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Dentin Biomodification Potency of Proanthocyanidins

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Introduction

With the advances in chemical and biomedical sciences, there has been a movement to remove mercury from dental materials. Mercury containing amalgams have been used in dental fillings for over 150 years, but as new materials with competitive properties arise, dentists now have the opportunity to use alternative materials[1]. Before exploring the properties of alternative materials, it is important to understand amalgams and why it has remained in use for several centuries. An amalgam by definition is "an alloy of mercury with another metal that is solid or liquid at room temperature according to the proportion of mercury present and is used especially in making tooth cements."[2] Essentially, an amalgam is a mercury, silver, tin, and copper mixture that forms a malleable material, ideal for filling cavities. At the chemical reaction progresses, the malleable material hardens and forms a durable material. Some of the benefits of amalgams include: being low cost, having low moisture sensitivity during application, having bacteriostatic properties, and having a greater durability comparted to resins.

Some of the topics that will be discussed in this paper are a brief history of dentistry, the development of amalgams, the development of resin composites, followed by current research into Proanthocyanidins. "Physical properties of particular importance in dental materials are dimensional stability, tensile and compressive strength, thermal conductivity, color and translucence. As well, the material should be biologically and chemically compatible in the mouth, inexpensive, and easy to prepare in the dentist office."[3] Finding a material that satisfies the demanding physical and chemical variables of a filling can be very difficult. Ideally, the material used in a filling would be as similar to the original tooth in all of these areas, but we have yet to create a compound capable of doing so. Many of the materials currently in use can excel in one of the categories, and yet fall short in another. Like while amalgams have high durability they fall short in cosmetic appearance. And while composite resins excel in cosmetic appearance, they fall short

in durability. Research into chemical additives, such as proanthocyanidins, show promise as they are able to directly address the specific chemical or physical shortcoming.

Amalgams

Amalgams are one of the earliest developed and longest lasting materials in the history of dental compounds. The first documentation of amalgams is from 659 A.D. in China during the Tang Dynasty.[4] It was not until nearly a millennia later that amalgams made their way to Europe. The earliest European documentation of amalgams occurred in 1525 in Germany. Although amalgams were around in 1525, it was not till the 1800's that amalgams became the primary restorative dental material in standard practice. The use of amalgams in dentistry continues to be a part of common practice in the modern age. In order for a material to continue to be in use over such a long period of time, without being substituted with an alternative, shows how effective amalgams are as a filling material. Some of the reasons why amalgams have remained around for so long, is they are low cost, highly durable, long lasting, low moisture sensitivity during application, and bacteriostatic. These properties are highly aligned with the properties of the natural tooth structure.

Low Copper Alloy

There are several types of amalgams, but the two types that will be discussed in this paper is low copper alloys and admixed alloys. The key on the following page shows some of the important components of amalgams, along with the standard abbreviations. In a low copper alloy, γ and β react with mercury to form γ, γ_1, and γ_2, as shown below. As the reaction begins, the amalgam starts out soft as the γ_1 and γ_2 phases begin formation. As time passes, the reaction forms γ_1 and γ_2 crystals, which in turn increases the strength and hardness of the material. The time frame between being soft and malleable to hard and no longer workable is called the working time. This limited time frame makes it especially important to work efficiently when filling a cavity.

Key:

γ : Ag3Sn (mechanically the strongest)

γ_1 : Ag2Hg3 (major matrix phase in set amalgam)

γ_2 : Sn8Hg (weakest phase, corrodes easily)

β : Ag5Sn

η' : Cu_6Sn_5

ε : Cu_3Sn

Low Copper Alloy

$Ag_3Sn, Ag_5Sn + Hg \rightarrow Ag_2Hg_3 + Sn_8Hg + Ag_3Sn$

i.e. $(\gamma + \beta) + Hg \rightarrow \gamma_1 + \gamma_2 + \gamma$

The major structural component of a low coper amalgam is γ_1. This is also known as the major matric phase of the amalgam. The silver from γ_1 has a solubility of 0.035% by weight in mercury, where the tin from γ_2 has a solubility of 0.6% by weight in mercury. The γ_1 forms first as it is less soluble in mercury compared to γ_2. The precipitation of γ_1, forms with a base centered cubic, BCC, structure. The BCC structure gives γ_1 structural integrity, making it ideal for dental fillings. Due to the tin in γ_2 being slightly more soluble than silver in γ_1, γ_2 precipitates after γ_1. Instead of having BCC structure, γ_2 has a hexagonal 3D structure, a significantly weaker structure compared to BCC. To provide reference on the strength of the two substances, γ_2 only has around 10% of the structural strength as compared to γ_1. Due to the relative weakness of γ_1, it is desirable to remove it from the amalgam.[5]

Typically, the amount of γ (Ag_3Sn) unreacted material, ranges from 27-35%. Whereas the amount of γ_1 (Ag_2Hg_3) and γ_2 (Sn_8Hg) range from 54-56% and 11-13% respectively. If there is always unreacted starting material, γ (Ag_3Sn), it may seem logical to add more liquid mercury so the γ (Ag_3Sn) can react to completion. While the sources I found did not explicitly state why more Hg is not added to the reaction, it is likely to prevent excess unreacted Hg from being present. Unreacted γ(Ag_3Sn) poses no biological threat to the patient whereas unreacted liquid Hg has many biological hazards.[5]

Admixed Alloy

High copper alloys, such as an admixed alloy, is able to remove the γ_2 phase from the compound by the addition of a copper compound, AgCu. AgCu reacts with the γ_2, forming Cu_6Sn_5, also referred to as η'. In removing the γ_2 compound and replacing it with η', the amalgam alloy has an improved structural strength, allowing it to have an increased durability and longevity as a filling.[6] While low copper alloys and admixed alloys have significant structural integrity, low cost, and

relatively simple application process, the presence of lead makes them less than ideal.

