MALAYSIAN JOURNAL OF
Medicine and Health Sciences

Potential of Epigallocatechin-3-gallate as Chelating Agent against Matrix Metalloproteinase Expression and as Cross-Linking Agent Towards Hybrid Layer in Dentin Collagen- a Review Article

Journal:	*Malaysian Journal of Medicine & Health Sciences*
Manuscript ID	MJMHS-2021-0603.R1
Manuscript Type:	Review Articles
Keywords:	EGCG, MMPs, Crosslink, Chelating, Collagen

Potential of Epigallocatechin-3-gallate as Chelating Agent against Matrix Metalloproteinase Expression and as Cross-Linking Agent Towards Hybrid Layer in Dentin Collagen- a Review Article

ABSTRACT

Adhesive dentistry's main assumption is to create a strong chemical bond between dental hard tissues and restorative composite material. One of the most important aspects of this interface is the hybrid layer. Unfortunately, due to physical and chemical causes, the hybrid layer wears away with time. Epigallocatechin-3-gallate (EGCG), a component extracted from green tea, has several roles in medical and dentistry field including as a crosslinking agent and as a chelating agent. Although there are several negative results, EGCG was proven to be able to preserve resin-dentin bonds without harming the restoration. As crosslinking agent and chelating agent, EGCG has the potential to enhance physical properties of dentin collagen and resin-dentin adhesion.

Keywords: *EGCG, MMPs, Crosslink, Chelatin, Collagen, Human and Medicine.*

INTRODUCTION

The adhesive system is one of the most revolutionary breakthroughs in the field of conservative dentistry. This system create minimally invasive restorations that require minimal preparation (1). Du (2) reported that resin adhesion to dentin had high bond strength immediately after application, but decreased by 50-60% after 1-2 years. To avoid the decrease of bond strength, a hybrid layer that is strong, stable and has high durability is required. Hybrid layer is a layer formed from resin monomers that infiltrate demineralized intratubular, intertubular and extratubular collagen fibrils (3,4). Several factors that affect the quality of the hybrid layer, one of which is enzym that involve in

demineralization of dentin collagen is Matrix metalloproteinase (MMP) on odontoblasts (1,3). MMP must be prevented for increasing the resin-dentin adhesion. The dentine resin attachment mechanism is a physical-mechanical attachment and a chemical reaction between the dentin bonding material and the collagen on the dentin surface. A stable bond can be achieved between the restorative material and the teeth (5). Dentin also contains water which can cause degradation of resin components, to increase resin-dentin adhesion, prevent water molecular retention are needed, thereby increasing collagen integrity, preventing resin degradation, and strengthening hybrid layer formation (6).

Epigallocatechin-3-gallate (EGCG) is one of the catechins with the highest percentage in green tea extract (49%), has a high affinity for metal ions which can inhibit the action of the enzyme (MMP) through the chelating process and increase the integrity and stability of collagen so it can increase the adhesion strength of the hybrid layer[2]. Another research stated by Albuquerque (7) that application of EGCG can increase the resistance of dentin-bonding attachments within 2 years. As a cross-linking agent, EGCG is also able to replace water molecules in the collagen bond chain by hydrogen bonding with the collagen peptide chain which can reduce collagen interaction with water so that collagen becomes more hydrophobic (8). Thus the monomer can better infiltrate the collagen fibrils and prevent water absorption for increase the monomer-dentin bond (9).

EGCG can also have a negative effect when mixed with adhesives at a certain concentration. Yu (10) stated that the antioxidant effect of EGCG can change the degree of adhesive conversion. From the research results, it appears that the higher the concentration of EGCG used in the mixture, the lower the degree of conversion of the adhesive. This was confirmed by Du (2) who reported that giving 100-300 µg / mL of EGCG into the adhesive can cause EGCG to be trapped in the polymer linear chain after

the irradiation process which causes the adhesive polymerization to be inadequate. This is because the anti-free radical properties of EGCG can interfere with the free radical polymerization process of the adhesive so that research is needed to find the right EGCG concentration.

Even though it has quite a lot of therapeutic effects, until now EGCG materials in the field of dentistry still minimal. This literature study discusses the potential of EGCG as a cross-linking agent against dentine collagen and hybrid layer and chelating agent against MMPs.

Dentin

Dentin is a layer of tooth structure underneath the enamel. This layer consists of 65% inorganic components in the form of hydroxyapatite crystals, 30% organic components in the form of collagen and 5% water (11). The extracellular matrix of the dentin is made up of a complex three-dimensional network of collagen fibrils calcified by nanoapatite crystals. A central triple-helix area, a non-helical aminoterminal area (N-telopeptide), and a carboxyterminal area make up the collagen chain (C-telopeptide). The length of the collagen fibrils looks to have a hollow of 15-20 nm, which the resin monomer will penetrate and polymerize under 150,000-fold magnification. The mechanical retention of dentin adhering to collagen is the result of this situation (3,12).

Collagen chains in dentin are the most stable collagen compared to collagen in the body system (13). This is due to intramolecular and intermolecular cross-links formed by covalent connections between the C terminal on one collagen molecule and the N terminal on the collagen molecule next to it. By linking the spaces between collagen molecules that are filled with water, hydrogen bonds help to stabilize the triple helical chain (4).

This crosslink plays a role in the acid etching process during the bonding procedure, and prevent the collagen denaturation so that a hybrid layer can be formed (3).

Elasticity, hardness, visco-elasticity, and fracture coefficient are all mechanical properties of teeth. When exposed to external forces, visco-elasticity is used to measure materials with viscous and elastic properties. The storage modulus and loss modulus are the measuring indices employed (11,14).

Matrix metalloproteinase (MMP)

The MMP enzyme is an enzyme that basically can degrade all components of the dentin extracellular matrix. Almost all MMPs are secreted as enzyme precursors, namely zymogens, in which cysteine propeptide binds to its sulfihydrile groups until the active zinc ion region as the fourth ligand undergoes "cysteine change". In vitro the change from a form to an active form can be achieved by proteolytic elimination of the propeptide, randomizing the cysteine-zinc interactions, or modifying the sulfihydrile groups, allowing the interaction between the zinc active region and the water molecule and contact with the active site. In many cases, the activation process occurs gradually including the autocatalytic process. In vitro proMMPs can be activated by various chemical compounds and reactions, including thiol-modified compounds, denaturation, chaotropic compounds, reactive oxygen, and heating. MMP-2, MMP-8 and MMP-9 can also be activated by acidic pH followed by neutralization (12).

Hybrid Layer

Hybrid layer is the most vital part of adhesive based restoration. The quality of the hybrid layer determine the strength and durability of a restoration. Hybrid layer can

be interpreted as a layer formed due to resin infiltration between collagen and hydroxyapatite fibers which functions as a micromechanical retention of composite resin restorations to the dentin tissue. This layer consists of 50% collagen matrix and 50% resin. Hybrid layer serves to combine 2 different elements, namely hydrophilic dentin and hydrophobic composite material, protecting the dentin surface from micro-leakage and increasing dentin resistance to acid. The ideal hybrid layer is characterized by the presence of a collagen network that is bonded and reinforced with polymers (3,15).

Hybrid Layer Degradation

Adhesive are now starting to use hydrophilic monomers such as *Hydroxyethyl metacrylate* (HEMA) as a hydrophobic monomer solvent to increase the wetting aility of the adhesive abd prevent phase changers that occur when dimacrylate-based adhesive are applied to the dentin matrix which tends to be moist. Resin monomers which is hydrophilic in nature is very suspectible to hydrolysis due to the presence of ester bonds in the HEMA component. In addition, the increase in the HEMA component in the adhesive has been shown to increase water absorption in polymerized polymers, which causes a decrease in the mechanical properties of the hybrid layer.[3] HEMA is able to provide good adhesions and is not easily degraded so that it can produce long-lasting restoration (16).

Epigallocathechin Gallate (EGCG)

EGCG is a polyphenol that belongs to the catechin group, which able to inhibit the opening of sodium ion channels so that it has the potential to be anti-inflammatory. In addition, EGCG also has antioxidant properties that can reduce ROS by binding to ROS

(17,18). EGCG is composed of 2- phenylchromane framework which is substituted in chain number 3,5,7,31,41 with a hydroxyl group. During biosynthesis, if the B-ring comes from sinton gallic acid, the catechin will be substituted with the 51st position of the hydroxyl group, namely "gallo" catechins which will esterify with gallic acid to form "gallate". Levorotatory compounds (2R, 3R) are called "epi" while dextrorotatory compounds (2S, 3R) are called "catehchin" so that when combined, they will become epigallocatechin gallate (EGCG). EGCG compound has the chemical formula (2R, 3R) -5,7-dihydroxy-2- (3,4,5-trihydroxyphenly) -3,4-dihydro-2H-chromen-3-yl-3,4,5-trihydroxybenzoate (19). Judging from its chemical structure, EGCG is said to have the most potent antioxidative properties.

EGCG can close TLR4 which can increase the production of TNF-α and hydroxyl and gallat groups that will bind to free radical ions so that it can prevent oxidation reactions that can cause tissue damage (9,20). EGCG can also carry out antioxidant activity, EGCG will bind free metal ions such as Fe2 + and Zn2 + ions and make them more stable so that the catalyzed reactions can be inhibited (1).

Demineralization in the caries process has been shown not only to be caused by contact with acids from bacteria, but also due to the collagenolytic and gelatinolytic activity of the proteases present in the dentin organic matrix. The enzymes that play a role in this are MMP and cysteine cathepsin (21).

EGCG as Cross-Linking Agent Towards Hybrid Layer in Dentin Collagen

The technology regarding adhesive materials is still developing, one of the weakness is the decrease in the resin-dentin bond which is often associated with unstable

hybrid layer. On the other hand, dentin also has MMP proenzymes and cysteine catepsin which play a role in the destruction of collagen fibrils in the hybrid layer.

Collagen fibrils' stability, tensile strength, and viscoelasticity depend on the intermolecular cross-links from translation of collagen. This is indicated by an increase in the degree of cross-link which is accompanied by an increase in the elastic tension of collagen fibrils. Chu (9), reported that applying EGCG solution to the collagen surface resulted in more regular collagen fibrils with a larger diameter and smaller interfibriler space. The higher the concentration used, the greater the diameter of the collagen fibrils formed.

The cross-linking between EGCG and collagen chains can also affect the permeability of the hybrid layer to water molecules. This is evidenced by the research of Sun (8), which shows that the application of 0.1% EGCG to dentin after acid etching can increase the contact angle of the dentine surface to water.

