Published in final edited form as:

J Dent. 2013 January ; 41(1): 71–79. doi:10.1016/j.jdent.2012.10.006.

Effect of proanthocyanidins and photo-initiators on photo-polymerization of a dental adhesive

Yi Liu and Yong Wang

University of Missouri-Kansas City School of Dentistry, Kansas City, MO 64108, USA

Abstract

Objectives—To evaluate the effects of proanthocyanidins (PA) and photoinitiator type on the degree of conversion (DC) and polymerization rate (PR) of a model dental adhesive.

Methods—Three types of photo-initiation systems were introduced into the Bis-GMA/HEMA co-monomer mixture, resulting in four resin formulations including CQ/A (0.5 wt% CQ and EDMAB), CQ/A/I-1 (0.5 wt% CQ, EDMAB and DPIHP), CQ/A/I-2 (1.0 wt% CQ, EDMAB and DPIHP), and TPO (2.1 wt% TPO). For each resin formulation, adhesives containing 0, 2.5%, 5% and 10% of PA with respect to the weight of resin were produced after mixing the resin with various amount of PA/ethanol solution. When light-cured, the RP and DC of each adhesive was determined using ATR- FTIR spectroscopy.

Results—Across and within the initiator groups, the DC followed the general trend of CQ/A < CQ/A/I-1 < CQ/A/I-2 < TPO and 0-PA > 2.5-PA > 5-PA > 10-PA, respectively. The change of PR with respect to photo-initiation systems and PA content was in a similar but less pronounced pattern.

Conclusion—PA hampered the polymerization of all adhesives regardless of photoinitiators used. The initiator formulations CQ/A/I-2 and TPO are better fit for PA-containing adhesives, both leading to > 65% DC in the presence of 5% PA.

Clinical significance—The inclusion of PA in dental adhesives has been limited by its interference with the light-curing of adhesive resins. This study found photo-initiation formulations that could maintain a satisfactory degree of monomer conversion while a significant amount of PA is incorporated.

Keywords

Dental adhesive; Proanthocyanidins; Degree of conversion; ATR-FTIR

1. Introduction

Composite dental fillings were first introduced into the field of restorative dentistry in the 1960s¹. Despite over 50 years of development, the durability of composite restorations is still no match to that of the traditional amalgam fillings^{2,3}. One of the leading causes for

© 2012 Elsevier Ltd. All rights reserved.

Corresponding Author: Dr. Yong Wang, University of Missouri-Kansas City, School of Dentistry, 650 E. 25th St., Kansas City, MO 64108, Fax: 816-235-5524, Phone: 816-235-2043, Wangyo@umkc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

the failure of composite fillings is the loss of marginal integrity^{3, 4}, suggesting that the composite-dentin interface is a major issue. While the use of dental adhesives has greatly improved the interfacial strength in the short term⁵, its long-term stability leaves a lot to be desired. The basis for adhesives' bonding is the adhesive/dentin hybrid layer, which is composed of adhesive polymers and demineralized collagen fibrils that form micro-interlocked entanglement⁶. Unlike mineralized collagen fibrils found in the intact dentin, collagen fibrils in the hybrid layer are partially exposed due to a number of reasons such as the incomplete resin infiltration and unsatisfactory degree of polymerization^{7, 8}. As a result, collagen fibrils in the hybrid layer are vulnerable to hydrolytic and enzymatic degradation, which could in turn contribute to the breakdown of the dentin-composite interface. In addition, recent studies have revealed that the acid etching of dentin activates the otherwise dormant host-derived matrix metalloproteinases (MMPs)⁹, further increasing the risk of collagen degradation. Based on the above information, the importance of exploring materials and techniques to enhance dentin collagen's biological stability becomes self-evident.

One common practice to improve collagen's resistance toward hydrolytic and enzymatic degradation is to treat it with cross-linking agents. Indeed, a number of studies have established that dentin collagen presented enhanced mechanical and biological properties upon cross-linking^{10–17}. Among all the cross-linking agents reported, a group of naturally-occurring polyphenolic compounds, proanthocyanidins (PA) is of particular interest, because not only is it highly efficient in cross-linking collagen, it is also non-toxic and readily available^{18, 19}. As such, it is warranted to investigate the effect of PA on the performance of current dental adhesive systems, and to design an optimal approach for its use.