Admix Alloy

$\gamma(Ag_3Sn) + Ag\text{-}Cu\ (eutectic) + Hg \rightarrow \gamma 1\ (Ag_2Hg_3) + \eta'\ (Cu_6Sn_5) +$ unreacted $\gamma\ (Ag_3Sn)$ + unreacted Ag-Cu (eutectic)

i.e. $\gamma + Ag\text{-}Cu + Hg \rightarrow \gamma_1 + \eta' + \gamma_1 + Ag\text{-}Cu$

Amalgam Disadvantages

One drawback of amalgams is they often require a more invasive filling procedure. Figure 1 below[7] is of a tooth requiring a filling. The drop-like shape on the occlusion surface of the tooth represents decay. If this tooth were to receive an amalgam filling, all of the area between the black lines would require removal. The area of removal is in the shape of a dove tail due to amalgams lacking the capability to bond to the tooth surface. While cements can be used when applying amalgams, it is not very common practice. The shape of the hole is critical to the longevity of the filling. Without the proper shape, the amalgam filling can easily fall out. Unfortunately, the requirement of a dove tail shaped hole also means that healthy tooth structure is often removed in the process, resulting in a more invasive procedure. While occlusion (biting surface) fillings can be treated by the methods described above, lingual, buccal, labial, and vestibular surfaces are more difficult to treat and can often be even more invasive. There is less enamel and dentin on the sides of the teeth, which makes it more difficult to create a dove tail shaped hole without compromising the strength of the remaining healthy tooth structure. Often these types of fillings are connected to occasional fillings to provide structural integrity.[5]

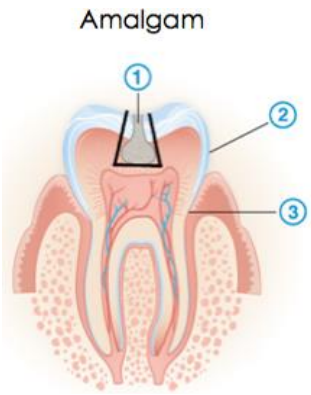

Figure 1: 1 indicates the site of decay. 2 refers to the enamel structure. 3 refers to the dentin structure. The black line represents the tooth structure needing to be removed for an amalgam filling.[7]

While more invasive fillings are a drawback of amalgam fillings, the main factor pushing for an alternative method of treatment is the cosmetic factor. Amalgams have a grey silver color as a metallic substance, which comes in stark contrast to the coloring of the tooth. Ideally, the filling would be as similar as possible to the original tooth

in biocompatibility, durability, and appearance. The need for more cosmetically appealing materials is one of the reasons why resins were developed. [6]

Resins

There are four main components of resins: the polymer matrix, inorganic filler, coupling agent, and the indicator and accelerator. The most types of polymer matrix are shown below, Bis-GMA: 2,2-UDMA: Urethane dimethacrylate (figure 2)[8] and Bis[4-(2-hydroxy-3-methacryloxypropoxy) phenyl]propane (figure 3)[9]. The double bonds on the ends of these compounds allow the polymerization reaction to occur once activated by free radical initiators. While there are many other polymerization compounds, these are the most popular compounds for dental resins currently.

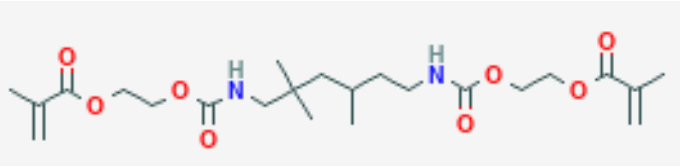

Figure 2: UDMA: Urethane dimethacrylate, a common polymer matrix.[8]

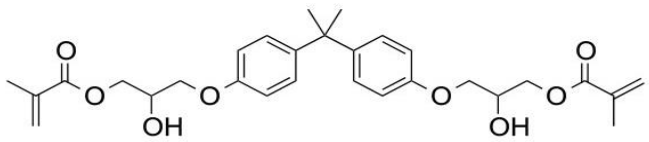

Figure 3: Bis-GMA: 2,2-Bis[4-(2-hydroxy-3-methacryloxypropoxy) phenyl]propane is a common polymer matrix.[9]

When selecting a polymer matric it is important to choose one that minimizes shrinking, demonstrates biocompatibility, and has a quick setting time.

The second component of a resin filling is the inorganic filler particles. The filler is composed of either quartz, glass, or a sol-gel derived ceramic. The filler comes in a variety of sizes, from 23-30 um (macro fills) to 1-100nm (Nano fills). Typically, a hybrid mixture is used, containing some macro and nano filler. The variety in size allow for more effective packing and greater structural integrity. The inorganic filler serves three main functions: reinforcing the resin matrix, providing a degree of translucency, and controlling the volume shrinkage during polymerization. Figure 4, shown below, shows the variety in size of composite fillers.[10]

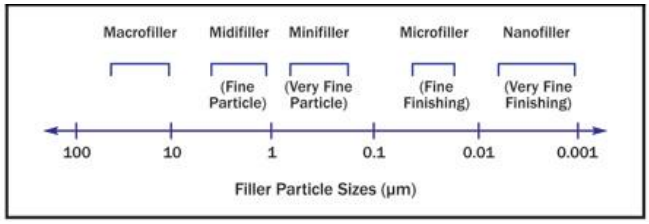

Figure 4: Composite filler ranges versus particle size. This chart demonstrates the wide range of particle size.[10]

The third component of resins is the coupling agent. The role of the coupling agent is to form a bridge between the inorganic filler and the

organic polymer matrix. Silicon containing compounds, like MPTS: 3-Methacryloxypropyltrimethoxysilane (shown in figure 5)[11], are used to form the bridging bonds. The methoxy groups hydrolyze to form hydroxyl groups through acid or base catalyzed reactions. The hydroxyl groups then bind covalently to the hydroxyl groups of the inorganic filler. During the final curing step, the methacryloxy groups react with the resins, completing the bridging bond, as shown in figure 6.[5] Without a coupling agent, the organic and inorganic compounds in the filling would be unable to bind to each other.

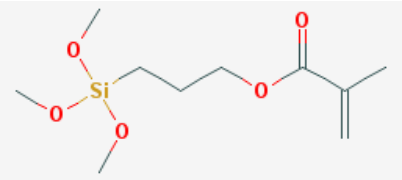

Figure 5: MPTS: 3-Methacryloxypropyltrimethoxysilane is a common coupling agent, responsible for forming bridging bonds between the organic matrix and the inorganic filler.[11]

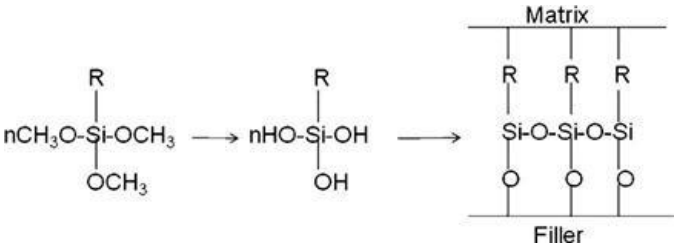

Figure 6: Silicon compounds, like MPTS, form a bridging bond between the organic matrix and the inorganic filler. The bridging bonds are necessary for the strength of the filling and integration of the resin components.[5]

The fourth and final component to a filling is the indicator and accelerator. The function of the indicator and accelerator is to initiate the polymerization reaction of the polymer through free radicals. There are two main categories of accelerators: light initiated and chemical activation (figure 7). Champhorquinone is an accelerator that has peak wavelength absorption of 465nm. At this wavelength, the wavelength of light is blue. Champhorquinone is the reason why dentists shine a blue light on a filling once completed. When Champhorquinone absorbs light at this wavelength, it generated a free radical, which can in turn initiate the polymerization reaction. The other method of curing a filling is through chemical activation. This is where an amine is mixed in with the organic polymer, inorganic filler, and the coupling agent right before filling the cavity. The disadvantage of this method is as soon as the material is mixed, the material begins to polymerize and set. This can be difficult for the dentist as he has greater time restrictions. The ability to choose when the material begins to set, as with light initiated accelerators, is preferable.[6]