A different point was stated by Chu (9), who reported that administering EGCG 0.64% could reduce the contact angle of the dentin surface against water and allow maximum surface wetting. From the research method used, the two studies with different results used different methods. A dentin collagen membrane was immersed in EGCG solution for 1 hour at room temperature. Meanwhile, Sun (8), used pieces of dentin that had been polished and demineralized using acid etching and applied EGCG for 120 seconds. This difference in the material used allows for differences in research results even though they both use water droplets.

To determine the degree of attachment between the adhesive and dentin, most studies (n = 23) used a tensile bond strength test (TBS) and 4 journals evaluated nanoleakage analysis. Among the 17 studies that tested TBS with positive results, 10 of

them use EGCG solution with varying concentrations on dentin before bonding application. According to Yu (10), EGCG can maintain adhesion stability through inhibitory activity of MMPs. Meanwhile, Maria Fonesca (14), EGCG has hydrophobic properties through aromatic groups and hydrophilic through polar hydroxyl groups. The hydrophobic group is able to induce Van der Waals bonds with hydrophobic molecules in the resin. While the hydrophilic group hydrogen bonds with proteins in the collagen chain. Gerhardt (22), reported that the administration of EGCG solution with a concentration of 2% before the bonding application showed lower TBS rates than controls both immediately after application and 6 months later. Gerhardt added that these results could possibly due to the formation of a precipitate in the EGCG solution. Yang (23), reported that giving EGCG in ethanol and water solvents with a concentration of 0.02% showed a lower degree of nanolekakage than the 0.1% concentration, this research in line with another research that stated EGCG with the smallest concentration (0.0065%) is able to inhibit protease activity but its effectiveness drops significantly when it reaches a certain concentration. This raises the theory that the EGCG used in the treatment is highly dependent on the concentration used. Regarding this, Du (2), provided an explanation that giving EGCG in high concentrations can reduce the degree of adhesive conversion, because during the polymerization period, EGCG trapped in the adhesive can interfere with the formation of polymer linear chains.

EGCG as a cross-link agent and the wetting ability of the bonding material caused by the EGCG solvent, which is water. The use of different solvents can also causes the differences in test results, Chemical bonding using aceton solvent can produce a stronger bond between the bonding material and dentin collagen when compared to using ethanol solvent (15). In a study conducted by Soetojo (24), compared HEMA water solvent

expresses less MDA and has a good biocompability when compared to HEMA with ethanol or aceton solvent. Water solvents are considered to provide a wetting effect that can act as plasticisers of dentin collagen fibrils and keep collagen from collapsing.Water molecules in collagen make diffusion of the bonding material with ethanol and acetone solvents into collagen fibrils easier, but the water molecules will also diffuse into the solvent, so the concentration of the adhesive solvent and the concentration of BisGMA decreases which causes the infiltration of BisGMA to be inadequate (25). Yang (23), used ethanol solvent in his EGCG solution. The use of ethanol as a solvent to replace water can increase the TBS of most hydrophobic resins such as BisGMA / TEGDMA by making the total ethanol-matrix cohesive force approaching the ethanol-BisGMA / TEGDMA solution so that the collagen matrix will immediately form interpeptid hydrogen bonds. This evidence showed high number of TBS and nanoleakage of the EGCG-ethanol group compared to the EGCG-water group.

EGCG as Chelating Agent against MMPs' Expression

The use of MMP inhibitors is thought to increase the bond strength of the adhesive to dentin. EGCG also functions as a chelating agent, EGCG interacts with MMP by exploiting the nature of catechins which have high affinity for metal ions. Yang (23) states that the Zn ion which is the chemical structure of MMP can be bound by EGCG so that MMP is no longer recognized by the collagen matrix so that collagen degradation can be inhibited. But Cheng (26) have a different theory that stated EGCG inhibits the gelatinolytic activity of MMP by binding EGCG with the catalytic area or the area close to the catalytic area where gelatinolytic activity is occurring.

Based on journal search results, most studies (n = 15) regarding the application of EGCG to dentin collagen did show positive results both on TBS, nanoleakage and on the morphology of collagen fibrils. However, there is one randomized clinical trial that showed negative results. Costa (27), reported that administering 0.1% EGCG solution for 60 seconds before application of the bonding to the cervical lesion restoration procedure without caries (NCCL) did not show any difference in terms of retention, margin adaptation, secondary caries and post-restoration sensitivity after 24 months with or without the procedure. This result explains that although EGCG does not have a positive effect on the restoration results, EGCG also does not have a negative effect that can worsen the restoration. Therefore, further evaluation is needed regarding concentrations, solvents and application methods that can be used to increase the resin-dentin adhesion in the hybrid layer.

CONCLUSION

The application of EGCG can have a positive effect on the morphology of dentinal collagen fibrils, inhibit MMPs activity in dentinal collagen, decrease the hydrophilic properties of dentinal collagen fibrils, increase the tensile bond strength (TBS) of the hybrid layer and reduce the nanoleakage of the hybrid layer in dentin collagen.

ACKNOWLEDGEMENT

The authors would like to thank the staff of Endodontic Division of Faculty of Dental Medicine, Universitas Airlangga. The authors declare no potential conflicts of interest with respect to the authorship and publication of this article.

REFERENCES

1. Münchow E.A., Bottino M.C. Recent Advances in Adhesive Bonding: The Role of Biomolecules, Nanocompounds, and Bonding Strategies in Enhancing Resin Bonding to Dental Substrates. Curr Oral Heal Reports. 2017;4(3):215-27. doi:10.1007/s40496-017-0146-y.
2. Du X., Huang X., Huang C., Wang Y., Zhang Y. Epigallocatechin-3-gallate (EGCG) enhances the therapeutic activity of a dental adhesive. J Dent. 2012;40(6):485-92. doi:10.1016/j.jdent.2012.02.013.
3. Breschi L., Maravic T., Cunha S.R., *et al.* Dentin bonding systems: From dentin collagen structure to bond preservation and clinical applications. Dent Mater. 2018;34(1):78-96. doi:10.1016/j.dental.2017.11.005.
4. Tezvergil-Mutluay A., Pashley D., Mutluay M.M. Long-Term Durability of Dental Adhesives. Curr Oral Heal Reports. 2015;2(4):174-81. doi:10.1007/s40496-015-0070-y
5. Soetojo A., Purnama D., Lunardhi C.G.J., Widjiastuti I. Cytotoxicity Test of 4-Methacryloxyethyl Trimellitic Anhydrride-based Dentin Bonding Material Using Acetone Solution in Dental Pulp Fibroblast. J Int Oral Health. 2019;(11):191-6.
6. Fialho M.P.N., Hass V., Nogueira R.P., *et al.* Effect of epigallocatechin-3- gallate solutions on bond durability at the adhesive interface in caries-affected. J Mech Behav Biomed Mater. 2019;91:398-405. doi:10.1016/j.jmbbm.2018.11.022.
7. Albuquerque N.L.G., Neri J.R., Lemos M.V.S., Yamauti M., De Sousa F.F.O., Santiago S.L. Effect of polymeric microparticles loaded with catechin on the physicochemical properties of an adhesive system. Oper Dent. 2019;44(4):E202-11. doi:10.2341/18-112-L.
8. Sun Q., Gu L., Quan J., *et al.* Epigallocatechin-3-gallate enhance dentin biomodification and bond stability of an etch-and-rinse adhesive system. Int J Adhes. 2018;80:115-21. doi:10.1016/j.ijadhadh.2017.11.001.
9. Chu C., Deng J., Xiang L., *et al.* Evaluation of epigallocatechin-3-gallate (EGCG) cross-linked collagen membranes and concerns on osteoblasts. Mater Sci Eng C. 2016;67:386-94. doi:10.1016/j.msec.2016.05.021.
10. Yu H.H., Zhang L., Yu F., Li F., Liu Z.Y., Chen J.H. Epigallocatechin-3-gallate and epigallocatechin-3-O-(3-O-methyl)-gallate enhance the bonding stability of

an etch-and-rinse adhesive to dentin. Materials (Basel). 2017;10(2). doi:10.3390/ma10020183.

11. Garg N., Garg A. *Textbook of Endodontics*. 3rd ed. New Delhi: Jaypee Brothers Medical Publishers; 2014
12. Zirta U.A., Juanita A.G, Nurrohman H. The role of matrix metalloproteinases in dentin caries. J Indones Dent Assoc. 2009;58(2):25-31.
13. Pashley D, Tay F. Mechanical stability of resin-dentin bonds. Dent Biomater Imaging, Test Model. 2008;112-161. doi:10.1533/9781845694241.112.
14. Maria F.B., Camara B.D., Mara da S.T., Luis Souto B.A., Paulo Moisés de O.H., Eduardo de Paiva G.S. Mechanical-physicochemical properties and biocompatibility of catechin-incorporated adhesive resins. J Appl Oral Sci. 2019;27:1-11. http://dx.doi.org/10.1590/1678-7757-2018-0111.
15. Zubaidah N, Effendy R, Soetojo A, Estiyaningsih T, Tanzil MI, Khotimah K.cDifference of chemical bonds between UDMA bonding agents with ethanol solvent and acetone solvent on dentin collagen. Pesqui Bras Odontopediatria Clín Integr. 2021;21:e0116. https://doi.org/10.1590/pboci.2021.030.
16. Saraswati W., Widjiastuti I., Rukmo M., Wahjuningrum D.A.cThe Expression of HMGB1 in Dentin Pulp Complex Induced by Resin Monomer HEMA. Int Medical Device and Tech Conf. 2017.
17. Ismiyatin K., Soetojo A., Wahluyo S., Purwanto B., Rahayu R.P., Mukono I.S. Topical Epigallocatechin-3-gallate hydrogels regulated inflammation and pain. J Int Dent Med Res. 2019;12(1):54-60.
18. Ismiyatin K., Soetoyo A., Wahluyo S., Safitri I. Therapeutic Efficacy of Topical Epigallocatechin-Gallate as a New Therapeutic Strategy for Inhibition of Pain Conduction on Rat Models with Acute Pulpal Inflammation. Int Med Device Technol Conf. 2017;107-10.
19. Legeay S., Rodier M., Fillon L., Faure S., Clere N. Epigallocatechin Gallate: A Review of Its Beneficial Properties to Prevent Metabolic Syndrome. Nutr. 2015;7(7). doi:10.3390/nu7075230.
20. Ismiyatin K., Wahluyo S., Pureanto D.A., Rahayu R.p., Soetojo A., Mukono I.S. Effect of Topical Epigallocatechin-Gallate on Lipopolysaccharide-induced Pulpa Inflammation in Rat Models. Iranian Endodontic Journal. 2018;13(4):528-33.

