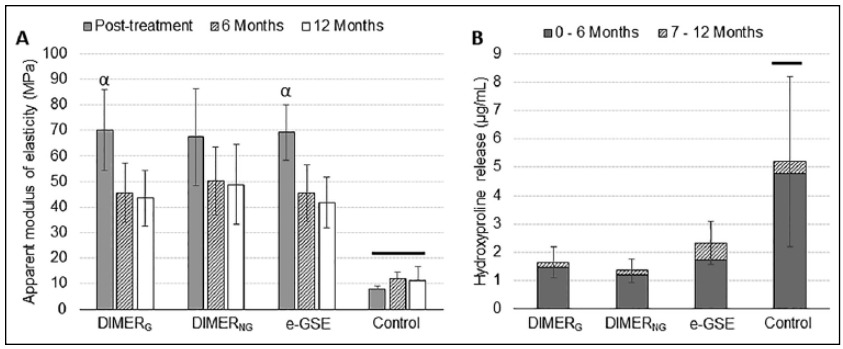


**Figure 2.** Studies of dentin matrix's apparent modulus of elasticity (E) and total hydroxyproline released in the media. (**A**) The apparent modulus of elasticity of groups $DIMER_G$, $DIMER_{NG}$, and e-GSE immediately after treatment and after 6 and 12 mo of storage. (**B**) Cumulative hydroxyproline release after 12 mo of storage. Symbol ($\alpha$) is used to show significant differences between posttreatment and 6 mo for $DIMER_G$ ($P < 0.001$) and e-GSE ($P < 0.001$); bars depict differences in apparent modulus of elasticity and hydroxyproline release between control (Hepes) and $DIMER_G$, $DIMER_{NG}$, and e-GSE ($P < 0.001$). Error bars depict standard deviation. e-GSE, enriched oligomeric grape seed extract.

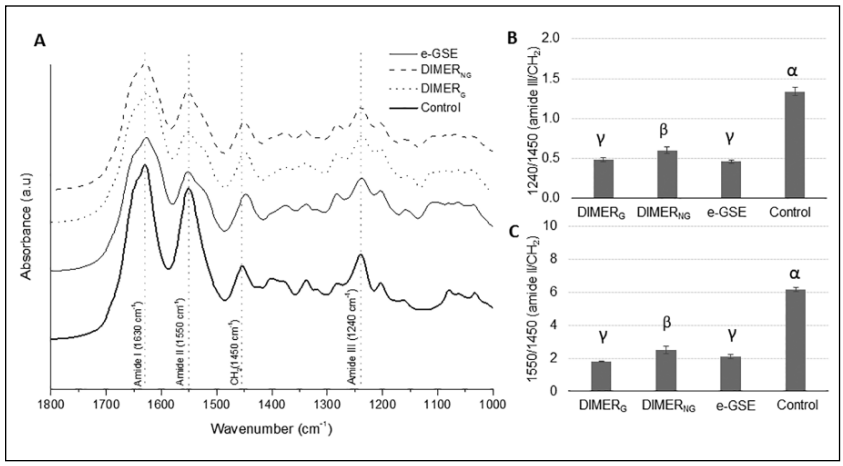


**Figure 3.** FTIR chemical characterization dentin collagen. (**A**) FTIR spectra of collagen and collagen biomodified by $DIMER_G$, $DIMER_{NG}$, e-GSE, where peaks assigned to collagen are depicted by the dashed lines. Ratios calculated from the areas under the peak assigned to (**B**) amide III over $CH_2$ scissoring (1,240/1,450 $cm^{-1}$) and (**C**) amide II over $CH_2$ (1,550/1,450 $cm^{-1}$). Symbols ($\alpha$, $\beta$, $\gamma$) depict statistically significant differences between groups ($P < 0.05$). Error bars depict standard deviation. e-GSE, enriched oligomeric grape seed extract; FTIR, Fourier-transform infrared spectroscopy.