There are two options when incorporating PA in an adhesive system: as a primer, or as an additive to the adhesive. In the former approach, PA is applied and rinsed off prior to the application of adhesive, and therefore has little to no effect on the light-curing of adhesive resin. The limitation of this approach is the time constraint when applying PA as a primer, as well as the lack of sustained release of PA to exert its protective effect on dentin collagen over a long time period. The reported application time varied from 10 min to 1 h, which is not feasible in clinical setting^{10–14}. In comparison, the latter approach could afford a sustained release of PA, but the presence of PA during light-curing might interfere with polymerization of adhesive monomers. After all, PA is a known radical scavenger¹⁹, and it is only natural to anticipate that such radical-consuming capability could be detrimental to the light-curing process which relies on the generation and propagation of radical species to make the resin set. In addition, the release of PA could lead to resin breakdown and reduce integrity of the bonding interface when the resin is poorly cured.

Not surprisingly, when Green et al.¹³ examined the hybrid layer formed with PA-containing adhesives, they found that it presented more porous morphology as compared to its PA-free counterpart, presumably due to a lower degree of conversion of adhesive resins. In addition, Hechler et al.²⁰ examined both approaches as stated above, they found that when PA was directly mixed with the adhesive, the microtensile bond strength was lower than when PA was used as a primer after one year of collagenase digestion. In another study²¹, Epasinghe et al. also reported a lower bond strength when a higher dose of PA was included in the adhesive. These results, however, are still circumstantial evidences that PA might adversely interfere with the radical polymerization of dental adhesives. Consequently, this work aimed to directly study dental resin's polymerization behavior as a function of PA concentration and different initiator system. By examining the degree of conversion (DC) and polymerization rate (PR) of PA-containing model adhesive upon light-curing, the null hypothesis tested was that the polymerization behavior of the adhesive is not affected by the presence of PA or photo-initiator system used.

2. Materials and Methods

2.1. Reagents

Bisphenol A glycidyl dimethacrylate (Bis-GMA), 2-hydroxyethyl methacrylate (HEMA), camphorquinone (CQ), trimethylbenzoyl-diphenylphosphine oxide (TPO), ethyl (4-dimethyl amino) benzoate (EDMAB), diphenyliodonium hexafluorophosphate (DPIHP) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Ethanol was purchased from Pharmco-AAPER (Brookfield, CT, USA). Grape seed extract proanthocyanidin (PA) was generously donated by MegaNatural (Madera, CA, USA). All reagents were used as received.

2.2. Formulation of PA-containing model adhesives

Model adhesives with compositions similar to commercial dental adhesives such as Single Bond were used in the presented research¹³. The protocol involved the preparation of a photo-initiator-containing neat resin and a PA-containing ethanol solution prior to mixing them together to make the final PA-containing model adhesive. The neat resin consisted of Bis-GMA and HEMA at a Bis-GMA/HEMA mass ratio of 55/45. Three CQ-based photo-initiator formulations (CQ/A, CQ/A/I-1 and CQ/A/I-2), and one TPO-based formulation (TPO) were added to the neat resin at selected concentrations as shown in Table 1. Specifically, CQ/A is a traditional two-component CQ-based initiator system with tertiary amine (A) EDMAB as the coinitiator, CQ/A/I-1 is a three-component system with extra iodonium salt (I) DPIHP, CQ/A/I-2 shares the same composition as CQ/A/I-1 but with twice the amount of initiators, and TPO is a single-component system with TPO at the same molar concentration as CQ in CQ/A/I-2. Each of the four resultant initiator-containing neat resins was then mixed with 0 % (w/w), 3.75 % (w/w), 7.5 % (w/w), and 15 % (w/w) PA/ethanol solution, respectively, at a resin/solution mass ratio of 60/40. This concluded the formulation of the control pure adhesive (0-PA), as well as adhesives containing 2.5 % (2.5-PA), 5 % (5-PA), and 10 % of PA (10-PA) with respect to the mass of neat resins. All adhesive samples were prepared in a room with amber lights, and vortexing and sonication were required to ensure solutions to be well-mixed.

2.3. Polymerization and collection of real-time FTIR spectra

Photo-curing of adhesive samples was monitored real-time using a Fourier transformed infrared (FTIR) spectrometer equipped with an attenuated total reflectance (ATR) attachment (Spectrum One, Perkin-Elmer, Waltham, MA, USA) at a resolution of 4 cm^{-1} . The ATR crystal was diamond with a transmission range between 650 and 4000 cm^{-1} . 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 following the complete evaporation of ethanol solvent. A 5-minute evaporation period was generally enough as evidenced by disappearance of the IR peak at $\sim 1045\text{ cm}^{-1}$ attributable to the C-O stretching of ethanol. 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) for an exposure time of 40 s, and continued for a total of 10 min. When curing, the power output of the dental light was set to 600 mW/cm^2 , which was calibrated with a visible curing light meter (Cure Rite, EFOS Inc., Williamsville, NY, USA), and the distance between the curing light and cover slip was kept at approximately 2 mm.