21. Van Doren SR. Matrix metalloproteinase interactions with collagen and elastin. Matrix Biol. 2015;44-46:224-231. doi:10.1016/j.matbio.2015.01.005.
22. Gerhardt K.M.F., Oliveira C.A.R., França F.M.G., Basting R.T., Turssi C.P., Amaral F.L.B. Effect of epigallocatechin gallate, green tea extract and chlorhexidine application on long-term bond strength of self-etch adhesive to dentin. Int J Adhes Adhes. 2016;71:23-7. doi:10.1016/j.ijadhadh.2016.08.005.
23. Yang H., Guo J., Deng D., Chen Z., Huang C. Effect of adjunctive application of epigallocatechin-3-gallate and ethanol-wet bonding on adhesive-dentin bonds. J Dent. 2016;44:44-49. doi:10.1016/j.jdent.2015.12.001.
24. Soetojo A., Cahyadi E.K.H., Prasetyo E.A. Malondialdehyde Expressions on Pulp Odontoblast Cells after Application 2-hydroxylethul methacrylate mixed with water, ethanol and aceton solvent. Saudi Endod J. 2019;(9):96-100.
25. Prasetya A.I., Kunarti S., Soetojo A., Prasetyo E.A. Chemical Bond Strength Difference between 4-Meta Bonding Agents with Ethanol and Acetone Solvent on Type I Collagen. Journal of International Dental and Medical Research. 2017;11(2):567-71.
26. Cheng XW, Kuzuya M, Kanda S, et al. Epigallocatechin-3-gallate binding to MMP-2 inhibits gelatinolytic activity without influencing the attachment to extracellular matrix proteins but enhances MMP-2 binding to TIMP-2. Arch Biochem Biophys. 2003;415(1):126-132. doi:10.1016/S0003-9861(03)00221-2.
27. Costa C., Albuquerque N., Mendonça J.S., Loguercio A.D., Saboia V., Santiago S.L. Catechin-based Dentin Pretreatment and the Clinical Performance of a Universal Adhesive: A Two-year Randomized Clinical Trial. Oper Dent. 2020;45(5):473-483. doi:10.2341/19-088-C.

Potential of Epigallocatechin-3-gallate as Chelating Agent against Matrix Metalloproteinase Expression and as Cross-Linking Agent Towards Hybrid Layer in Dentin Collagen- a Review Article

ABSTRACT

Adhesive dentistry's main assumption is to create a strong chemical bond between dental hard tissues and restorative composite material. One of the most important aspects of this interface is the hybrid layer. Unfortunately, due to physical and chemical causes, the hybrid layer wears away with time. Epigallocatechin-3-gallate (EGCG), a component extracted from green tea, has several roles in the medical and dentistry field including as a crosslinking agent and as a chelating agent. Although there are several negative results, EGCG was proven to be able to preserve resin-dentin bonds without harming the restoration. As a crosslinking agent and chelating agent, EGCG has the potential to enhance the physical properties of dentin collagen and resin-dentin adhesion. **The purpose of this study was to see how EGCG, as a cross-linking agent, affected dentinal collagen and hybrid layers, as well as how chelating chemicals affected MMPs.**

Keywords: *EGCG, MMPs, Crosslink, Chelating, Collagen, Human and Medicine.*

INTRODUCTION

The adhesive system is one of the most revolutionary breakthroughs in the field of conservative dentistry. This system creates minimally invasive restorations that require minimal preparation (1). Du (2) reported that resin adhesion to dentin had high bond strength immediately after application, but decreased by 50-60% after 1-2 years. To avoid the decrease of bond strength, a hybrid layer that is strong, stable, and has high durability is required. A hybrid layer is a layer formed from resin monomers that infiltrate

demineralized intratubular, intertubular, and extra tubular collagen fibrils (3,4). Several factors that affect the quality of the hybrid layer, one of which is enzyme that involve in the demineralization of dentin collagen is Matrix metalloproteinase (MMP) on odontoblasts (1,3). MMP must be prevented for increasing the resin-dentin adhesion. The dentine resin attachment mechanism is a physical-mechanical attachment and a chemical reaction between the dentin bonding material and the collagen on the dentin surface. A stable bond can be achieved between the restorative material and the teeth (5). Dentin also contains water which can cause degradation of resin components, to increase resin-dentin adhesion, prevent water molecular retention are needed, thereby increasing collagen integrity, preventing resin degradation, and strengthening hybrid layer formation (6).

The adhesive system is one of the most revolutionary breakthroughs in the field of conservative dentistry. This system creates minimally invasive restorations that require minimal preparation (1). Du (2) reported that resin adhesion to dentin had high bond strength immediately after application, but decreased by 50-60% after 1-2 years. To avoid the decrease of bond strength, a hybrid layer that is strong, stable, and has high durability is required. A hybrid layer is a layer formed from resin monomers that infiltrate demineralized intratubular, intertubular, and extra tubular collagen fibrils (3,4). Several factors affect the quality of the hybrid layer, one of which is the enzyme that involves in the demineralization of dentin collagen is Matrix metalloproteinase (MMP) on odontoblasts (1,3). MMP must be prevented for increasing the resin-dentin adhesion. The dentine resin attachment mechanism is a physical-mechanical attachment and a chemical reaction between the dentin bonding material and the collagen on the dentin surface. A stable bond can be achieved between the restorative material and the teeth (5). Dentin

also contains water which can cause degradation of resin components, to increase resin-dentin adhesion, prevent water molecular retention are needed, thereby increasing collagen integrity, preventing resin degradation, and strengthening hybrid layer formation (6).Epigallocatechin-3-gallate (EGCG) is one of the catechins with the highest percentage in green tea extract (49%), has a high affinity for metal ions which can inhibit the action of the enzyme (MMP) through the chelating process and increase the integrity and stability of collagen so it can increase the adhesion strength of the hybrid layer2. Another research stated by Albuquerque (7) that the application of EGCG can increase the resistance of dentin-bonding attachments within 2 years. As a cross-linking agent, EGCG is also able to replace water molecules in the collagen bond chain by hydrogen bonding with the collagen peptide chain which can reduce collagen interaction with water so that collagen becomes more hydrophobic (8). Thus the monomer can better infiltrate the collagen fibrils and prevent water absorption to increase the monomer-dentin bond (9).

EGCG can also have a negative effect when mixed with adhesives at a certain concentration. Yu (10) stated that the antioxidant effect of EGCG can change the degree of adhesive conversion. From the research results, it appears that the higher the concentration of EGCG used in the mixture, the lower the degree of conversion of the adhesive. This was confirmed by Du (2) who reported that giving 100-300 µg / mL of EGCG into the adhesive can cause EGCG to be trapped in the polymer linear chain after the irradiation process which causes the adhesive polymerization to be inadequate. This is because the anti-free radical properties of EGCG can interfere with the free radical polymerization process of the adhesive so that research is needed to find the right EGCG concentration.

Even though it has quite a lot of therapeutic effects, until now EGCG materials in the field of dentistry are still minimal. This literature study discusses the potential of EGCG as a cross-linking agent against dentine collagen and hybrid layer and chelating agent against MMPs.

Dentin

Dentin is a layer of tooth structure underneath the enamel. This layer consists of 65% inorganic components in the form of hydroxyapatite crystals, 30% organic components in the form of collagen, and 5% water (11). The extracellular matrix of the dentin is made up of a complex three-dimensional network of collagen fibrils calcified by nanoapatite crystals. A central triple-helix area, a non-helical aminoterminal area (N-telopeptide), and a carboxy-terminal area make up the collagen chain (C-telopeptide). The length of the collagen fibrils looks to have a hollow of 15-20 nm, which the resin monomer will penetrate and polymerize under 150,000-fold magnification. The mechanical retention of dentin adhering to collagen is the result of this situation (3,12).

Collagen chains in the dentin are the most stable collagen compared to collagen in the body system (13). This is due to intramolecular and intermolecular cross-links formed by covalent connections between the C terminal on one collagen molecule and the N terminal on the collagen molecule next to it. By linking the spaces between collagen molecules that are filled with water, hydrogen bonds help to stabilize the triple-helical chain (4). This crosslink plays a role in the acid etching process during the bonding procedure and prevents collagen denaturation so that a hybrid layer can be formed (3).

Elasticity, hardness, visco-elasticity, and fracture coefficient are all mechanical properties of teeth. When exposed to external forces, visco-elasticity is used to measure

materials with viscous and elastic properties. The storage modulus and loss modulus are the measuring indices employed (11,14).

Matrix metalloproteinase (MMP)

The MMP enzyme is an enzyme that basically can degrade all components of the dentin extracellular matrix. Almost all MMPs are secreted as enzyme precursors, namely zymogens, in which cysteine propeptide binds to its sulfhydryl groups until the active zinc ion region as the fourth ligand undergoes "cysteine change". In vitro the change from a form to an active form can be achieved by proteolytic elimination of the propeptide, randomizing the cysteine-zinc interactions, or modifying the sulfhydryl groups, allowing the interaction between the zinc active region and the water molecule and contact with the active site. In many cases, the activation process occurs gradually including the autocatalytic process. In vitro proMMPs can be activated by various chemical compounds and reactions, including thiol-modified compounds, denaturation, chaotropic compounds, reactive oxygen, and heating. MMP-2, MMP-8, and MMP-9 can also be activated by acidic pH followed by neutralization (12).

Hybrid Layer

The hybrid layer is the most vital part of the adhesive-based restoration. The quality of the hybrid layer determines the strength and durability of a restoration. The hybrid layer can be interpreted as a layer formed due to resin infiltration between collagen and hydroxyapatite fibers which functions as micromechanical retention of composite resin restorations to the dentin tissue. This layer consists of 50% collagen matrix and 50% resin. The hybrid layer serves to combine 2 different elements, namely hydrophilic dentin, and hydrophobic composite material, protecting the dentin surface from micro-leakage

and increasing dentin resistance to acid. The ideal hybrid layer is characterized by the presence of a collagen network that is bonded and reinforced with polymers (3,15).