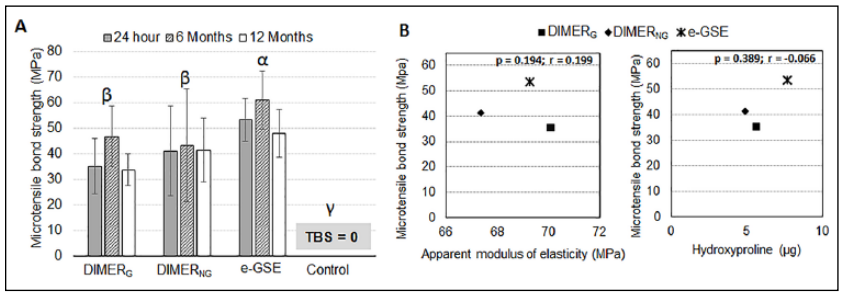


**Figure 4.** Bioadhesive properties of the dentin-resin interface and correlation studies. (**A**) TBS of the experimental groups. All priming solutions resulted in stable adhesion after 24-h, 6-mo, and 12-mo aging in SBF ($P > 0.05$). Solid bar depicts statistically significant difference between groups e-GSE, $DIMER_G$, and $DIMER_{NG}$ ($P < 0.05$). (**B**) Graphs showing that there was no statistically significant correlation between TBS and apparent modulus of elasticity ($P = 0.194$) or TBS and hydroxyproline release ($P = 0.389$). Error bars depict standard deviation. e-GSE, enriched oligomeric grape seed extract; SBF, simulated body fluid; TBS, microtensile bond strength.

e-GSE treatment, followed by $DIMER_{NG}$ treatment, when compared with control ($P < 0.001$; Fig. 3B). A significant decrease in the $A_{1550}/A_{1450}$ ratio (Fig. 3C) was observed in the $DIMER_G$, $DIMER_{NG}$, and e-GSE groups when compared with the control group ($P < 0.001$). The bands in ~1,630 $cm^{-1}$ and ~1,552 $cm^{-1}$ for amide I and amide II, respectively, showed a slight shift for the $DIMER_G$ and e-GSE groups (~1,625 to 1,627 $cm^{-1}$). The ~1,240-$cm^{-1}$ band (amide III) did not shift among groups, although the intensity of this band was lower for PAC-treated groups as compared with control.

## *Dentin-Resin Interface*

*Studies of Dentin-Resin Bioadhesion.* The results of TBS are depicted in Figure 4A. No interactions were observed between the factors time and dentin treatment ($P = 0.737$). The e-GSE group exhibited significantly higher bond strength than both $DIMER_G$ ($P = 0.001$) and $DIMER_{NG}$ ($P = 0.015$) groups. No statistically significant difference was observed between the $DIMER_G$ and $DIMER_{NG}$ groups ($P = 0.670$). Aging in artificial saliva up to 12 mo did not affect the TBS of the experimental groups ($P = 0.378$), while no specimens remained bonded in the control group (TBS = 0). No significant correlation was observed between TBS and the apparent modulus of elasticity ($P = 0.194$, $r = 0.199$) or between TBS and HYP release ($P = 0.389$, $r = –0.066$), as shown in Figure 4B. Subtle differences in fracture pattern among groups were compatible with the bioadhesive performance (Appendix Fig. 2).

*Adhesive Resin Degree of Conversion in a Macro-Hybrid Layer Model.* The degrees of conversion of the adhesive resin in the macro-hybrid layers were as follows: 72.2% ± 4.2% for $DIMER_G$, 73.8% ± 4.2% for $DIMER_{NG}$, and 72.9% ± 1.6% for e-GSE treatment. The degree of conversion of the experimental resin within the macrohybrid layer was in the range of 74.3% ± 5.0%. There were no statistically significant differences in the resin degree of conversion among control, $DIMER_G$, $DIMER_{NG}$, and e-GSE ($P = 0.715$).