2.4. Degree of conversion and polymerization rate

The degree of conversion (DC) was assessed according to the changes in absorbance ratio between the peak at $\sim 1637\text{ cm}^{-1}$ (methacrylate C=C stretching) and that at $\sim 1608\text{ cm}^{-1}$ (phenyl C=C stretching) before and after light-curing. A two-point baseline correction and maximum peak height protocol was employed to measure the band intensities. For each adhesive formulation, an initial value of absorbance ratio for the uncured state was determined by averaging the ratio values obtained from the first 20 spectra. The degree of conversion (DC) was then calculated by the following equation:

$$DC = \left(1 - \frac{\text{Absorbance}_{1637\text{ cm}^{-1}}^{\text{cured}}}{\text{Absorbance}_{1608\text{ cm}^{-1}}^{\text{cured}}} \div \frac{\text{Absorbance}_{1637\text{ cm}^{-1}}^{\text{uncured}}}{\text{Absorbance}_{1608\text{ cm}^{-1}}^{\text{uncured}}} \right) \times 100\%$$

For each adhesive formulation, the last 20 spectra of time-resolved spectra were employed to generate a single mean DC value, and the three mean values were averaged to obtain the final DC. The polymerization rate (PR) was determined using the maximum slope of linear region of the DC-time plots by using the least square linear fitting. The polymerization rate reported was the average of the three individual slopes for the individual runs for a given specimen group.

2.5. Statistical analysis

Data were expressed as means $\pm$ standard deviation. Comparisons of degree of conversion and polymerization rate between groups were analyzed by two-way ANOVA and Turkey's post hoc tests with 95% confidence level.

3. Results

Representative time-resolved FTIR spectra of one model adhesive (CQ/A/I-1) in the absence of PA are shown in Figure 1 to illustrate the peak intensity changes at $\sim 1608\text{ cm}^{-1}$ and $\sim 1637\text{ cm}^{-1}$ during polymerization. The absorbance peak at $\sim 1608\text{ cm}^{-1}$ arises from the stretching mode of aromatic C=C bonds of benzene rings (Figure 1d), which could be found in both Bis-GMA and PA. The intensity of this peak stays unchanged throughout polymerization because the highly stable benzene rings are not susceptible to radical attack. The peak at $\sim 1637\text{ cm}^{-1}$, on the other hand, originates from the stretching mode of C=C double bond found in Bis-GMA and HEMA (Figure 1d). During polymerization the C=C double bonds are converted to C-C single bonds, and as a result, the intensity of the peak at $\sim 1637\text{ cm}^{-1}$ decreases as polymerization proceeds.

The time-resolved DC curves of all adhesives are shown in Figure 2. All curves feature a sigmoid pattern, with the DC increased dramatically upon light curing, reaching a plateau within 40 s, and continuing to increase slightly throughout the recording period (10 min). It is evident that the increase in the amount of PA resulted in the decrease of DC regardless of the initiation systems.

Shown in Figure 3 and Table 2 are the DCs at the end of the observation period (10 min) for all adhesives with different initiator systems and amount of PA. Among the initiator groups, an overall trend can be observed with $\text{CQ/A} < \text{CQ/A/I-1} < \text{CQ/A/I-2} < \text{TPO}$ at all PA concentrations except 0-PA, where $\text{CQ/A} < \text{CQ/A/I-1} = \text{CQ/A/I-2} < \text{TPO}$ as the difference between 0-PA CQ/A/I-1 and 0-PA CQ/A/I-2 is short of statistical significance ($p = 0.31$). Within each of the initiator groups, the gradual increase in the amount of PA generally leads to a gradual decrease of DC, while a significant and substantial drop in DC occurs at 10-PA for all four initiator systems.

The change of polymerization rate (PR) with respect to initiator systems and PA concentrations is slightly different from that of DC (Figure 4, Table 2). Among the initiator groups, PR shows a similar trend like DC, namely $CQ/A < CQ/A/I-1 < CQ/A/I-2 < TPO$ with exceptions at 0-PA for CQ/A/I-1 and CQ/A/I-2 ($p = 0.73$) and at 5-PA for CQ/A and CQ/A/I-1 ($p = 0.17$). Within each of the initiator groups, the pattern of the changes of PR varies by the nature of initiator systems. More specifically, for CQ/A, the peak PR value was recorded when 5% PA was present, significantly higher than that of 0-PA, 2.5-PA and 10-PA (Table 2). In contrast, the peak PR value for CQ/A/I-1, CQ/A/I-2 and TPO was observed when PA was absent, and the increase of PA concentration led to the decrease of PR, resulting in the lowest PR at 10% PA.