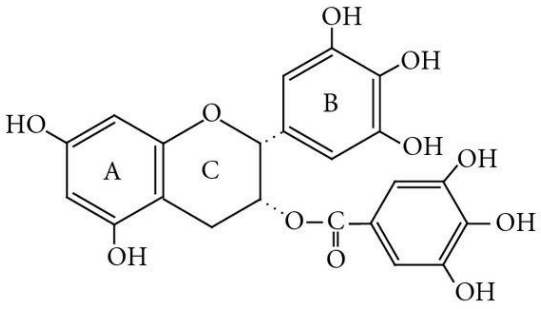
Hybrid Layer Degradation

The adhesive is now starting to use hydrophilic monomers such as Hydroxyethyl methacrylate (HEMA) as a hydrophobic monomer solvent to increase the wetting ability of the adhesive and prevent phase changes that occur when the diacrylate-based adhesive is applied to the dentin matrix which tends to be moist. Resin monomers which are hydrophilic in nature are very susceptible to hydrolysis due to the presence of ester bonds in the HEMA component. In addition, the increase in the HEMA component in the adhesive has been shown to increase water absorption in polymerized polymers, which causes a decrease in the mechanical properties of the hybrid layer.3 HEMA can provide good adhesions and is not easily degraded so that it can produce long-lasting restoration (16).

Epigallocathechin Gallate (EGCG)

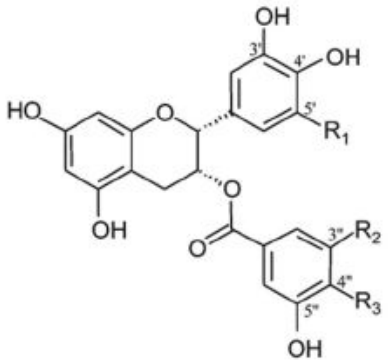
EGCG is a polyphenol that belongs to the catechin group, which able to inhibit the opening of sodium ion channels so that it has the potential to be anti-inflammatory. In addition, EGCG also has antioxidant properties that can reduce ROS by binding to ROS (17,18). EGCG is composed of 2- phenylchromane framework which is substituted in chain number 3,5,7,31,41 with a hydroxyl group. During biosynthesis, if the B-ring comes from sinton gallic acid, the catechin will be substituted with the 51st position of the hydroxyl group, namely "gallo" catechins which will esterify with gallic acid to form "gallate". Levorotatory compounds (2R, 3R) are called "epi" while dextrorotatory compounds (2S, 3R) are called "catehchin" so that when combined, they will become epigallocatechin gallate (EGCG). EGCG compound has the chemical formula (2R, 3R) -

5,7-dihydroxy-2- (3,4,5-trihydroxyphenly) -3,4-dihydro-2H-chromen-3-yl-3,4,5-trihydroxybenzoate (19). Judging from its chemical structure, EGCG is said to have the most potent antioxidative properties.



EGCG

Fig 1. EGCG



Substances	R_1	R_2	R_3
(–)-epigallocatechin-3-*O*-gallate (EGCG)	OH	OH	OH
(–)-epigallocatechin-3-*O*-(3-*O*-methyl)-gallate (EGCG-3Me)	OH	OCH_3	OH

Fig. 2. The Chemical Structure of EGCG and EGCG-3Me

EGCG can close TLR4 which can increase the production of TNF-α and hydroxyl and gallate groups that will bind to free radical ions so that it can prevent oxidation

reactions that can cause tissue damage (9,20). EGCG can also carry out an antioxidant activity, EGCG will bind free metal ions such as Fe2 + and Zn2 + ions and make them more stable so that the catalyzed reactions can be inhibited (1).

Demineralization in the caries process has been shown not only to be caused by contact with acids from bacteria but also due to the collagenolytic and gelatinolytic activity of the proteases present in the dentin organic matrix. The enzymes that play a role in this are MMP and cysteine cathepsin (21).

Table 1. Studies on the efficacy of EGCG

Tittle	author	Result
Evaluation of epigallocatechin-3-gallate (EGCG) cross-linked collagen membranes and concerns on osteoblasts	Chu, Chenyu Deng, Jia Xiang, Lin Wu, Yingying Wei, Xiawei Qu, Yili Man, Yi	Immersion of collagen membranes in 0.64%, 0.064%, and 0.0064% (w/v) EGCG solutions for 1 hour resulted in collagen that looks more compact, fibrils are more organized, increases fibril diameter, and narrows the space between fibrils. The higher the concentration of EGCG used, the more hydrophilic the dentinal collagen is. EGCG can also increase the modulus of elasticity and strength of collagen.
Epigallocatechin-3-gallate enhance dentin biomodification and bond stability of an etch-and-rinse adhesive *sistem*	Sun, Qiurong Gu, Lisha Quan, Jingjing Yu, Xiaoran Huang, Zihua Wang, Ruoxun Mai, Sui	Application of EGCG 0.1% for 120 seconds before bonding can increase Tensile Bond Strength (TBS) significantly compared to no treatment. EGCG can increase the contact angle by increasing the degree of hydrophobicity.
Effect of epigallocatechin-3-gallate solutions on bond durability at the adhesive interface in caries-affected dentin	Fialho, Melissa Proença Nogueira Hass, Viviane Nogueira, Rodrigo Proença França, Fabiana Mantovani Gomes Turssi, Cecilia Pedroso Basting, Roberta Tarkany	Application 20µl of 0.02%, 0.2%, 0.5% EGCG for 60 seconds did not show a significant difference in FFB. Both the CHX and EGCG groups had lower TBS than the control. The application of 0.02% EGCG had the lowest degree of nanoleakage compared to other concentrations, CHX, and control although not significant
Effect of polymeric microparticles loaded with catechin on the physicochemical properties of an adhesive *sistem*	Albuquerque, Nadine Luísa Guimarães Neri, Jiovanne Rabelo Lemos, Marcelo Victor Sidou Yamauti, Monica De Sousa, Francisco Fabio Oliveira Santiago, Sérgio Lima	Addition of PLGA (poly(D-L lactide-coglycolide) Acid) containing EGCG in the form of microparticles into bonding as much as 0.5%, 1.0%, and 2.0% w/w manually and the description of the application of 0.1% and 1% EGCG solutions before the bonding application showed an increase in TBS after 12 months is characterized by decreased degradation of collagen fibrils
Epigallocatechin-3-gallate and epigallocatechin-3-O-(3-O-methyl)-gallate enhance the bonding stability of an etch-and-rinse adhesive to dentin	Yu, Hao Han Zhang, Ling Yu, Fan Li, Fang Liu, Zheng Ya Chen, Ji Hua	The addition of EGCG-Me 600 µg/mL to the adhesive applied to teeth that had been etched in 2 layers showed the highest TBS.
Effect of epigallocatechin	Gerhardt, K. M.F.	There is no significant difference in tensile bond

EGCG as Cross-Linking Agent Towards Hybrid Layer in Dentin Collagen

The technology regarding adhesive materials is still developing, one of the weaknesses is the decrease in the resin-dentin bond which is often associated with an unstable hybrid layer. On the other hand, dentin also has MMP proenzymes and cysteine cathepsin which play a role in the destruction of collagen fibrils in the hybrid layer.

Collagen fibrils' stability, tensile strength, and viscoelasticity depend on the intermolecular cross-links from a translation of collagen. This is indicated by an increase in the degree of cross-link which is accompanied by an increase in the elastic tension of collagen fibrils. Chu (9), reported that applying EGCG solution to the collagen surface resulted in more regular collagen fibrils with a larger diameter and smaller interfibrillar space. The higher the concentration used, the greater the diameter of the collagen fibrils formed.

The cross-linking between EGCG and collagen chains can also affect the permeability of the hybrid layer to water molecules. This is evidenced by the research of

Sun (8), which shows that the application of 0.1% EGCG to dentin after acid etching can increase the contact angle of the dentine surface to water.

A different point was stated by Chu (9), who reported that administering EGCG 0.64% could reduce the contact angle of the dentin surface against water and allow maximum surface wetting. From the research method used, the two studies with different results used different methods. A dentin collagen membrane was immersed in EGCG solution for 1 hour at room temperature. Meanwhile, Sun (8), used pieces of dentin that had been polished and demineralized using acid etching and applied EGCG for 120 seconds. This difference in the material used allows for differences in research results even though they both use water droplets.

To determine the degree of attachment between the adhesive and dentin, most studies (n = 23) used a tensile bond strength test (TBS), and 4 journals evaluated nanoleakage analysis. Among the 17 studies that tested TBS with positive results, 10 of them use EGCG solution with varying concentrations on dentin before bonding application. According to Yu (10), EGCG can maintain adhesion stability through the inhibitory activity of MMPs. Meanwhile, Maria Fonesca (14), EGCG has hydrophobic properties through aromatic groups and hydrophilic through polar hydroxyl groups. The hydrophobic group can induce Van der Waals bonds with hydrophobic molecules in the resin. While the hydrophilic group hydrogen bonds with proteins in the collagen chain. Gerhardt (22), reported that the administration of EGCG solution with a concentration of 2% before the bonding application showed lower TBS rates than controls both immediately after application and 6 months later. Gerhardt added that these results could be due to the formation of a precipitate in the EGCG solution. Yang (23), reported that giving EGCG in ethanol and water solvents with a concentration of 0.02% showed a lower

degree of nanolekakage than the 0.1% concentration, this research is in line with another research that stated EGCG with the smallest concentration (0.0065%) can inhibit protease activity but its effectiveness drops significantly when it reaches a certain concentration. This raises the theory that the EGCG used in the treatment is highly dependent on the concentration used. Regarding this, Du (2), explained that giving EGCG in high concentrations can reduce the degree of adhesive conversion, because, during the polymerization period, EGCG trapped in the adhesive can interfere with the formation of polymer linear chains.

EGCG as a cross-linking agent and the wetting ability of the bonding material caused by the EGCG solvent, which is water. The use of different solvents can also cause differences in test results, Chemical bonding using acetone solvent can produce a stronger bond between the bonding material and dentin collagen when compared to using ethanol solvent (15). A study conducted by Soetojo (24), compared HEMA water solvent expresses less MDA and has good biocompatibility when compared to HEMA with ethanol or acetone solvent. Water solvents are considered to provide a wetting effect that can act as plasticizers of dentin collagen fibrils and keep collagen from collapsing. Water molecules in collagen make the diffusion of the bonding material with ethanol and acetone solvents into collagen fibrils easier, but the water molecules will also diffuse into the solvent, so the concentration of the adhesive solvent and the concentration of BisGMA decreases which causes the infiltration of BisGMA to be inadequate (25). Yang (23), used ethanol solvent in his EGCG solution. The use of ethanol as a solvent to replace water can increase the TBS of most hydrophobic resins such as BisGMA / TEGDMA by making the total ethanol-matrix cohesive force approaching the ethanol-BisGMA / TEGDMA solution so that the collagen matrix will immediately form interpeptid hydrogen bonds.