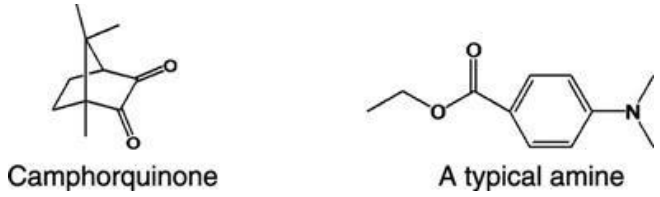

Figure 7: Champhorquinone absorbs light at 465nm to generate free radicals, which can initiate the polymerization reaction. Amines begin reacting with the polymer upon contact, making it more difficult to time the setting of the resin material.[5]

Benefits and Disadvantages of Resins

One of the major benefits of resin fillings is the ability to have less invasive fillings. Unlike amalgam fillings, resins bond directly to the dentin matrix through a bonding agent. Figure 8 shows how the tooth structure prior to applying a resin filling is limited to the area of decay.[7] This varies drastically from the amalgam filling where a dovetail shaped hole was necessary to hold the amalgam in place. In limiting the removed tooth structure to the area of decay, the process of applying a filling is less invasive and a greater amount of the healthy tooth structure can remain intact. While the procedure is less invasive, the actual filling has a shorter lifespan. Where an amalgam lasts from 10-15 years, a resin composite filling has a limited lifespan of 5-8 years.[12] In particular, resins in high stress locations, such as occlusion surfaces, tend to underperform long term in comparison to amalgams.[13] While there are many benefits of resins over amalgams, there is room for improvement in durability and biocompatibility. It is for this reason, there has been a push in researching alternative materials and improved methods for resin fillings. For the time being, many insurance companies provide coverage for resin fillings up to the cost of the amalgam, but any additional cost must be paid out of pocket. While resins continue to be more expensive than amalgams, it is possible that if resins improve to the point of outperforming amalgams in durability and longevity, then insurance companies could provide additional financial support for the application of resins over amalgams.

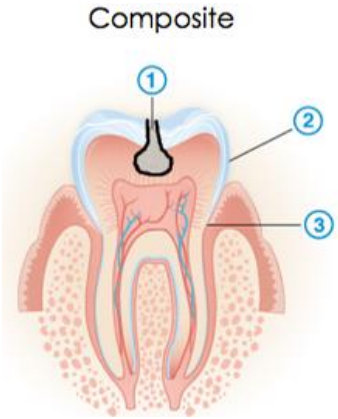

Figure 8: 1 indicates the site of decay. 2 refers to the enamel structure. 3 refers to the dentin structure. The black line immediately surrounding the site of decay represents the tooth structure needing to be removed for a composite filling.[7]

Proanthocyanidins

While the effects of proanthocyanidins, PACs, have been known for many years, it was not

until recently that they have researched into applying them to dentistry. PACs antimicrobial, antiadhesion, antioxidant, anti-inflammatory properties[14] made it a good candidate for potential dental applications.[15] As a flavonoid, PACs are present in many biological materials including berries and pine bark. Typically, berries and fruits had the largest quantities of proanthocyanidins, with the exception of citrus fruits, where vegetables and roots lacked proanthocyanidins.[14] Proanthocyanidins are composed of repeating flavan-3-ol monomeric building blocks. The number of favan-3-ols and orientation of bonding determine the chemical properties of the PAC. The proanthocyanidin shown below in figure 9[16]

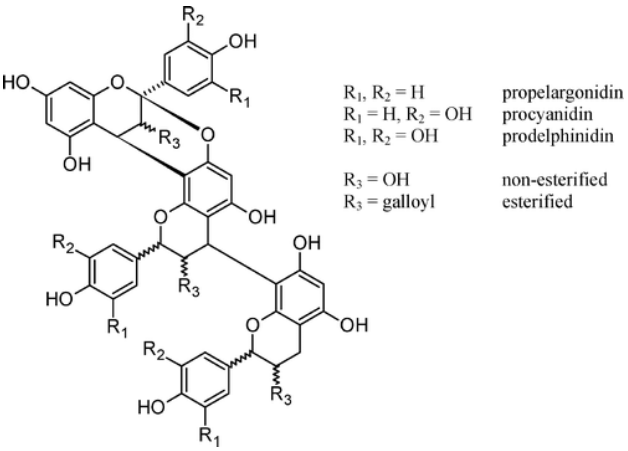

Figure 9: The trimer shown above is an example of a simple proanthocyanidin. The monomer of the PAC polymer is flavan-3-ol. In this case, the polymer consists of three flavan-3-ol monomers.[16]

provides an example of a typical PAC consisting of three flavan-3-ols, also known as a trimer.

Types or Proanthocyanidins

There are three different types of proanthocyanidins (PACs): procyanidins, oligomeric proanthocyanidins (OPACs), and polymeric proanthocyanidins (polymeric PACs). These categories are based on the hydroxylation patterns of the A and B rings of the flavanone skeleton. Procyanidins are PACs containing (-) epicatechin or (+) catechin as their building blocks. OPACs are composed of (-) epicatechin (2,3 cis) and (+) catechin (2,3 trans). Polymeric PACs, commonly referred to as tannins, form complex aggregates. Polymeric PACs, tannins, are typically insoluble in polar organic solvents and result in the precipitation of proteins.[17] Figure 10 shows a few of the possible PAC isomers possible and the degree of diversity in binding as well as the diversity in number of bonded flavan-3-ols.[17] Due to the high degree of diversity it can be exceptionally difficult to isolate a specific PAC with