4. Discussion

The hypothesis that the polymerization behavior of the adhesive is not affected by the presence of PA or photo-initiator systems has to be rejected. In this study, the DC and PR of a Bis-GMA/HEMA model adhesive with three different photoinitiator systems were examined as a function of the amount of PA. The results indicated that the incorporation of PA into the model adhesive negatively influenced the photo-polymerization behavior such as DC and PR, which was also significantly dependent on the three photo-initiator system used.

The first initiation system was the classical two-component CQ/amine system (CQ/A), which has been widely used in a variety of commercial dental adhesives²². In this system, CQ plays the role of a photosensitizer, and the tertiary amine (EDMAB in our case) is a hydrogen-donating coinitiator. Upon light-curing, CQ absorbs visible light and enters into the excited triplet state (CQ*, Scheme 1a), which subsequently extracts a hydrogen atom from the α -carbon of the tertiary amine (A). The resulted aminyl free radical (A•) is the actual reactive radical species and goes on to kick off the chain polymerization, whereas the CQ ketyl radical (CQH•) is mainly involved in chain termination through radical-radical recombination^{23, 24}. In the absence of PA, this CQ/amine photoinitiation system registered a DC at 64.07 ± 0.64 (Figure 3, Table 2). When an increasing amount of PA was added to the adhesive, the DC gradually descended into the sub-60% territory (blue line in Figure 3).

Based on the above results, the negative effect of PA on DC is evident. Proanthocyanidins (PA), and plant polyphenolics in general, are well-known antioxidants¹⁹. One major pathway for PA's capability to quench reactive oxidative radicals is hydrogen atom transfer²⁵. In this process, PA donates one or more H atoms from the phenolic functional groups, and PA itself is converted to a less reactive radical stabilized by intra-molecular hydrogen bonding or resonance structure (Scheme 2a). When involved in a radical polymerization, it hampers the chain initiation and propagation by donating hydrogen to initiation species (A• for CQ-based system) and propagation species (M•), terminating the chain reaction (Schemes 2b, 2c).

Among the myriad of factors that lead to the failure of dental adhesives, an unsatisfactory degree of conversion (DC) of resin monomers is no doubt one of the major contributors. A low DC could result in a number of detrimental events, such as lower mechanical strength of the adhesive, incomplete encapsulation of collagen fibrils, and poor marginal sealing. In the absence of PA, the widely-used CQ/amine photoinitiation system reached a DC at ~65% (Table 2), which is an acceptable value for most of light-cured adhesives²⁶. Using 65% as the target DC value, we examined the DC of the model adhesive with other different photoinitiator systems when an increasing amount of PA is incorporated.

Since the traditional CQ/amine system did not meet our criteria of a satisfactory DC in the presence of PA, we moved on to the second type of initiation system. The three-component CQ/amine/iodonium salt system (CQ/A/I-1 and CQ/A/I-2) emerged in the last decade as an improvement over the classical CQ/amine system^{22, 24, 27–30}. The additional component, iodonium salt (DPHIP in our case) is a hydrogen-accepting compound that can revert the chain-terminating CQ ketyl radical (CQH•) to the original initiator CQ, while generating a highly active and initiation-capable phenyl radical (Ph•)²⁷ (Scheme 1b). The triad of consumption of terminating radicals, re-generation of photosensitizer and production of additional reactive radicals synergistically increases the efficiency of the three-component initiation system. Indeed, our study showed that the DC of CQ/A/I-1 improved to near 70% in the absence of PA (Figure 3, Table 2), as compared to ~65% for its CQ/A counterpart. The addition of PA caused the decrease in DC of the CQ/A/I-1 system (red line in Figure 3), but it maintained above 65% in the presence of up to 2.5% PA.

Using the same CQ/amine/iodonium salt system, the amount of its constituents (CQ/A/I-2) was doubled in an effort to further increase the DC of PA-containing adhesives. Interestingly, the increased amount of initiators had no effect on the DC in the absence of PA (Figure 3, Table 2). This is an indication that the polymerization process is diffusion-controlled for the CQ/A/I system at the investigated initiator concentrations. When PA was incorporated, however, the DC of CQ/A/I-2 became significantly higher than that of CQ/A/I-1, suggesting that PA has retarded the polymerization to a point that the reaction shifts from diffusion-controlled to thermodynamically-controlled. Similar to other initiation systems, the increase in the amount of PA in CQ/A/I-2 led to the decrease in DC (green line in Figure 3), although the pattern between 0-PA and 2.5-PA was different. Nevertheless, the trend could have been the same if the DC of 0-PA CQ/A/I-2 were not limited by diffusion (dashed green line, Figure 3). The DC of CQ/A/I-2 maintained above 65% in the presence of up to 5% PA, which was an improvement over CQ/A/I-1.