This evidence showed a high number of TBS and nanoleakage of the EGCG-ethanol group compared to the EGCG-water group.

EGCG as Chelating Agent against MMPs' Expression

The use of MMP inhibitors is thought to increase the bond strength of the adhesive to dentin. EGCG also functions as a chelating agent, EGCG interacts with MMP by exploiting the nature of catechins which have a high affinity for metal ions. Yang (23) states that the Zn ion which is the chemical structure of MMP can be bound by EGCG so that MMP is no longer recognized by the collagen matrix so that collagen degradation can be inhibited. But Cheng (26) has a different theory that stated EGCG inhibits the gelatinolytic activity of MMP by binding EGCG with the catalytic area or the area close to the catalytic area where gelatinolytic activity is occurring.

Based on journal search results, most studies (n = 15) regarding the application of EGCG to dentin collagen did show positive results both on TBS, nanoleakage, and the morphology of collagen fibrils. However, there is one randomized clinical trial that showed negative results. Costa (27), reported that administering 0.1% EGCG solution for 60 seconds before application of the bonding to the cervical lesion restoration procedure without caries (NCCL) did not show any difference in terms of retention, margin adaptation, secondary caries, and post-restoration sensitivity after 24 months with or without the procedure. This result explains that although EGCG does not have a positive effect on the restoration results, EGCG also does not have a negative effect that can worsen the restoration. Therefore, further evaluation is needed regarding concentrations, solvents, and application methods that can be used to increase the resin-dentin adhesion in the hybrid layer.

CONCLUSION

MMP activity on dentinal collagen can be inhibited by EGCG, which has a favorable influence on the shape of dentinal collagen fibrils.

ACKNOWLEDGEMENT

The authors would like to thank the staff of Endodontic Division of Faculty of Dental Medicine, Universitas Airlangga. The authors declare no potential conflicts of interest with respect to the authorship and publication of this article.

REFERENCES

1. Münchow EA, Bottino MC. Recent Advances in Adhesive Bonding: The Role of Biomolecules, Nanocompounds, and Bonding Strategies in Enhancing Resin Bonding to Dental Substrates. Curr Oral Heal Reports. 2017;4(3):215-27. doi:10.1007/s40496-017-0146-y.
2. Du X, Huang X, Huang C, Wang Y, Zhang Y. Epigallocatechin-3-gallate (EGCG) enhances the therapeutic activity of a dental adhesive. J Dent. 2012;40(6):485-92. doi:10.1016/j.jdent.2012.02.013.
3. Breschi L, Maravic T, Cunha SR, *et al.* Dentin bonding systems: From dentin collagen structure to bond preservation and clinical applications. Dent Mater. 2018;34(1):78-96. doi:10.1016/j.dental.2017.11.005.
4. Tezvergil-Mutluay A, Pashley D, Mutluay MM. Long-Term Durability of Dental Adhesives. Curr Oral Heal Reports. 2015;2(4):174-81. doi:10.1007/s40496-015-0070-y
5. Soetojo A, Purnama D, Lunardhi CGJ, Widjiastuti I. Cytotoxicity Test of 4-Methacryloxyethyl Trimellitic Anhydrride-based Dentin Bonding Material Using Acetone Solution in Dental Pulp Fibroblast. J Int Oral Health. 2019;(11):191-6.
6. Fialho MPN, Hass V, Nogueira RP, *et al.* Effect of epigallocatechin-3- gallate solutions on bond durability at the adhesive interface in caries-affected. J Mech Behav Biomed Mater. 2019;91:398-405. doi:10.1016/j.jmbbm.2018.11.022.

7. Albuquerque NLG, Neri JR, Lemos MVS, Yamauti M, De Sousa FFO, Santiago SL. Effect of polymeric microparticles loaded with catechin on the physicochemical properties of an adhesive system. Oper Dent. 2019;44(4):E202-11. doi:10.2341/18-112-L.
8. Sun Q, Gu L, Quan J, *et al.* Epigallocatechin-3-gallate enhance dentin biomodification and bond stability of an etch-and-rinse adhesive system. Int J Adhes. 2018;80:115-21. doi:10.1016/j.ijadhadh.2017.11.001.
9. Chu C, Deng J, Xiang L, *et al.* Evaluation of epigallocatechin-3-gallate (EGCG) cross-linked collagen membranes and concerns on osteoblasts. Mater Sci Eng C. 2016;67:386-94. doi:10.1016/j.msec.2016.05.021.
10. Yu HH, Zhang L, Yu F, Li F, Liu Z.Y, Chen JH. Epigallocatechin-3-gallate and epigallocatechin-3-O-(3-O-methyl)-gallate enhance the bonding stability of an etch-and-rinse adhesive to dentin. Materials (Basel). 2017;10(2). doi:10.3390/ma10020183.
11. Garg N, Garg A. *Textbook of Endodontics*. 3rd ed. New Delhi: Jaypee Brothers Medical Publishers; 2014
12. Zirta UA, Juanita AG, Nurrohman H. The role of matrix metalloproteinases in dentin caries. J Indones Dent Assoc. 2009;58(2):25-31.
13. Pashley D, Tay F. Mechanical stability of resin-dentin bonds. Dent Biomater Imaging, Test Model. 2008;112-161. doi:10.1533/9781845694241.112.
14. Maria FB, Camara BD, Mara da ST, Luis Souto BA, Paulo Moisés de OH, Eduardo de Paiva GS. Mechanical-physicochemical properties and biocompatibility of catechin-incorporated adhesive resins. J Appl Oral Sci. 2019;27:1-11. http://dx.doi.org/10.1590/1678-7757-2018-0111.
15. Zubaidah N, Effendy R, Soetojo A, Estiyaningsih T, Tanzil MI, Khotimah K.cDifference of chemical bonds between UDMA bonding agents with ethanol solvent and acetone solvent on dentin collagen. Pesqui Bras Odontopediatria Clín Integr. 2021;21:e0116. https://doi.org/10.1590/pboci.2021.030.
16. Saraswati W, Widjiastuti I, Rukmo M, Wahjuningrum DA. The Expression of HMGB1 in Dentin Pulp Complex Induced by Resin Monomer HEMA. Int Medical Device and Tech Conf. 2017.
17. Ismiyatin K, Soetojo A, Wahluyo S, Purwanto B, Rahayu RP, Mukono IS. Topical

Epigallocatechin-3-gallate hydrogels regulated inflammation and pain. J Int Dent Med Res. 2019;12(1):54-60.

18. Ismiyatin K, Soetoyo A, Wahluyo S, Safitri I. Therapeutic Efficacy of Topical Epigallocatechin-Gallate as a New Therapeutic Strategy for Inhibition of Pain Conduction on Rat Models with Acute Pulpal Inflammation. Int Med Device Technol Conf. 2017;107-10.
19. Legeay S, Rodier M, Fillon L, Faure S, Clere N. Epigallocatechin Gallate: A Review of Its Beneficial Properties to Prevent Metabolic Syndrome. Nutr. 2015;7(7). doi:10.3390/nu7075230.
20. Ismiyatin K, Wahluyo S, Pureanto DA, Rahayu RP, Soetojo A, Mukono IS. Effect of Topical Epigallocatechin-Gallate on Lipopolysaccharide-induced Pulpa Inflammation in Rat Models. Iranian Endodontic Journal. 2018;13(4):528-33.
21. Van Doren SR. Matrix metalloproteinase interactions with collagen and elastin. Matrix Biol. 2015;44-46:224-231. doi:10.1016/j.matbio.2015.01.005.
22. Gerhardt KMF, Oliveira CAR, França FMG, Basting RT, Turssi CP, Amaral FLB. Effect of epigallocatechin gallate, green tea extract and chlorhexidine application on long-term bond strength of self-etch adhesive to dentin. Int J Adhes Adhes. 2016;71:23-7. doi:10.1016/j.ijadhadh.2016.08.005.
23. Yang H, Guo J, Deng D, Chen Z, Huang C. Effect of adjunctive application of epigallocatechin-3-gallate and ethanol-wet bonding on adhesive-dentin bonds. J Dent. 2016;44:44-49. doi:10.1016/j.jdent.2015.12.001.
24. Soetojo A., Cahyadi EKH, Prasetyo EA. Malondialdehyde Expressions on Pulp Odontoblast Cells after Application 2-hydroxylethul methacrylate mixed with water, ethanol and aceton solvent. Saudi Endod J. 2019;(9):96-100.
25. Prasetya AI, Kunarti S, Soetojo A, Prasetyo EA. Chemical Bond Strength Difference between 4-Meta Bonding Agents with Ethanol and Acetone Solvent on Type I Collagen. Journal of International Dental and Medical Research. 2017;11(2):567-71.
26. Cheng XW, Kuzuya M, Kanda S, et al. Epigallocatechin-3-gallate binding to MMP-2 inhibits gelatinolytic activity without influencing the attachment to extracellular matrix proteins but enhances MMP-2 binding to TIMP-2. Arch Biochem Biophys. 2003;415(1):126-132. doi:10.1016/S0003-9861(03)00221-2.

27. Costa C, Albuquerque N, Mendonça JS, Loguercio AD, Saboia V, Santiago SL. Catechin-based Dentin Pretreatment and the Clinical Performance of a Universal Adhesive: A Two-year Randomized Clinical Trial. Oper Dent. 2020;45(5):473-483. doi:10.2341/19-088-C.

MALAYSIAN JOURNAL OF
Medicine and Health Sciences

Editorial Board

Malaysian Journal of Medicine & Health S... 03/04/21

Malaysian Journal of Medicine & Health Sciences...

To: ie elline, ellineistanto Indosat,

Reply-To: sploh@upm.edu.my

Details

02-Apr-2021

Dear Dr. Elline:

Your manuscript entitled "Nanohydroxiapatite Using Chicken Eggshell Waste And Its Characterization" has been successfully submitted online and is presently being given full consideration for publication in the Malaysian Journal of Medicine & Health Sciences.

Your manuscript ID is MJMHS-2021-0338.

Please mention the above manuscript ID in all future correspondence or when calling the office for questions. If there are any changes in your street address or e-mail address, please log in to ScholarOne Manuscripts at https://mc.manuscriptcentral.com/mjmhs and edit your user information as appropriate.

You can also view the status of your manuscript at any time by checking your Author Center after logging in to https://mc.manuscriptcentral.com/mjmhs.

Thank you for submitting your manuscript to the Malaysian Journal of Medicine & Health Sciences.