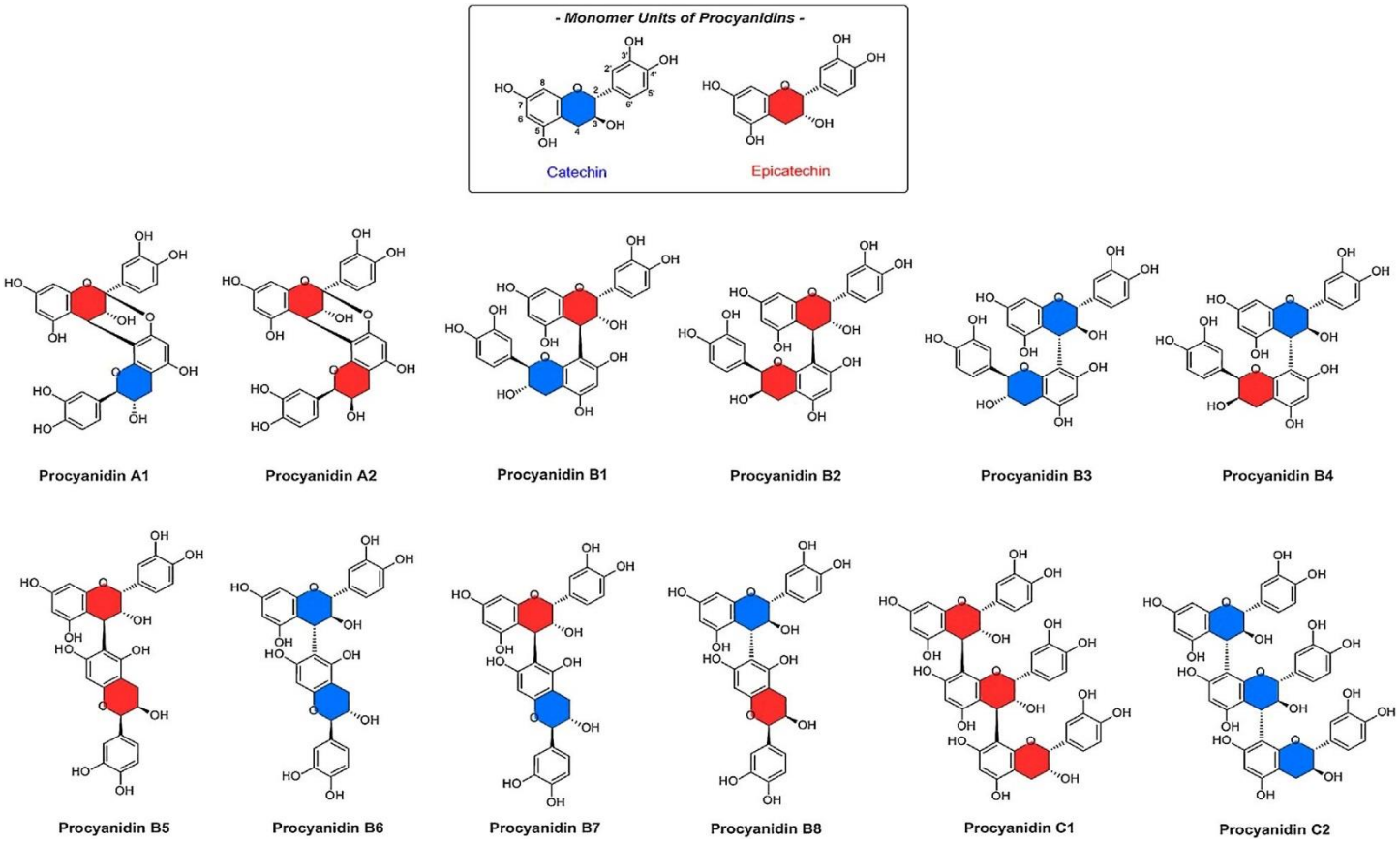

Figure 10: The figure above shows a few of the many isomeric configurations of PACs. PACs can differ in bond orientation and number of flavan-3-ol monomers. As a result, the degree a variability creates a variety in chemical properties.[17]

the same number of flavan-3-ol monomers and the same bonding configuration.

Adhesion studies

The Journal of Dental Research published a paper in 2016, written by the Department of Restorative Dentistry, College of Dentistry at UIC, on *Biostability of the Proanthocyanidins – Dentin Complex and Adhesion Studies.* The journal explored the application of proanthocyanidins as a potential adhesive substance to strengthen the bonding phase of a resin filling. Figure 11 shows the step by step process of filling a cavity with a composite resin filling.[18] The bonding phase is a critical step in the resin filling process, as the strength of the bond can determine the life length of the filling. If the bond is not effective, or wears out, then the filling can fail. "The mechanism of adhesion relies on the formation of a hybrid zone, with micromechanical interlocking between the resin and the collagen-rick dentin matrix. The degradation of unprotected collagen at the resin-dentin adhesive interface by activation of host-

Figure 11: The figure above shows the step-by-step procedure for composite resin fillings. One area of research is looking into applying PACs during the bonding phase.[18]

derived enzymes is also associated with early failure of resin composite restorations (Mazzoni et al. 2012)." In other words, the interface between resin and dentin is critical to the overall durability and life of a filling. The most frequent cause of failed adhesive restorations is secondary caries and marginal breakdown. If the bonding of a resin to the dentin of the tooth were to become stronger, then this could lower the number of failed restorations, and improve the lifespan of composite fillings.[19]

The proanthocyanidins used in the 2016 adhesion study by the college of UIC were extracted from grape seeds of *Vitis Vinifera.*[19] The higher-order oligomeric and polymeric PACs were removed by 2-phase partitioning procedure, resulting in an enriched grape seed extract (e-GSE). The e-GSE was applied to a dentin matrix during the bonding phase of the resin restoration procedure. After curing and setting, the physical properties of the e-GSE filling was tested and compared to a control filling. One of the most notable results was the drastic change in dentin elasticity. Figure 12, shows the effect e-GSE has on short and long-term elasticity.[19] The viscoelastic properties of collagen were altered, becoming more elastic and less viscus. When a low concentration of e-GSE was applied, there was a gradual increase in elasticity (E_r). While if a high concentration of e-GSE were applied, such as 30% e-GSE, then there was a rapid increase in E_r.[19]

The other notable result of this experiment is the microtensile bond strength results (Figure 13). The graph shows the effect of hydrophobicity and age on the s-GSE treated filling versus the control. The short-term data, 24 hours, shows the e-GSE to have a significantly higher bond strength than the control. This trend continues when