The third type of photoinitiator system (TPO) contains one single component: trimethylbenzoyl-diphenylphosphine oxide (TPO). Photolysis of TPO affords 2,4,6-trimethylbenzoyl (TMB•) and diphenylphosphonyl (DPP•) radicals (Scheme 1c), the latter of which being the initiating species³¹. Since the phosphorous-based initiating species DPP• is 1–2 orders of magnitude more reactive than its carbon-based counterpart³² (such as A• in CQ-based system), TPO has been found to be an even more efficient photoinitiator than CQ-based systems^{33, 34}. Consistent with the findings in the literature, our study showed that TPO is indeed the most efficient photoinitiator as indicated by its highest DC among all initiation systems used for 0-PA. More specifically, the DC was calculated to be close to 80% in the absence of PA (Figure 3, Table 2). In contrast, the DC of all other formulations (CQ/A, CQ/A/I-1 and CQ/A/I-2) was lower than 70%. Similar to the CQ-based systems, the addition of PA in TPO also caused the decrease in DC (purple line in Figure 3). But even at 5% PA, the DC of TPO was still higher than 70%, superior to all other photoinitiator formulations regardless PA-free or not. When the amount of PA was further increased to 10%, however, the DC of TPO dropped below 65%, indicating that the upper limit of PA content is 5–10% in order to obtain an acceptable DC for the TPO-based system.

PA's presence decreased the three-component (CQ/A/I-1, CQ/A/I-2) and single-component systems' (TPO) polymerization rate (PR) in a pattern similar to DC although the changes are less pronounced (Figure 4, Table 2). The change of PR with respect to PA content for CQ/amine two-component system (CQ/A), however, was distinctively different from that for CQ/A/I and TPO. More specifically, the PR curve of CQ/A features an up-and-down pattern as the PA concentration increases, with the peak PR found at 5% PA. It is believed that this phenomena is due to PA's ability to react with molecular oxygen in the presence of reduced transitional metals such as iron and copper ions³⁵. The grape seed extract PA used most

likely contains trace amount of these transitional metals from the manufacturing process³⁵. Since molecular oxygen is known to quench the camphorquinone triplet (CQ*)³⁶ which is essential to generating initiation species (Scheme 1a), the presence of PA could increase the quantum yield of CQ by eliminating the CQ*-consuming molecular oxygen. This pro-initiation behavior of PA counteracts with its anti-initiation radical-scavenging action, and the combined outcome is a non-monotonic function as shown for CQ/A. In contrast, in the CQ/EDMAB/DPIHP system (CQ/A/I-1, CQ/A/I-2), the iodonium salt DPIHP removes the product of the triplet CQ* initiating process (CQH• in Scheme 1b) and in effect leads to an already elevated quantum yield. In the TPO-based system (TPO), there is no photosensitizer to begin with. Consequently, for CQ/A/I and TPO, PA's radical-scavenging effect plays the dominant role in the initiation step, resulting in the pseudo-monotonic behavior of PR with respect to PA concentration. A more detailed kinetics study is certainly warranted to verify this hypothesis, but it is beyond the scope of the present work.

The results of this study corroborated that the incorporation of PA into dental adhesive adversely affected photo-polymerization. The DC of the adhesive decreased as a function of PA concentration. The DC was also dependent on the photo-initiators used. When the photo-initiator was the traditional CQ/A system, the DC was all below 65% in the presence of PA. Using the same CQ/A initiation system, it was previously reported that only 2% or less PA could be incorporated into the adhesive, which showed no adverse effect on dentin bond strength²¹. The addition of 3% PA would significantly lower the bond strength to ~50% of the original value²¹. Although it is unknown about the minimum concentration of PA needed for protection of collagen from degradation, so far 5% or more PA concentration has been used to show the collagen crosslinking capability in most of the recent studies^{11–13}. Based on the present study, this issue could be resolved by changing the concentration and type of photo-initiators. As shown in this study, when the photo-initiator was the CQ/amine/iodonium salt at high concentration (CQ/A/I-2) or TPO system, the DC could be maintained at 65% or more in the presence of up to 5% PA. Certainly, there are still questions that remain unanswered, such as: does a superior DC necessarily lead to a more stable interface? What is the optimal PA concentration to maintain not only a high DC but also a minimal effect of its release on the resin integrity? In future studies, we would like to answer these questions through comprehensive investigation of PA-containing adhesives' long-term interfacial morphology and physicochemical stabilities.