Sincerely,
Malaysian Journal of Medicine & Health Sciences Editorial Office

06-Apr-2021

Dear Dr. Elline:

Your manuscript, MJMHS-2021-0338, entitled "Nanohydroxiapatite Using Chicken Eggshell Waste And Its Characterization" has been unsubmitted to the Malaysian Journal of Medicine & Health Sciences. It may either have been unsubmitted at your request or because you did not complete all necessary parts of the submission.

1) References in text should be numbered in parenthesis [e.g. (1), (1,3), (1,3-5)] and cited consecutively in the order of appearance in the manuscript. Please refer to the sample manuscript. Please change format letter from small letter to normal letter.

2) Your title page does not follow MJMHS format. Please refer to the sample title page. Kindly remove the number next to the author's name since both authors came from same affiliation.

PLEASE RESUBMIT YOUR MANUSCRIPT USING THIS ARTICLE ID.

Please visit the instructions to authors to complete your submission and re-submit the manuscript for consideration of publication. You may contact the Editorial Office if you have further questions.

Sincerely,
Malaysian Journal of Medicine & Health Sciences Editorial Office

Sample-Manus...icle.pdf

Malaysian Journal of Medicine & Health Sciences 17 May 2021 16.17

Malaysian Journal of Medicine & Health Sciences - MJMHS-2021-...

To: ie_elline23@yahoo.co.id, ellineistanto@gmail.com, & 4 more

Details

Reply-To: mjmhs@upm.edu.my

17-May-2021

Dear Dr. Elline:

Your manuscript, MJMHS-2021-0338.R1, entitled "Nanohydroxiapatite Using Chicken Eggshell Waste And Its Characterization" has been unsubmitted to the Malaysian Journal of Medicine & Health Sciences. It may either have been unsubmitted at your request or because you did not complete all necessary parts of the submission.

1) Figure should be submitted in a TIFF/JPG file not in PDF.

2) Each figure and table should be submitted in an individual file. For example, if you have 7 tables, you have to submit 7 files separately. if you have 4 figure then, submit 4 files of figure separately.

PLEASE RESUBMIT YOUR MANUSCRIPT USING THIS ARTICLE ID.

Please visit the instructions to authors to complete your submission and re-submit the manuscript for consideration of publication. You may contact the Editorial Office if you have further questions.

Sincerely,
Malaysian Journal of Medicine & Health Sciences Editorial Office

07-Jun-2021

Dear Dr. Elline:

It is a pleasure to accept your manuscript entitled "Nanohydroxiapatite Using Chicken Eggshell Waste And Its Characterization" in its current form for publication in the Malaysian Journal of Medicine & Health Sciences. The comments of the reviewer(s) who reviewed your manuscript are included at the foot of this letter.

Thank you for your fine contribution. On behalf of the Editors of the Malaysian Journal of Medicine & Health Sciences, we look forward to your continued contributions to the Journal.

Sincerely,
Dr. Normala Ibrahim
Editor-in-Chief, Malaysian Journal of Medicine & Health Sciences
normala_ib@upm.edu.my

Associate Editor Comments to Author:

Associate Editor
Comments to the Author:
(There are no comments.)

Reviewer(s)' Comments to Author:

Reviewer: 1

Comments to the Author
(There are no comments.)

Reviewer: 2

Comments to the Author
(There are no comments.)

MALAYSIAN JOURNAL OF
Medicine and Health Sciences

Nanohydroxiapatite Using Chicken Eggshell Waste And Its Characterization

Journal:	*Malaysian Journal of Medicine & Health Sciences*
Manuscript ID	MJMHS-2021-0338.R1
Manuscript Type:	Supp: APASTB-2021
Keywords:	Nanohyroxyapatite, Chicken Eggshell Waste, Characterization

Abstract

Hydroxiapatite (HAp) is a multiused biomaterial and it can stimulates hard tissue repair. HAp is biocompatible, nontoxic and similar with bone and teeth structure. It can be synthesized from natural sources, such as eggshell waste. Eggshells waste contain almost 94% calcium carbonate, which preferable for producing CaO as calcium resource for synthesizing pure HAp powder with nanocrystalline form. Compared to other poultry eggshells, chicken eggshell has higher HAp composition. This study aims to review nano hydroxyapatite (nanoHAp) from chicken eggshell waste and its characterization using Scanning Electron Microscopy (SEM), Energy Dispersive X-ray spectroscopy (EDX) , X-ray Diffraction Analysis (XRD), and Fourier Transform (FTIR) Spectroscopy.

Key Words : Nanohydroxyapatite, Chicken Eggshell Waste, Characterization

Introduction

Hydroxyapatite is biomaterial that widely used. Its chemical properties has an excellent biocompatibility, bioactivity, stimulates growth of bone tissue and economically cost. There were several experiment concerning to produce best quality HAp because of its advantages. It was synthesized through various methods.(1-2) There are many natural sources of HAp, such as bovin bone, aquatic source, and eggshells.(3-4) Among them, Chicken eggshell is interesting to be reviewed because it contains 94% calcium carbonate and low cost biomaterials. It consists of higher hydroxyapatite than other poultry eggshells.(2,5)

Function Of Hydroxiapatite In Dentistry

In Implantology, calcium source from nanoHAp creates osseogenesis, inhibits the growth of bacteria, reduced inflammatory, and reconstruct bone defects.(6) In periodontology, HAp is used to fill bone loss in surgical procedure and binds chemically in osseointegration process.(7) Nowadays, HAp is also used in tissue engineering, It is known as excellent material approach for hard tissue reconstruction and repair. It support alginate or other polymers as reinforcement

and osteoconductive material to promote a successful tissue regeneration.(8-9) Another study performed gelatin,magnesium doped hydroxyapatite mixed with alginate thus they concluded that it is possible to achieve scaffolds with fine microscopic pore.(10) Earlier clinical tested the hypersensitivity effect of nanohydroxyapatite compared with Pro-Argin and fluoride varnish, and it showed nanohydroxyapatite effective as dentin desensitizing.(11)

Source of Hydroxyapatite

HAp can be synthesized from natural and synthetic source. Synthetic HAp was common material used in tissue engineering, bone regeneration and replacement. It was suitable to human hard tissue with 1,67 stoichiometry. The main of its composisition is calsium, so similar to HAp from living source. Natural HAp carries advantages such as reduces the impurity and production cost, also biological origin and overcomes environtment pollutant.(12-13) It can be synthesized from mammalian bone, clam shell, coral, poultry eggshell.(14-15) Chicken eggshell is one of common derived HAp with economically cost, and consumed tons yearly.(2) It also contains higher HAp (0,0950 g/g) than other poultry eggshells (0,3315 g/g - 0,0559 g/g).(5) Synthetic nanoHAp can achieved 534 nm particle size and natural HAp can be synthesized to 250–550 nm with appropriate milling process, so natural HAp is acceptable for bone replacement application.(16)

Review of HAp Characterization

Characterization of HAp derived from eggshell usually uses several tools , such as Scanning Electron Microscopy (SEM)-Energy Diffraction Spectroscopy (EDX) which can observes the morphological and elemental study. Heating process with appropriate method resulted in well shaped nano HAp particle with agglomerates shape creates pore in between.(2) Calcination at 1100 °C can performed smooth surface of agglomeration in spherical shape.(16) Figure 1 (Fig.1) showed SEM analysis of HAp at 2 hours aging time performed fluffy agglomerates

rounded edge morphology, and highly agglomerated HAp figure at 1000 °C calcination (Fig.2).(3,17)

Cristalline structure and composition phase can be determined by XRD analysis. XRD uses CuKa radiation with 40 - 45 kV voltage and data collects from $10^{o} < 2\theta < 80^{o}$.(12,17) The diffraction pattern performed compatible HAp characterization phase with crystal size range at 20.12 and 19.93 nm in eggshell HAp which calcinated in 1000°C and there were no secondary phase (Fig.3). It suitable with bone tissue HAp which has 15 nm crystallite size.(3)

FTIR used to observe the type of chemical bonds and functional group in samples.(12) Usually samples uses with KBr to make a pellet and it is tested with 4000-400 cm^{-1} wavenumber range.(18) Figure 4 showed adsorbance of H_2O in graph broad band at 1643.48 cm^{-1} and 3449.01 cm^{-1}. Vibration mode was known by observed peak at 878.26 cm^{-1} and 1460.87 cm^{-1} which indicates elimination CO_3^{2-} ion because of calcination process of HAp. Band at 633,14 confirmed the OH structure in HAp. Peak at 926.81 showed starching mode of PO^{3-}. From this graph it was confirmed crystalline phase.(2)

Several study concerned in HAp characterization are performed by Horta et al who synthesized nanohydroxyapatite by precipitation method using hen eggshell with 2 different aging time (Table I), Khandelwal and Prakash synthesized HAp powder from eggshell with wet chemical method also characterized it by SEM-EDX, XRD, FTIR, TGA-DTA (Thermogravimetric/ Differential Thermal Analysis) (Table II). Another study performed due to develop tissue engineering biomaterial by Agbabiaka et al who also characterized HAp from eggshell using three calcination temperature (Table III).(17) Hamidi et al characterized derived HAp from eggshell using calcination and ball milling method with different rotational speed and heat treatment temperature using SEM,XRD and FTIR. (Table IV).(16)

Conclusion

Hydroxiapatite is a biomaterial used due to its biocompatibility and the similarity with bone and teeth structure, whether it was synthetic or natural source. Eggshell waste is one of popular natural source which can be synthesized into nanohydroxiapatite. According to several research before, calcination using high temperature and appropiate ball milling process has considered to give several effect in achived proper HAp. Selection of suitable HAp characterization tools can performed appropriate interpretation result.

Conflict of Interest

Author declared no potential conflicts of interest with respect to the authorship and/or publication of this article.