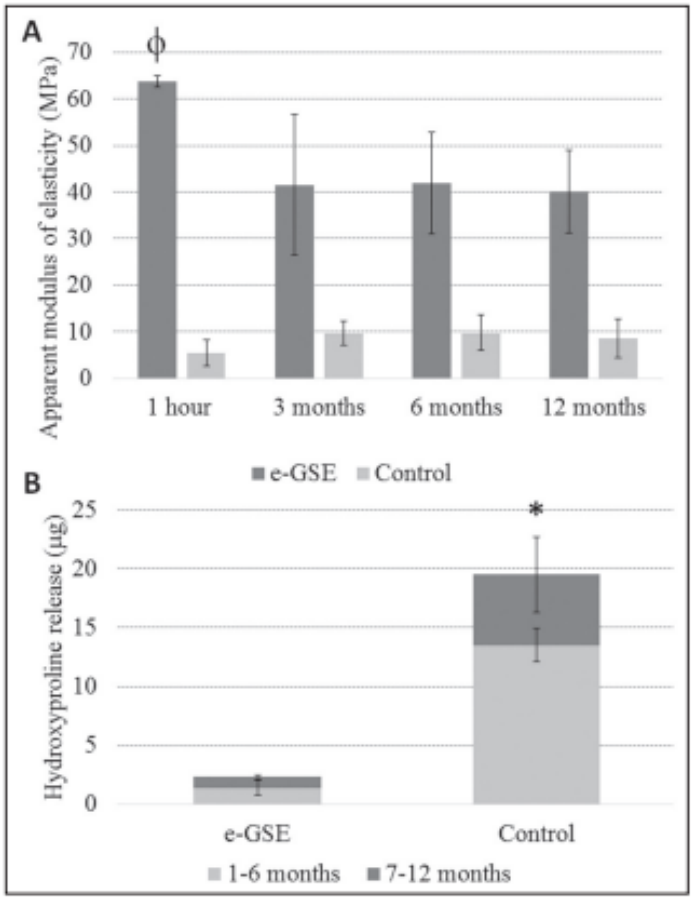

Figure 12: Results of the long-term apparent modulus of elasticity (A) of the dentin matrix and collagen solubilization by endogenous proteases reported as cumulative hydroxyproline (HYP; B) released in the 0 to 6 mo (lighter gray) and between 7 and 12 mos (darker gray).[19]

looking at the long-term, 1 year, bond strength test. Notably, the bond strength remains around 65 MPa with only a slight decrease in bond strength with age. In contrast, the control exhibits a greater degree of decreased bond strength over the time progression from 24 hours to 1 year. The terms H18, H6, and H0 refer to the concentrations of 2-hydroxyethyl methacrylate (HEMA), (18% HEMA, 6% HEMA, and 0% HEMA respectively). HEMA, a

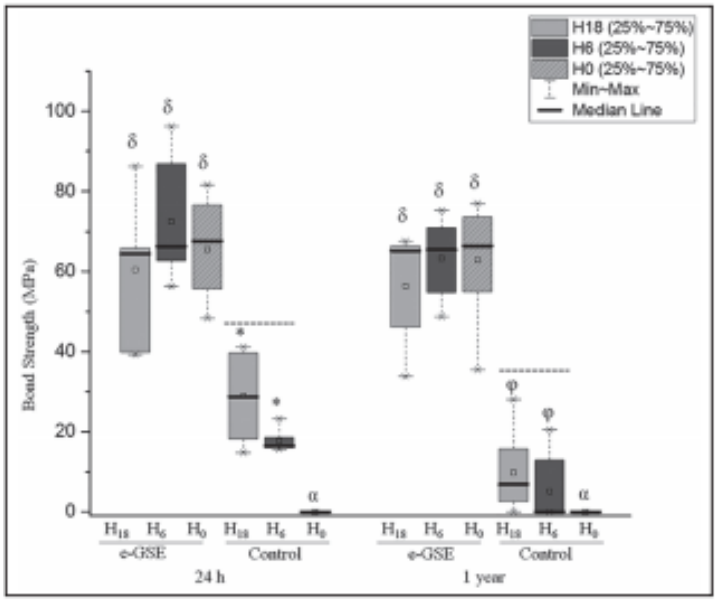

Figure 13: Results of the microtensile bond strength after 24 h and 1 y of artificial saliva. The e-GSE treated showed minimal influence by the varied hydrophilicity, whereas the control showed a significant influence by the hydrophilicity of the experimental adhesive resin.[19]

major component of the experimental resin composition, is a polymer matrix that has the ability to absorb significant volumes of water; meaning, the higher the concentration of HEMA in the polymer, the lower the concentration of residual water.[20] In other words, the presence of HEMA removes water from the filling, allowing more hydrophobicity sensitive resins to bond more effectively. The e-GSE resin bonds showed minimal changes as the HEMA concentration changed, meaning the presence of e-GSE decreases the sensitivity of the resin bonding to water. Whereas the control lacked e-GSE and exhibited a greater degree of decreased bond strength as the

concentration of HEMA decreased. Overall, the data collected suggest that PACs have potential as a bio adhesive between the dentin matrix and the resin complex. The improved elasticity and improved microtensile bond strength are key attributes of a successful bonding compound for the dentin resin interface.

Bacterial adhesion studies

A 2008 study, Cranberry Derived Proanthocyanidins Reduce Bacterial Adhesion to Selected Biomaterials, tested PACs as a potential inhibitor of bacterial binding. In particular, the study examined the potential to reduce gram positive bacteria binding to PTFE and PVC. This was the first time that cranberry PACs were shown to inhibit gram positive organisms from binding to a nonbiological particle.[21] While the PAC treated surfaces did not completely inhibit bacterial adhesion, it did limit the binding in ranges from 1.2 to 5.8 fold.[21] Meaning, 1.2-5.8 few particles bonded to PAC treated surfaces. The reduction in binding applied to both biomaterial and latex particles. One of the benefits of PAC surface treatment is it did not impart selective pressures for resistant bacteria, as the PAC acts in a way that does not negatively affect the bacteria.[21]

A 2011 study examined the potential application of proanthocyanidins as a potential treatment of periodontal disease. The three main bacteria responsible for periodontitis and gingivitis, a more mind form of periodontitis, are *Treponema denticola (gram +)*, *Tannerella forsythia (gram -)*, and *Porphyromonas gingivalis (gram -).*[22] The main bacteria involved in tooth decay is *Streptococcus mutans,* which happens to be a gram positive facultative anaerobe.[23] Seeing how PACs have shown potential as a adhesion inhibitor for gram positive bacteria, PACs show potential as a periodontal disease treatment. The findings for the 2011 study showed PACs to inhibit binding of *P. gingivalis, T. forsythia,* and *T. denticola*, including both gram positive and gram-negative bacterium. Once again, the PACS do not interfere with the growth of bacteria, rather they interfere with the bacterial biofilm formation. By interfering with the biofilm formation, selective pressures for resistant bacterium is avoided. In addition, PACS were

shown to reduce collagenase activity and invasion of the basement membrane of the periodontium.[22]

A study from the Journal of Agricultural and Food Chemistry, published in 2015, reported similar findings on the effects of proanthocyanidins on gingivitis and periodontitis related bacteria.[24] The study primarily focused on the anaerobic Gram-negative bacteria, *Fusobacterium nucleatum*, and its interaction with PACs, extracted from wild blueberries (*Vacciunium angustifolium Ait.*). Similar to previous studies, the PACs inhibited the development of biofilms by the periodontitis related bacteria. In addition, the PAC "reduced the secretion of cytokines and MMPs (matric metalloproteinases) by macrophages by blocking activation of the NF-kB signaling pathway."[24] The combination of preventing biofilm formation and interrupting cytokine and MMP pathways make PACs a promising candidate for periodontal related treatment.

The consumption of proanthocyanidins in one's normal diet, through cranberry juice and non-citrus fruits, are in too low of concentrations to have beneficial influences on one's oral health. But if PACs were purified, they could have potential as an alternative method of treatment for gingivitis and periodontal disease. Current methods for treatment for gingivitis and periodontitis involve antibiotics, which have the potential to encourage the selection of resistant bacteria. PACs offer a potential alternative treatment that discourages bacterial growth of gram-positive and gram-negative bacteria through biofilm reduction.