5. Conclusion

In this study, we have examined the effect of PA's presence on the polymerization behavior of a Bis-GMA/HEMA model adhesive with three different photoinitiators, including the CQ/amine, CQ/amine/iodonium salt, and TPO systems. It is found that PA hampers the monomer conversion and alters the polymerization kinetics in all adhesives regardless of photoinitiators used. The TPO system exhibits the most efficient polymerization as demonstrated by its considerably higher degree of conversion (DC). At a PA concentration as high as 5%, the CQ/A/I-2 or TPO system maintains its superiority in DC to the other initiation systems. These findings establish these two systems as a better fit to be used in PA-containing adhesives.

Acknowledgments

This investigation was supported in part by USPHS Research Grants R15-DE021023 from the National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD 20892. The authors would like to thank Dr. Ying Liu for her assistance with statistical analysis.

REFERENCES

1. Bowen RL. Adhesive bonding of various materials to hard tooth tissues. II. Bonding to dentin promoted by a surface-active comonomer. *J Dent Res*. 1965; 44(5):895–902. [PubMed: 5213019]
2. Van Nieuwenhuysen JP, D'Hoore W, Carvalho J, Qvist V. Long-term evaluation of extensive restorations in permanent teeth. *Journal of Dentistry*. 2003; 31(6):395–405. [PubMed: 12878022]
3. Spencer P, Ye Q, Park J, Topp EM, Misra A, Marangos O, et al. Adhesive/dentin interface: The weak link in the composite restoration. *Annals of Biomedical Engineering*. 2010; 38(6):1989–2003. [PubMed: 20195761]
4. De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M, et al. A critical review of the durability of adhesion to tooth tissue: Methods and results. *Journal of Dental Research*. 2005; 84(2):118–132. [PubMed: 15668328]
5. Inoue S, Vargas MA, Abe Y, Yoshida Y, Lambrechts P, Vanherle G, et al. Microtensile bond strength of eleven contemporary adhesives to dentin. *Journal of Adhesive Dentistry*. 2001; 3(3): 237–245. [PubMed: 11803711]
6. Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by the infiltration of monomers into tooth substrates. *Journal of Biomedical Materials Research*. 1982; 16(3):265–273. [PubMed: 7085687]
7. Wang Y, Spencer P. Quantifying adhesive penetration in adhesive/dentin interface using confocal Raman microspectroscopy. *Journal of Biomedical Materials Research*. 2002; 59(1):46–55. [PubMed: 11745536]
8. Wang Y, Spencer P. Hybridization efficiency of the adhesive/dentin interface with wet bonding. *Journal of Dental Research*. 2003; 82(2):141–145. [PubMed: 12562889]
9. Breschi L, Martin P, Mazzoni A, Nato F, Carrilho M, Tjäderhane L, et al. Use of a specific MMP-inhibitor (galardin) for preservation of hybrid layer. *Dental Materials*. 2010; 26(6):571–578. [PubMed: 20299089]
10. Bedran-Russo AKB, Pereira PNR, Duarte WR, Drummond JL, Yamaychi M. Application of crosslinkers to dentin collagen enhances the ultimate tensile strength. *Journal of Biomedical Materials Research - Part B Applied Biomaterials*. 2007; 80(1):268–272.
11. Bedran-Russo AKB, Pashley DH, Agee K, Drummond JL, Miescke KJ. Changes in stiffness of demineralized dentin following application of collagen crosslinkers. *Journal of Biomedical Materials Research - Part B Applied Biomaterials*. 2008; 86(2):330–334.
12. Castellan CS, Pereira PN, Grande RHM, Bedran-Russo AK. Mechanical characterization of proanthocyanidin-dentin matrix interaction. *Dental Materials*. 2010; 26(10):968–973. [PubMed: 20650510]
13. Green B, Yao X, Ganguly A, Xu C, Dusevich V, Walker MP, et al. Grape seed proanthocyanidins increase collagen biodegradation resistance in the dentin/adhesive interface when included in an adhesive. *Journal of Dentistry*. 2010; 38(11):908–915. [PubMed: 20709136]
14. Castellan CS, Bedran-Russo AK, Karol S, Pereira PNR. Long-term stability of dentin matrix following treatment with various natural collagen cross-linkers. *Journal of the Mechanical Behavior of Biomedical Materials*. 2011; 4(7):1343–1350. [PubMed: 21783144]
15. Xu C, Wang Y. Cross-linked demineralized dentin maintains its mechanical stability when challenged by bacterial collagenase. *Journal of Biomedical Materials Research - Part B Applied Biomaterials*. 2011; 96 B(2):242–248.
16. Xu C, Wang Y. Collagen cross linking increases its biodegradation resistance in wet dentin bonding. *The journal of adhesive dentistry*. 2012; 14(1):11–18. [PubMed: 21594232]
17. Tezvergil-Mutluay A, Mutluay MM, Agee KA, Seseogullari-Dirihan R, Hoshika T, Cadenaro M, et al. Carbodiimide cross-linking inactivates soluble and matrix-bound MMPs, in vitro. *Journal of Dental Research*. 2012; 91(2):192–196. [PubMed: 22058118]
18. Han B, Jaurequi J, Tang BW, Nimni ME. Proanthocyanidin: A natural crosslinking reagent for stabilizing collagen matrices. *Journal of Biomedical Materials Research - Part A*. 2003; 65(1):118–124. [PubMed: 12635161]