References

1. Sossa PAF, Giraldo BS, Garcia BCG, Parra ER, Arango PJA. Comparative study between natural and synthetic Hydroxyapatite: structural, morphological and bioactivity properties. Matéria (Rio J) [Internet]. 2018 Dec 6 [cited 2021 Mar 4];23(4). Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1517-70762018000400408&lng=en&tlng=en
2. Khandelwal H, Prakash S. Synthesis and Characterization of Hydroxyapatite Powder by Eggshell. JMMCE. 2016;04(02):119–26.
3. Horta M, Aguilar M, Moura F, Campos J, Ramos V, Quizunda A. Synthesis and characterization of green nanohydroxyapatite from hen eggshell by precipitation method. Materials Today: Proceedings. 2019;14:716–21.
4. Mohd Pu'ad NAS, Koshy P, Abdullah HZ, Idris MI, Lee TC. Syntheses of hydroxyapatite from natural sources. Heliyon. 2019 May;5(5):e01588.
5. Gintu AR, Salenussa MW, Wadu I, Hartini S. Sintesis Biokeramik Hidroksiapatit (Hap) Dari Kerabang Telur Ayam Kampung, Ayam Broiler, Dan Bebek Menggunakan Metode Pengendapan Basa Dan Hidrolisis.Bioma. 2017;6(2):11.
6. Bordea IR, Candrea S, Alexescu GT, Bran S, Băciuț M, Băciuț G, et al. Nano-hydroxyapatite use in dentistry: a systematic review. Drug Metabolism Reviews. 2020 Apr 2;52(2):319–32.
7. Pepla E, Besherat LK, Palaia G, Tenore G,, Guido Migliau. Nano-hydroxyapatite and its applications in preventive, restorative and regenerative dentistry: a review of literature. ADS [Internet]. 2014 [cited 2021 Mar 4]; Available from: http://www.annalidistomatologia.com/common/php/portiere.php?ID=152fe78586592c638a38bef5e4b82eb2
8. Sancilio S, Gallorini M, Di Nisio C, Marsich E, Di Pietro R, Schweikl H, et al. Alginate/Hydroxyapatite-Based Nanocomposite Scaffolds for Bone Tissue Engineering Improve Dental Pulp Biomineralization and Differentiation. Stem Cells Int. 2018 Aug 2;2018:1–13.
9. Sathiyavimal S, Vasantharaj S, LewisOscar F, Selvaraj R, Brindhadevi K, Pugazhendhi A. Natural organic and inorganic–hydroxyapatite biopolymer composite for biomedical applications. Progress in Organic Coatings. 2020 Oct;147:105858.

10. Panseri S, Montesi M, Dozio SM, Savini E, Tampieri A, Sandri M. Biomimetic Scaffold with Aligned Microporosity Designed for Dentin Regeneration. Front Bioeng Biotechnol [Internet]. 2016 Jun 8 [cited 2020 Sep 27];4. Available from: http://journal.frontiersin.org/Article/10.3389/fbioe.2016.00048/abstract
11. Wang L, Magalhães A, Francisconi-dos-Rios L, Calabria M, Araújo D, Buzalaf M, et al. Treatment of Dentin Hypersensitivity Using Nano-Hydroxyapatite Pastes: A Randomized Three-Month Clinical Trial. Oper. 2016 Jul 1;41(4):E93–101.
12. Hammood AS, Hassan SS, Alkhafagy MT. Comparison of Natural and Nano-synthetically-Produced Hydroxyapatite Powder. JOM. 2019 Jan;71(1):272–8.
13. Ketta M, Tůmová E. Eggshell structure, measurements, and quality-affecting factors in laying hens: a review. Czech J Anim Sci. 2016 Jul 24;61(07):299–309.
14. Yusril Yusuf, Dyah Uswatun Khasanah Firda Yanuar Syafaat Ishak Pawarangan, Mona Sari, Vicky Julius Mawuntu Yazida Rizkayanti. Hidroksiapatit Berbahan Dasar Biogenik. Gadjah Mada University Press; 2019.
15. Mozartha M. Hidroksiapatit Dan Aplikasinya Di Bidang Kedokteran Gigi. Cakradonya Dent J. 2015;7(2):807–68.
16. Hamidi AA, Salimi MN, Yusoff AHM. Synthesis and characterization of eggshell-derived hydroxyapatite via mechanochemical method: A comparative study. In Kaohsiung City, Taiwan; 2017 [cited 2021 May 12]. p. 020045. Available from: http://aip.scitation.org/doi/abs/10.1063/1.4981867
17. Agbabiaka OG, Oladele IO, Akinwekomi AD, Adediran AA, Balogun AO, Olasunkanm OG, et al. Effect of calcination temperature on hydroxyapatite developed from waste poultry eggshell. Scientific African. 2020 Jul;8:e00452.
18. Hikmawati D, Maulida HN, Putra AP, Budiatin AS, Syahrom A. Synthesis and Characterization of Nanohydroxyapatite-Gelatin Composite with Streptomycin as Antituberculosis Injectable Bone Substitute. International Journal of Biomaterials. 2019 Jun 25;2019:1–8.

Figure Legend

Fig.1 SEM analysis of HAp performed fluffy agglomerates rounded edge morphology (Horta et al., 2019)

Fig.2 SEM analysis of HAp with highly agglomerated morphology (Agbabiaka et al., 2020)

Fig.3 The XRD pattern performed compatible characterization phase in eggshell HAp which calcinated in 1000°C (Horta et al., 2019)

Fig.4 FTIR graph confirmed crystalline phase (Khandelwal and Prakash., 2016)

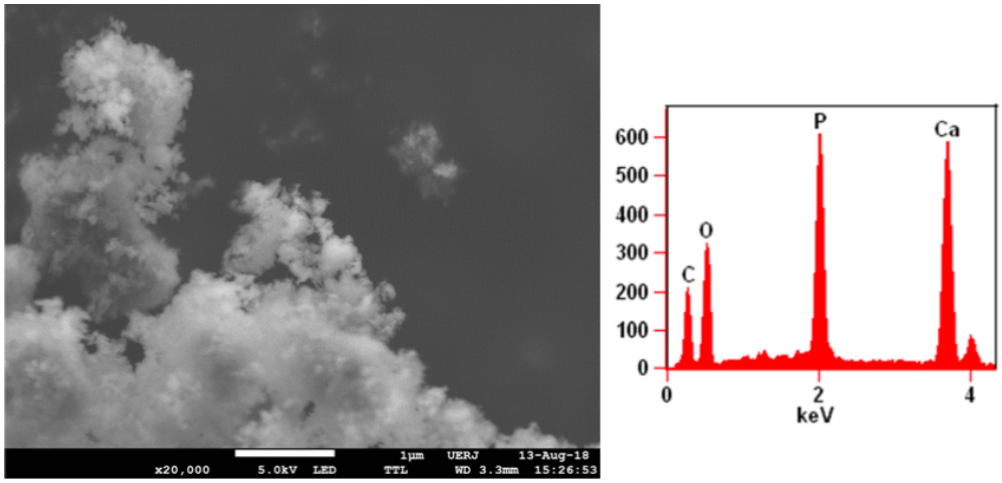


Fig.1 SEM analysis of HAp performed fluffy agglomerates rounded edge morphology (Horta et al., 2019)

298x142mm (72 x 72 DPI)

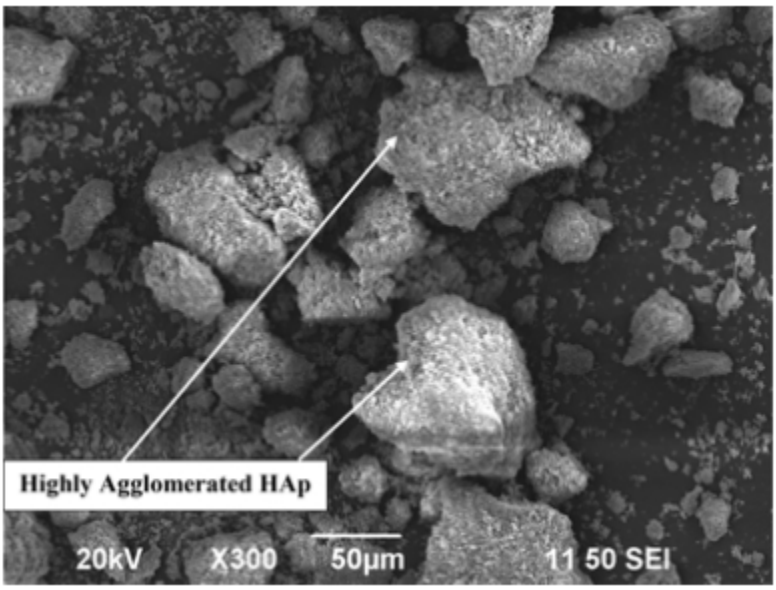


Fig.2 SEM analysis of HAp with highly agglomerated morphology (Agbabiaka et al., 2020)

136x102mm (72 x 72 DPI)

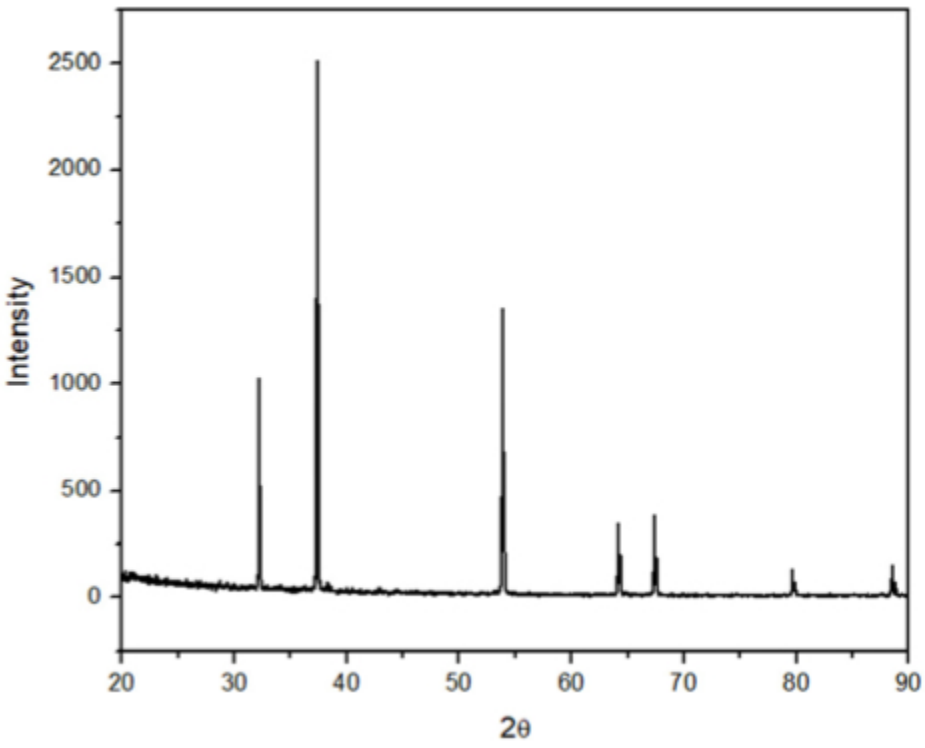


Fig.3 The XRD pattern performed compatible characterization phase in eggshell HAp which calcinated in 1000°C (Horta et al., 2019)