Negative effects of PACs

In researching the application of PACs to dental materials, there were a few studies that gave unfavorable results when considering using PACs for a clinical application. The American Journal of Dentistry published a paper in 2012, *Proanthocyanidins Alter Adhesive/Dentin Bonding Strengths when Included in a Bonding System*, that tested incorporating PACs into a dentin adhesive compound.[25] The adhesives were tested for microtensile bond strength and examined under a scanning electron microscope (SEM). Initially, the

microtensile bond strength, of the control outperformed the PAC treated adhesives and primers (Figure 14).[25] The PAC treated adhesive experimentally had a bond strength of 22.22 MPa, where the control had a bond strength of 41.18 MPa. The control continued to outperform the PAC treated samples all the way up to the 4-week mark. While the control decreased in bond strength, the PAC treated adhesives and primers continue to increase in bond strength over time. Past the 4-week mark, the PAC treated samples showed superior bond strength.[25] One of the reasons why this can be a negative effect for PACs is if the bond is unable to mature quickly, the filling may have a chance to fail early on before reaching complete bond maturation. A weak initial microtensile bond strength has the potential to have clinical problems early on. Long-term, the PAC treatment may be beneficial, but if the filling cannot endure the early stresses and fails because of it, then the long-term properties are irrelevant.

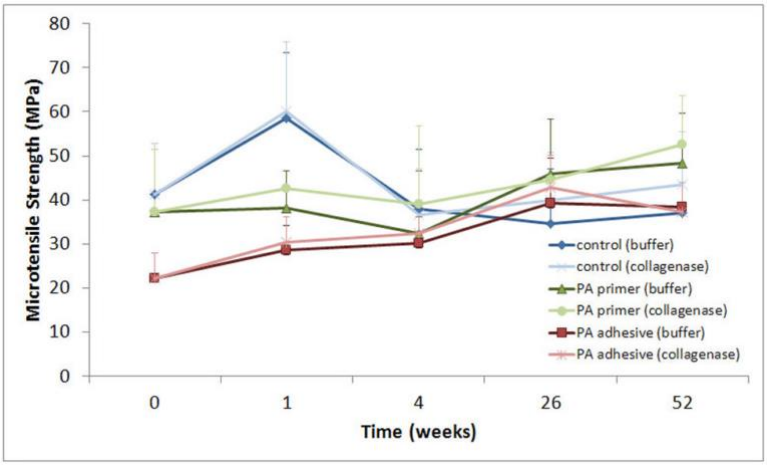

Figure 14: The relationship between mean microtensile bond strength values with storage time and medium.[25]

Another study from 2012, *Effect of proanthocyanidin incorporation into dental adhesive resin-dentine bond strength*, explored the application of PAC addition to adhesive resins. This study concluded the addition of PACs had negative effects on the bond strength when incorporated with a concentration of higher than 2% by weight. At 3% PAC by weight or higher in adhesive material, the bond began to show adverse effects. The article concluded by saying "The addition of proanthocyanidin to an experimental adhesive has no adverse effect on the immediate resin-dentin bond strength when the concentration of proanthocyanidin in the adhesive is less than or equal to 2%."[26] The study does not indicate PACs have any positive influence on bond strength. It only concludes that PACs have negative influences on the bond strength when the concentration rises above 2%. The shortcoming of this test is it failed to test the long-term effect of PAC treatment. It

agrees with the 2012 article, mentioned just prior, by showing the short term decreased in bond strength of the PAC treated adhesive.

One of the other shortcomings of PACs is they have the potential to interfere with the polymerization process. The study examined the effects of PACs in Bis-GMA/HEMA co-monomer mixtures with camphorquinone (CQ) as the polymer accelerator. Three different varieties of CQ were used, including CQ/A, CQ/A/I-1, and CQ/A/I-2. The four resin formulations were as follows 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 of these four resin formulations a variety of PAC concentrations were added (0%, 2.5%, 5%, and 10% by weight), such that in the end there were 16 unique samples. The variety in resin formulas, light activated accelerator, and PAC concentration allowed a diverse study to be performed on the effect PACs have on dental adhesives. The degree of conversion as measured in real time, shown in figure 15, by measuring the absorbance ratio of the between the peak at ~1637