## Discussion

The study revealed key PAC properties that contribute to a strong and stable bioadhesion between resin-based materials and the sustained interaction with the dentin matrix. The dimeric fractions increased the dentin matrix's modulus of elasticity and prevented biodegradation in the same manner as their precursor material, e-GSE. Long-term evaluation of the biomodified dentin matrix's biomechanics revealed that biomodification with galloylated PACs is less durable, targeting the roles of the galloyl moieties in terms of both reactivity and stability of the biomodified dentin matrix. The studies also revealed that dentin matrices treated with e-GSE, composed of PACs with DP up to 7, show stronger adhesion as compared with treatment with the dimeric fractions $DIMER_G$ and $DIMER_{NG}$. The FTIR-based chemical profiling of the biomodified dentin matrix revealed changes in peak intensities mediated by PAC interactions with type I collagen. There was no correlation between dentin-resin bioadhesion and the outcomes of dentin ECM biomechanics and biostability (Fig. 4B), leading to the conclusion that dimeric fractions and their precursor, e-GSE, exert different mechanisms of biomodification associated with improvement of the dentin-resin biointerface.

Dentin biomodification with $DIMER_G$, $DIMER_{NG}$, and e-GSE increased the apparent modulus of elasticity of the dentin matrix (Fig. 2A) up to 7-fold. However, in both e-GSE and $DIMER_G$, an initial drop in the modulus of elasticity of the dentin matrix was observed at the 6-mo time point, with modulus of elasticity stabilization for the remaining time. While the galloylated PAC fraction elicited strong immediate bioactivity, it did not sustain the dentin biomodification as compared with nongalloylated PACs ($DIMER_{NG}$). The high biomodification potency of galloylated compounds was first reported for monomeric galloylated compounds (EGCG), where a direct correlation was observed between potency and the number of phenolic hydroxyl (–OH) groups (Vidal, Leme, et al. 2014). However, in subsequent studies, the monomeric nongalloylated counterpart (EGC) did not enhance the mechanical properties, thus limiting any further relationships. The present findings show that the phenolic –OH groups of the galloyl motif increase the compound's reactivity and hydrophilicity (Iglesias et al. 2010; Vidal, Leme, et al. 2014; Aydin et al. 2019); however, the sustainability of the bioactivity was found to be diminished, which is plausible considering that the galloyl motif is prone to hydrolysis of the ester bond linkage (Lee et al. 2010; Krook and Hagerman 2012).

While higher release of HYP was observed for the e-GSE group from 7 to 12 mo, there were no significant differences in the cumulative HYP release among $DIMER_G$, $DIMER_{NG}$, and e-GSE over 12 mo. All treatments resulted in statistically lower HYP release when compared with the control group (Fig. 2B). Both dimeric fractions were able to reduce collagen degradation, meaning that smaller molecular weight compounds are effective in preventing collagen degradation (Vidal, Leme, et al. 2014). All biomodification agents induced cross-links within the different levels of hierarchy of the dentin matrix, likely reinforcing and hiding specific binding sites of endogenous proteases in the type I collagen (Vidal, Leme, et al. 2014). Additionally, PACs with lower molecular weight could aid by improving the wettability and infiltration of the biomodification solution within the dentin ECM (Jenkins et al. 2013). Regardless of the treatment, higher amounts of HYP were found in the storage solution during the first 6 mo of storage, likely due to a higher initial activity of endogenous proteases.

PAC primers did not induce secondary structural changes to type I collagen, as depicted by lack of shifts in ~1,240 $cm^{-1}$ (amide III). However, lower intensity was observed for all PAC-modified groups (ratio $A_{1240}/A_{1450}$), which can be attributed to spectral traces of treatment reagents (Liu, Dusevich, and Wang 2014). e-GSE, $DIMER_{NG}$, and $DIMER_G$ induced cross-linking in the dentin matrix, as evidenced by the decrease in the ratio between the amide II and $CH_2$ scissoring bands ($A_{1550}/A_{1450}$), resulting in a reduction in $–NH_2$ and consequently the band amide II (Silva Junior et al. 2015; Oliveira et al. 2019). Specific for the dimeric fractions, the $A_{1550}/A_{1450}$ ratio was significantly different from each other probably due to the higher reactivity of the galloylated compounds present in $DIMER_G$ when compared with $DIMER_{NG}$.