19. Quideau S, Deffieux D, Douat-Casassus C, Pouységu L. Plant polyphenols: Chemical properties, biological activities, and synthesis. *Angewandte Chemie - International Edition*. 2011; 50(3):586–621.
20. Hechler B, Yao X, Wang Y. Proanthocyanidins Alter Adhesive/Dentin Bonding Strengths when Included in a Bonding System. *American Journal of Dentistry*. 2012 in press.
21. Epasinghe DJ, Yiu CKY, Burrow MF, Tay FR, King NM. Effect of proanthocyanidin incorporation into dental adhesive resin on resin-dentine bond strength. *Journal of Dentistry*. 2012; 40(3):173–180. [PubMed: 22155037]
22. Wang Y, Spencer P, Yao X, Ye Q. Effect of coinitiator and wafer on the photoreactivity and photopolymerization of HEMA/camphorquinone-based reactant mixtures. *Journal of Biomedical Materials Research - Part A*. 2006; 78(4):721–728. [PubMed: 16739171]
23. Angiolini L, Caretti D, Salatelli E. Synthesis and photoinitiation activity of radical polymeric photoinitiators bearing side-chain camphorquinone moieties. *Macromolecular Chemistry and Physics*. 2000; 201(18):2646–2653.
24. Kunio I, Takeshi E. A review of the development of radical photopolymerization initiators used for designing light-curing dental adhesives and resin composites. *Dental Materials Journal*. 2010; 29(5):481–501. [PubMed: 20859059]
25. Wright JS, Johnson ER, DiLabio GA. Predicting the activity of phenolic antioxidants: Theoretical method, analysis of substituent effects, and application to major families of antioxidants. *Journal of the American Chemical Society*. 2001; 123(6):1173–1183. [PubMed: 11456671]
26. Powers, J.; Sakaguchi, R. *Craig's Restorative Dental Materials*. 12th Edition. St. Louis: C.V. Mosby, Co; 2006.
27. Padon KS, Scranton AB. Mechanistic investigation of a three-component radical photoinitiator system comprising methylene blue, N-methyldiethanolamine, and diphenyliodonium chloride. *Journal of Polymer Science, Part A: Polymer Chemistry*. 2000; 38(11):2057–2066.
28. Grotzinger C, Burget D, Jacques P, Fouassier JP. Visible light induced photopolymerization: Speeding up the rate of polymerization by using co-initiators in dye/amine photoinitiating systems. *Polymer*. 2003; 44(13):3671–367.
29. Kim D, Stansbury JW. Kinetic pathway investigations of three-component photoinitiator systems for visible-light activated free radical polymerizations. *Journal of Polymer Science, Part A: Polymer Chemistry*. 2009; 47(3):887–898.
30. Cook WD, Chen F. Enhanced photopolymerization of dimethacrylates with ketones, amines, and iodonium salts: The CQ system. *Journal of Polymer Science, Part A: Polymer Chemistry*. 2011; 49(23):5030–5041.
31. Kajiwar A, Konishi Y, Morishima Y, Schnabel W, Kuwata K, Kamachi M. Timeresolved electron spin resonance study on radical polymerization with (2,4,6- trimethylbenzoyl)diphenylphosphine oxide. Direct estimation of rate constants for addition reactions of diphenylphosphonyl radicals to vinyl monomers. *Macromolecules*. 1993; 26(7):1656–1658.
32. Sluggett GW, Turro C, George MW, Koptug IV, Turro NJ. (2,4,6-Trimethylbenzoyl)diphenylphosphine oxide photochemistry. A direct time-resolved spectroscopic study of both radical fragments. *Journal of the American Chemical Society*. 1995; 117(18):5148–5153.
33. Neumann MG, Schmitt CC, Ferreira GC, Corrêa IC. The initiating radical yields and the efficiency of polymerization for various dental photoinitiators excited by different light curing units. *Dental Materials*. 2006; 22(6):576–584. [PubMed: 16289725]
34. Leprince JG, Hadis M, Shortall AC, Ferracane JL, Devaux J, Leloup G, et al. Photoinitiator type and applicability of exposure reciprocity law in filled and unfilled photoactive resins. *Dental Materials*. 2011; 27(2):157–164. [PubMed: 21067803]
35. Danilewicz JC. Review of reaction mechanisms of oxygen and proposed intermediate reduction products in wine: Central role of iron and copper. *American Journal of Enology and Viticulture*. 2003; 54(2):73–85.
36. Yagci Y, Jockusch S, Turro NJ. Photoinitiated polymerization: Advances, challenges, and opportunities. *Macromolecules*. 2010; 43(15):6245–6260.