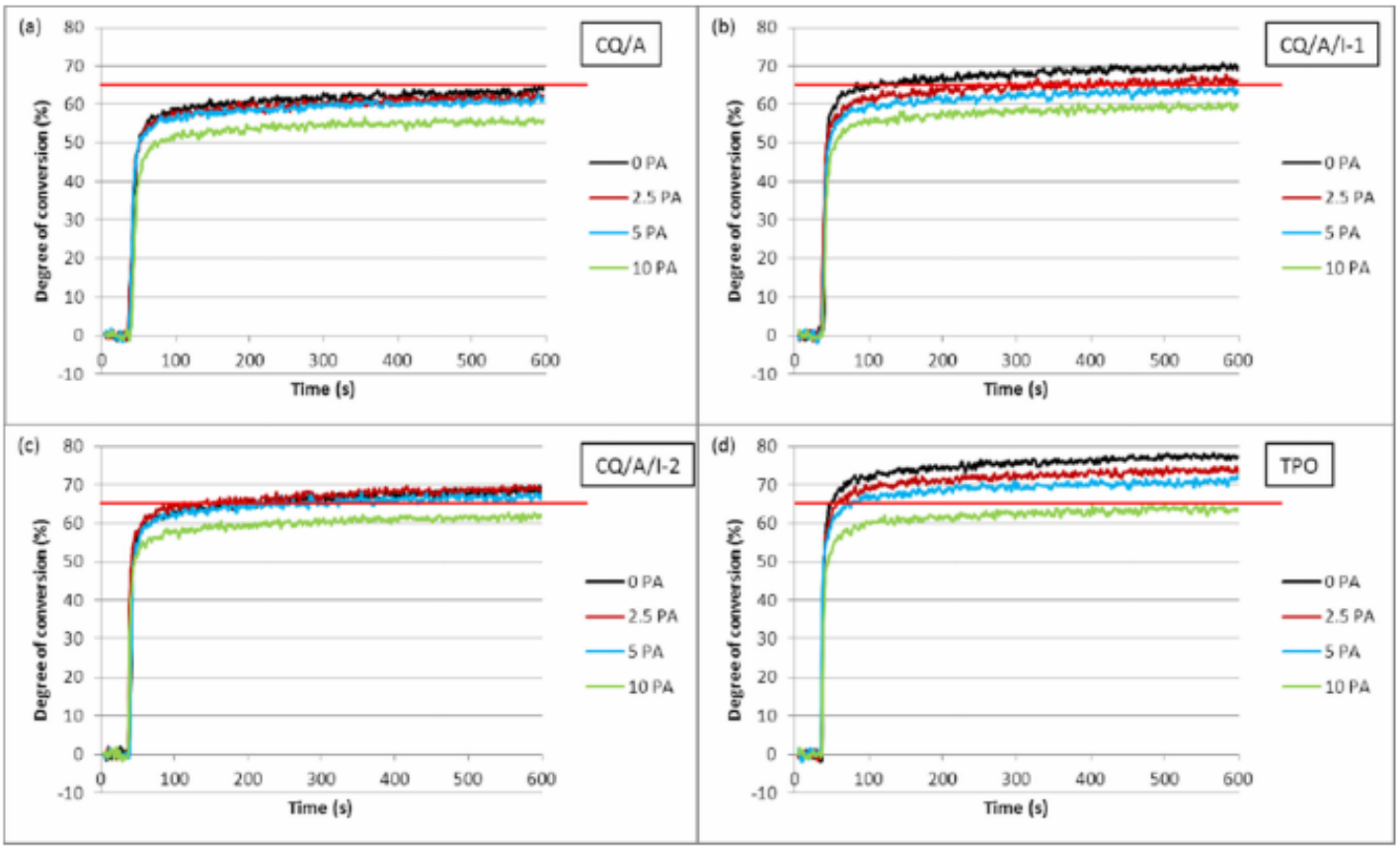

Figure 15: Real-time degree of conversion (DC) of adhesives with the four resin compounds is shown above. The red line marks the point of 65% polymerization.

cm^{-1} (methacrylate C=C stretching) and that at ~1608 cm^{-1} (phenyl C=C stretching) before and after curing.[27] The spectra showed that, regardless of the resin compound mixture, the addition of PACs had a negative effect on the polymerization process. Out of the mixtures, CQ/A/I-2 and TPO mixtures had the highest degree of conversion, making these two compounds the better compounds for PAC containing adhesives.[28]

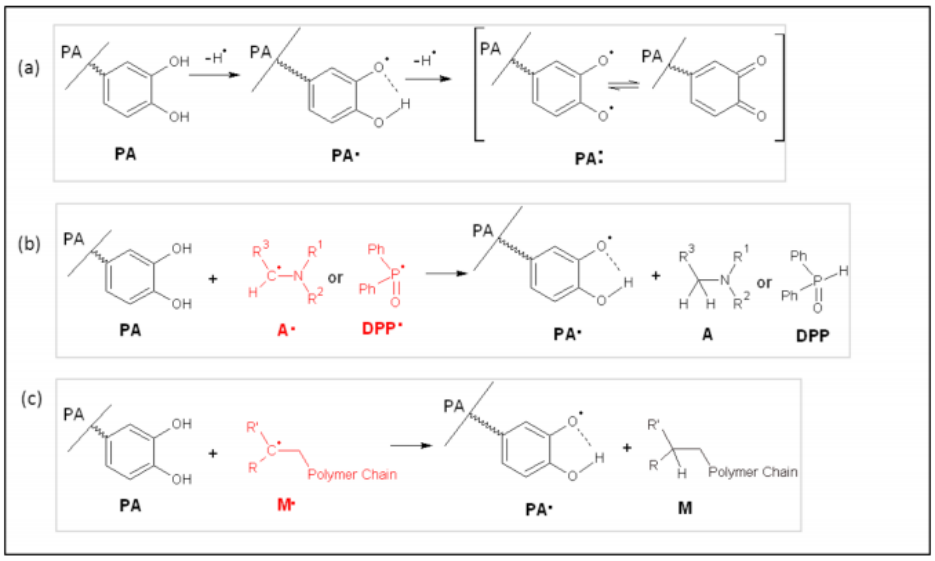

Figure 16: (a) General mechanism of PACs hydrogen-donating capability; (b) Termination of initiation species by hydrogen atom transfer from PACs; (c) Termination of propagation species by hydrogen atom transfer from PACs.[27]

The potential for PACs to interfere with the polymerization process is of major concern as this can compromise the overall structural integrity of the filling. The reason behind the decreased degree in polymerization can be observed in the role of proanthocyanidins as antioxidants. One of the pathways that allow PACs to disable reactive oxidative radicals is through hydrogen transfer. The PAC donates a hydrogen from its phenolic functional group, stopping the oxidative radical through becoming a less reactive radical. The loss of the hydrogen is less significant to the overall function of the molecule due to PACs ability to stabilize itself through resonance structures. Relating this to the polymerization process of resin composites, the PAC interacts with the free radical of the polymer, donating a hydrogen, interrupting the polymerization process.[28] This process is shown below in figure 16. Part (a) of the figure shows the general mechanism for PACs hydrogen donating process. Part (b) shows the termination of initiation species when hydrogen atoms are transferred from PACs. The final part, (c), shows the process in which the polymerization propagation is terminated through the transfer of a hydrogen atom from the PAC.

Discussion:

When examining the potential for a new material in a medical application, there is a great number of variables that need to be taken into consideration prior to any sort of clinical application. This is especially true when it comes to materials in dentistry, as the material needs to be biocompatible, capable of enduring high physical stresses, maintain mineralization in a wet environment, endure enzymatic degradation, along with many other variables. The materials used in the past, such as amalgams, were successful in dealing with the high stress environment while maintaining bacteriostatic properties. The main drawbacks of amalgams are the cosmetic appearance and the presence of lead in the mouth. While the presence of lead in the reacted amalgam form is safe, as shown by the FDA and multiple studies, it is still viewed as a less than ideal substance. Outside of the health of a patient, a great deal of lead that originated from dental offices manages to leach into the environment, causing many environmental problems. The unideal properties of lead combined with the cosmetic disadvantage has driven research into alternative dental filling materials.

The development of composite resin fillings has allowed for a significantly improved material from a cosmetic perspective, but it is unable to maintain the same durability of the amalgams. The research discussed in this paper is directed to improve the overall strength of resin related materials in order to improve the life span of resin fillings. One of the recent areas of research is the application of proanthocyanidins in a dental setting. PACs antimicrobial, antiadhesion, antioxidant, anti-inflammatory properties[14] make it an ideal candidate.

Conclusion

The three areas of research with proanthocyanidins include: dentin collagen cross-linking, resin and dentin matrix bonding interaction, and bacterial influence. The current research surrounding PACs influence on dentin collagen cross-linking, shows that PACs are able to consistently improve the structural integrity of dentin through cross-linking.

The research on the application of PACs in the bonding phase of a resin composite filling is mixed. Some of the research indicates the PACs have a positive outcome on the strength of the filling, while others suggest PACs to have a negative influence. Part of the difficulty in combining the results from these studies is the PACs were collected from alternative sources, consist of varying isomeric bonding configurations, contain different number of monomers, and the concentration when applied varies. While some studies suggest a concentration above 2% has negative effects, another study shows the PACs to have a positive influence. The variety in results and high degree in variables suggest additional research is necessary in order to come to a definitive conclusion. The mixture of positive and negative results suggest that PACs have the potential to improve the bonding interface, but the correct concentration of an ideal PAC isomer is necessary.