All PAC primers promoted robust bioadhesion (Fig. 4A). Notably, the control group had no adhesion with the same hydrophobic adhesive resin ($H_0$). The amphiphilic nature of PACs is due to their hydrophilic polar hydroxyl groups, paired with the hydrophobic aromatic rings (Iglesias et al. 2010) to promote the adhesion between the hydrophobic resin ($H_0$) and the dentin ECM (Leme-Kraus et al. 2017). Moreover, the adhesion mediated by the priming solutions $DIMER_G$, $DIMER_{NG}$, and e-GSE remained stable after 6 and 12 mo. Priming of the dentin matrix with a PAC-rich solution can overcome problems leading to long-term degradation of the dentin-resin interface (Leme-Kraus et al. 2017). Comparisons among the biomodification primers revealed that priming solutions consisting of $DIMER_G$ and $DIMER_{NG}$ did not accomplish the same adhesion strength as the precursor e-GSE. Gravimetrically, the fraction $DIMER_G$ accounted for 2.6% w/w of the e-GSE, whereas $DIMER_{NG}$ accounted for 6.4% w/w of the e-GSE (Phansalkar et al. 2015). The intermediary order oligomers present in e-GSE likely contributed to the bioadhesive properties (Leme-Kraus et al. 2017), as both the reducing and chelating capacities increase with the increased DP of PACs (Iglesias et al. 2010).

To exclude any possible influence of PACs bound to the dentin matrix on the polymerization of the adhesive system, a macromodel of the hybrid layer was used to determine the degree of conversion of the adhesive resin. The results showed that the application of a PAC primer before the infiltration of the dentin matrix by the adhesive system did not impair the polymerization of the adhesive resin. An earlier study found a low degree of conversion of a commercially available adhesive system containing galloylated monomers (EGCG; Du et al. 2012). In the present study, PACs were not incorporated into the adhesive resin blend; instead, their use as rinse-out primer enabled unbound PACs to be removed. This approach can reinforce and stabilize the anchoring ECM and underlying dentin

(Leme et al. 2015) and minimize the risk of secondary caries adjacent to the restoration margins (Kim et al. 2017).

In conclusion, while the use of galloylated PACs for dentin biomodification results in high initial activity, the stability of the biomodified matrix is diminished as compared with nongalloylated PACs, likely due to hydrolysis of the ester bond linkage of 3-*O*-galloylated motif. All PAC-rich biomodification agents induced collagen cross-links in the dentin ECM. However, between the dimeric fractions, higher reactivity was depicted by the galloylated fraction, $DIMER_G$. Galloylation did not affect the stability of dentin-resin adhesion of dimeric PACs. Higher-order (higher DP) oligomeric PACs, as in e-GSE, promoted stronger dentin-resin adhesion. Finally, the lack of correlation between enhanced dentin biomechanics concurrent with lower biodegradability and the poorer bioadhesive performance by PAC dimers indicated the diverse mechanisms of interactions with the dentin matrix and the importance in defining PACs–dentin structure activity relationships.

## Author Contributions

A.A. Leme-Kraus, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; R.S. Phansalkar, M.C. dos Reis, B. Aydin, Y. Alania, contributed to data acquisition and analysis, critically revised the manuscript; A.B.S. Sousa, contributed to data acquisition, critically revised the manuscript; J. McAlpine, S.N. Chen, contributed to data analysis, critically revised the manuscript; G.F. Pauli, contributed to conception and data interpretation, critically revised the manuscript; A.K. Bedran-Russo, contributed to conception, design, data analysis and interpretation, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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