Figure 1.

(a) Time-resolved FTIR spectra of the adhesive (CQ/A/I-1) in the absence of PA. Light bulb: light-curing; (b) Representative spectrum before light-curing; (c) Representative spectrum after light-curing; (d) Functional groups responsible for the peaks at ~1637 cm⁻¹ and ~1608 cm⁻¹

Figure 2.

Real-time degree of conversion (DC) of adhesives with (a) CQ/A, the CQ/EDMAB photoinitiator; (b) CQ/A/I-1, the CQ/EDMAB/DPIHP photoinitiator; (c) CQ/A/I-2, the CQ/EDMAB/DPIHP photoinitiator with doubled initiator amount; and (d) TPO, the TPO photoinitiator. Red line: 65% of DC

Figure 3.
Degree of conversion (DC) of all adhesives as a function of PA concentration.

Figure 4.
Polymerization rate (PR) of all adhesives as a function of PA concentration.

Scheme 1.

(a) Mechanism of the photoinitiation of CQ/EDMAB (CQ/A) system; (b) Function of DPIHP in the CQ/EDMAB/DPIHP (CQ/A/I) system; (c) Mechanism of the photoinitiation of TPO system. (Red: reactive species)

Scheme 2.

(a) General mechanism of PA's hydrogen-donating capability; (b) Termination of initiation species by hydrogen atom transfer from PA; (c) Termination of propagation species by hydrogen atom transfer from PA. (Red: reactive species)

Table 1

Formulations of the four photoinitiators studied (weight with respect to Bis-GMA/HEMA resin)

Denotation	Components			
	CQ	EDMAB	DPIHP	TPO
CQ/A	0.5 wt%	0.5 wt%	-	-
CQ/A/I-1	0.5 wt%	0.5 wt%	0.5 wt%	-
CQ/A/I-2	1.0 wt%	1.0 wt%	1.0 wt%	-
TPO	-	-	-	2.1 wt%

Table 2

Degree of conversion (DC) and polymerization rate (PR) of adhesives with different initiator systems and various amount of PA (n=3).

	Degree of Conversion (DC, %)			
	0-PA (0/60/40)*	2.5-PA (1.5/60/38.5)	5-PA (3/60/37)	10-PA (6/60/34)
CQ/A	64.07 ± 0.64 ^{a,A}	62.16 ± 0.30 ^{a,b,A}	60.9 ± 0.50 ^{b,A}	56.94 ± 1.59 ^{c,A}
CQ/A/I-1	69.53 ± 0.35 ^{a,B}	66.00 ± 0.66 ^{b,B}	63.53 ± 0.43 ^{c,B}	59.45 ± 0.52 ^{d,A,B}
CQ/A/I-2	68.44 ± 1.06 ^{a,B}	69.00 ± 0.27 ^{a,C}	67.26 ± 0.35 ^{a,C}	61.49 ± 0.84 ^{b,B,C}
TPO	77.57 ± 0.63 ^{a,C}	74.11 ± 0.34 ^{b,D}	71.28 ± 0.19 ^{c,D}	63.98 ± 0.72 ^{d,C}
	Polymerization Rate (PR, %/s)			
	0-PA (0/60/40)	2.5-PA (1.5/60/38.5)	5-PA (3/60/37)	10-PA (6/60/34)
CQ/A	4.92 ± 0.08 ^{a,A}	5.19 ± 0.25 ^{a,A}	6.49 ± 0.23 ^{b,A}	4.98 ± 0.45 ^{a,A}
CQ/A/I-1	16.76 ± 0.21 ^{a,B}	9.96 ± 0.51 ^{b,B}	9.12 ± 0.42 ^{b,c,A}	7.86 ± 0.73 ^{c,B}
CQ/A/I-2	17.45 ± 0.86 ^{a,B}	13.19 ± 0.57 ^{b,C}	15.03 ± 2.23 ^{a,b,B}	12.35 ± 0.33 ^{b,C}
TPO	24.43 ± 1.34 ^{a,C}	20.29 ± 1.66 ^{a,b,D}	19.64 ± 1.58 ^{b,c,C}	15.92 ± 1.82 ^{c,D}

* Ratio in parenthesis indicates the PA/resin/ethanol weight ratio of each formulation.

** Values with same lower case letters are statistically equivalent within the same initiator formulation.

*** Values with same capital letters are statistically equivalent within the same PA concentration.