While there are no current clinical products containing PACs currently, I believe that PACs have great potential for clinical applications. A great deal of research is necessary prior to developing a usable product. These studies listed in this paper provide a good first step in the process. While experiments have been performed on isolating PACs and selecting the ideal oligomeric compound, more experimental data is needed to find the ideal compound.

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Bibliography

(1) ERICKSON, B. E. FDA REVISITS DENTAL AMALGAMS. *Chem. Eng. News Arch.* **2011**, *89* (5), 40–41.

(2) Merriam-Webster https://www.merriam-webster.com/dictionary/amalgam (accessed Oct 31, 2017).

(3) Williams, K. R. Behind the Scenes at the Toothpaste Aisle: The Chemistry of Dental Materials. *J. Chem. Educ.* **2009**, *87* (10).

(4) The History of Dental Amalgams - How They Have Changed Over Time http://www.caringtreechildrensdentistry.com/the-history-of-dental-amalgams/ (accessed Dec 6, 2017).

(5) Sakaguchi, R. L.; Powers, J. M. *Craig's Restorative Dental Materials*; Elsevier/Mosby, 2012.

(6) Sakaguchi, R. L.; Powers, J. M. *Craig's Restorative Dental Materials*; Mosby.

(7) Dental Restoration - Freestate Dental - Lawrence, KS http://www.freestate.dental/index.php/dental-services/49-dental-restoration (accessed Dec 9, 2017).

(8) Bis-hema ipdi [INCI] | C24H38N2O8 - PubChem https://pubchem.ncbi.nlm.nih.gov/compound/59609175#section=2D-Structure (accessed Dec 9, 2017).

(9) Bis-gma | C29H36O8 - PubChem https://pubchem.ncbi.nlm.nih.gov/compound/15284#section=Top (accessed Dec 9, 2017).

(10) Composite Resin Materials: Nano-What? | Dentistry Today http://www.dentistrytoday.com/restorative-134/1781--sp-1635805017 (accessed Nov 25, 2017).

(11) 3-Methacryloxypropyltrimethoxysilane | 2530-85-0 http://www.chemicalbook.com/ChemicalProductProperty_EN_CB6240961.htm (accessed Dec 9, 2017).

(12) Nam, J.-W.; Phansalkar, R. S.; Lankin, D. C.; Mcalpine, J. B.; Leme-Kraus, A. A.; Vidal, C. M. P.; Gan, L.-S.; Bedran-Russo, A.; Chen, S.-N.; Pauli, G. F. Absolute Configuration of Native Oligomeric Proanthocyanidins with Dentin Biomodification Potency.

(13) Dental amalgam or resin composite fillings? - Delta Dental https://www.deltadentalins.com/oral_health/amalgam.html (accessed Dec 6, 2017).

(14) Hellström, J. K.; Törrönen, A. R.; Mattila, P. H. Proanthocyanidins in Common Food Products of Plant Origin. *J. Agric. Food Chem.* **2009**, *57* (17), 7899–7906.

(15) Nam, J.-W.; Phansalkar, R. S.; Lankin, D. C.; Bisson, J.; Mcalpine, J. B.; Leme, A. A.; Vidal, C. M. P.; Ramirez, B.; Niemitz, M.; Bedran-Russo, A.; et al. Subtle Chemical Shifts Explain the NMR Fingerprints of Oligomeric Proanthocyanidins with High Dentin Biomodification Potency.

(16) grape-seed-extract-diagrams-2.png (880×389) https://annp.ca/wp-content/uploads/2015/08/grape-seed-extract-diagrams-2.png (accessed Dec 9, 2017).

(17) Bedran-Russo, A. K.; Pauli, G. F.; Chen, S.-N.; McAlpine, J.; Castellan, C. S.; Phansalkar, R. S.; Aguiar, T. R.; Vidal, C. M. P.; Napotilano, J. G.; Nam, J.-W.; et al. Dentin Biomodification: Strategies, Renewable Resources and Clinical Applications. *Dent. Mater.* **2014**, *30* (1), 62–76.

(18) composite_restoration_procedure.jpg (323×300) http://healthlifemedia.com/healthy/wp-content/uploads/2016/01/composite_restoration_procedure.jpg (accessed Dec 5, 2017).

(19) Leme-Kraus, A. A.; Aydin, B.; Vidal, C. M. P.; Phansalkar, R. M.; Nam, J. W.; McAlpine, J.; Pauli, G. F.; Chen, S.; Bedran-Russo, A. K. Biostability of the Proanthocyanidins-Dentin Complex and Adhesion Studies. *J. Dent. Res.* **2017**, *96* (4), 406–412.

(20) Chu, H.-H.; Ou, E.-D. Emulsion Polymerization of 2-Hydroxyethyl Methacrylate and Partition of Monomer between Particles and Water Phase. *Polym. Bull.* **2000**, *44* (3), 337–344.

(21) Eydelnant, I. A.; Tufenkji, N. Cranberry Derived Proanthocyanidins Reduce Bacterial Adhesion to Selected Biomaterials.

(22) Feghali, K.; Feldman, M.; Dang La, V.; Santos, J.; Grenier, D. Cranberry Proanthocyanidins: Natural Weapons against Periodontal Diseases. *J. Agric. Food Chem* **2012**, *60*, 5728–5735.

(23) Loesche, W. J. *Microbiology of Dental Decay and Periodontal Disease*; University of Texas Medical Branch at Galveston, 1996.

(24) Lagha, A. Ben; Dudonne, S.; Desjardins, Y.; Grenier, D. Wild Blueberry (Vaccinium Angustifolium Ait.) Polyphenols Target Fusobacterium Nucleatum and the Host Inflammatory Response: Potential Innovative Molecules for Treating Periodontal Diseases.

(25) Hechler, B.; Yao, X.; Wang, Y. Proanthocyanidins Alter Adhesive/Dentin Bonding Strengths When Included in a Bonding System.
(26) Epasinghe, D. J.; Yiu, C. K. Y.; Burrow, M. F.; Tay, F. R.; King, N. M. Effect of Proanthocyanidin Incorporation into Dental Adhesive Resin on Resin–dentine Bond Strength. *J. Dent.* **2012**, *40* (3), 173–180.
(27) Effect of Proanthocyanidins and Photo-Initiators on Photo-Polymerization of a Dental Adhesive. *J. Dent.* **2013**, *41* (1), 71–79.
(28) Liu, Y.; Wang, Y. Effect of Proanthocyanidins and Photo-Initiators on Photo- Polymerization of a Dental Adhesive.