168x134mm (72 x 72 DPI)

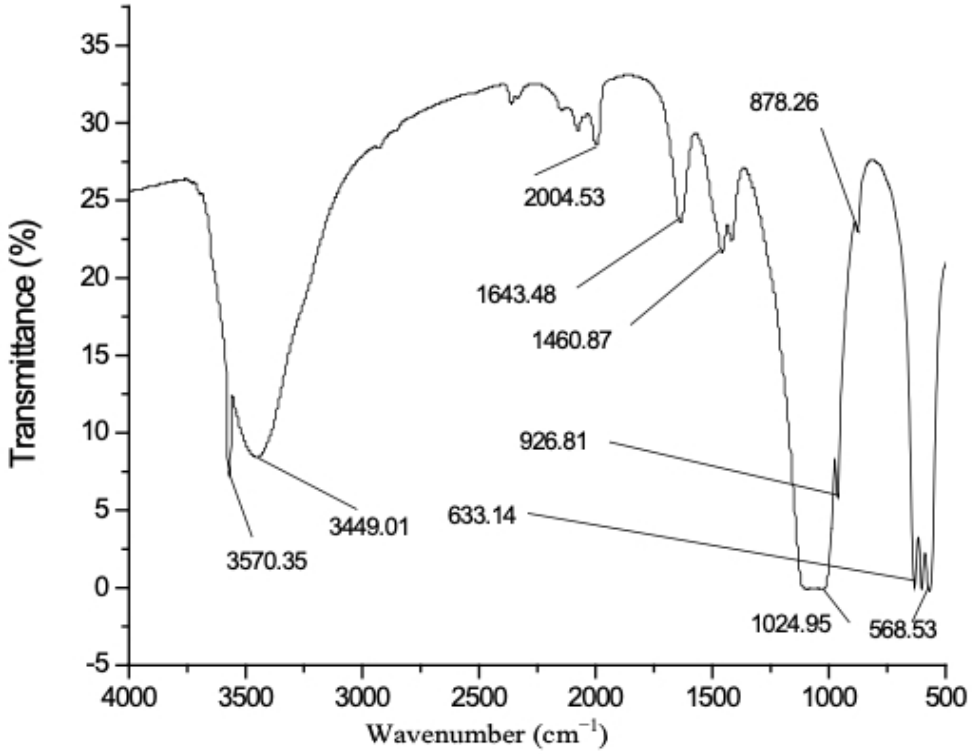


Fig.4 FTIR graph confirmed crystalline phase (Khandelwal and Prakash., 2016)

185x142mm (72 x 72 DPI)

Table I. Horta et al HAp characterization result

Characterization Method	**Remarks**	
	Precipitation method using 2 aging time	
	1 hour	2 hours
SEM	lamellar, such as plates like	Fluffy morphology (Fig.1)
EDX	Ca/P ratio : 1.59	Ca/P ratio : 1.49
XRD	complete decomposition of $CaCO_3$ to CaO monoclinic hydroxyapatite phase with no secondary phases average crystallite size: 20.12	complete decomposition of $CaCO_3$ to CaO monoclinic hydroxyapatite phase with no secondary phases average crystallite size: 19.93
Specific Surface BET	73.17 m^2/g	68.15m2/g
Concluding remark : Aging time variation performed no significant differences in the characteristics of the materials, Chicken eggshell were very promising for biomedic applications		

Table II. Khandelwal and Prakash HAp characterization result

Characterization Method	Remarks
	Wet Chemical method with 900°C calcination for 2 hours
SEM	Microcrystalline molecule with irregular agglomerates shapes with pores
EDX	Ca/P ratio : 1.68 and it was acceptable.
XRD	2θ range 15℃ to 80℃. Intense reflection peak between 31.8 - 32.5 of 2θ values, confirmed of the apatite phase, with 31,5 nm average particle size
FTIR	H_2O adsorbance at 1643.48 cm^{-1} and 3449.01 cm^{-1} Vibration mode CO_3^{2-} ion at 878.26 cm^{-1} and 1460.87 cm^{-1}, confirmed elimination of CO_3^{2-} because of calcination process OH stretching bond at 3570.35 cm^{-1} due to water adsorbance , at 633.14 confirmed OH in HAp. Peak at 926,81 confirmed HAp $PO4^{3-}$ starching mode at 565.53 showed crystalline phase (Fig.4)
TGA-DTA	At 1400°C temperature obtained thermal stability without major loss of weight HAp samples
Concluding remark : HAp powder which Calcinate at 900°C can performed pure and single apatit phase, with Ca/P ratio 1,68	

Table III. Agbabiaka et al characterization result

Characterization Method	**Remarks**		
	800^{O}C calcination	900^{O}C calcination	1000^{O}C calcination
SEM	crystallites flake /crystallites agglomerate like arbitrary flower structure	Agglomerates in spherical shape	Irregular agglomerated shape (Fig.2)
EDX	Ca/P:0.55, below stoichiometry ratio	Ca/P :1.26 with monetite and calcium hydrogen phosphate hydrate phase	Ca/P ratio : 1.65 , there were complete hydroxyapatite phase
XRD	The temperature wasn't suitable so there were no HAp phase identified	Main phase was monetite and hydroxyapatite. There were incomplete process of CaO during calcination, so $Ca_4H_2(P_3O_{10})_2$ phase wasn't available	There were strong peak HAp phase
Concluding remark: HAp 1000^{O}C performed strong peak of HAp in XRD analysis, EDX result also similar to stoichiometry ratio,morphology were vary depends on the synthesis of HAp,			

Table IV. Hamidi characterization result

Characterization Method	**Remarks**			
	200 rpm (800°C and 1100°C)		400 rpm (800°C and 1100°C)	800 rpm (800°C and 1100°C)
SEM	800°C : irregular form with spherical shape, small and large particles clusters with mean particle size: 263 nm. 1100 °C : mean particles 513 nm (Fig.1)		Large agglomerates with fine particles , mean particles 257 nm	smooth surface of agglomeration in spherical shape.
FTIR	1 reaction process	2 reaction process	1 reaction process	1 reaction process
	H_2O adsorbtion at 3600 and 2600 cm^{-1} At temperature 800 and 1100 ° C: major peak at 3435 cm^{-1} PO_4^{3-} weak stretching peak at 963 cm^{-1}	Lower H_2O adsorbtion at 3448 cm^{-1}(less water molecule) Streching OH group : peak at 3642cm^{-1} (HAp is present in the sample) PO_4^{3-} asymmetrical stretching vibration modes 602	H_2O adsorbtion at 3436 cm^{-1} Streching OH group : peak at 3567 (HAp is present in the sample) PO_4^{3-} asymmetrical stretching vibration modes : 566cm^{-1}	H_2O adsorbtion at 3435cm^{-1} Streching OH group : peak at3569 cm^{-1} (HAp is present in the sample) PO_4^{3-} asymmetrical stretching vibration modes: 1040 cm^{-1} , and and 471
XRD	800°C : Low crystallite size (19.00 nm) 1100 °C : high HA crystallite size (34.89 nm)	HAp crystal size 0 nm (no HAp phase)	Suitable crystal size	Suitable crystal size
Concluding remark: higher rotational speed forms chemically more complete HAp, heat treatment to 1100 °C results in stoichiometric HAp and promoted the particles to agglomerate and form bigger size due to increase of degree of crystallinity				

The author has explained the study's objectives perspicuously to see how EGCG, as a cross-linking agent, affected dentinal collagen and hybrid layers, as well as how chelating chemicals affected MMPs. Although the references used are appropriately cited by the author and the title is relevant to the abstract, the number of references cited is inadequate to be considered a full review article (can rewrite as a mini-review). The author can elaborate more about the current technology of cross-linking agents and chelating agents and highlight the significance of EGCG can play a role in improving. Thus, the number of references can be increased (10~20) to make the mini-review more impactful and significant to the particular audience especially in dentistry. Despite the inadequate references, this study might give new insights regarding potential EGCG to be employed in the adhesive system of conservative dentistry. However, some minor improvements are described below:

Introduction Page 5, Line 52, please use a better reference format as Du (2) does not reflect all authors; instead, use citation as Du et al.

Page 6, Line 44-50. Readers can detect repetition from the previous paragraph. Please paraphrase the highlighted part accordingly.

The author mentioned several factors that affect quality without explaining what is the several factors before emphasizing MMP.

Body Please see the highlighted sentence and correct the format accordingly and check the rest of the manuscript.

Reference Please use better reference format such as APA as a standard

Conclusion The summary is too short, please elaborates the conclusion, and emphasizes why EGCG is favourable.

REVIEW ARTICLE

Potential of Epigallocatechin-3-gallate as Chelating Agent against Matrix Metalloproteinase Expression and as Cross-Linking Agent Towards Hybrid Layer in Dentin Collagen: A Review

Kun Ismiyatin[1], Setyabudi Goenharto[1], Windi Irsya[2], Paramita Tanjung Sari[2], Olivia Vivian Widjaja[2], Ria Puspita Sari[2]

[1] Department of Conservative Dentistry, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, 60132, Indonesia
[2] Resident of Conservative Dentistry Department, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, 60132 Indonesia

ABSTRACT

Adhesive dentistry's main assumption is to create a strong chemical bond between dental hard tissues and restorative composite material. One of the most important aspects of this interface is the hybrid layer. Unfortunately, due to physical and chemical causes, the hybrid layer wears away with time. Epigallocatechin-3-gallate (EGCG), a component extracted from green tea, has several roles in the medical and dentistry field including as a crosslinking agent and as a chelating agent. Although there are several negative results, EGCG was proven to be able to preserve resin-dentin bonds without harming the restoration. As a crosslinking agent and chelating agent, EGCG has the potential to enhance the physical properties of dentin collagen and resin-dentin adhesion. The purpose of this study was to see how EGCG, as a cross-linking agent, affected dentinal collagen and hybrid layers, as well as how chelating chemicals affected Matrix metalloproteinase (MMPs).

Keywords: Epigallocatechin-3-gallate (EGCG), Matrix metalloproteinase (MMPs), Crosslink, Chelatin, Collagen

Corresponding Author:
Kun Ismiyatin, M.Kes., Sp.KG(K)
Email: kun-is@fkg.unair.ac.id

INTRODUCTION

The adhesive system is one of the most revolutionary breakthroughs in the field of conservative dentistry. This system creates minimally invasive restorations that require minimal preparation (1). Du (2) reported that resin adhesion to dentin had high bond strength immediately after application, but decreased by 50-60% after 1-2 years. To avoid the decrease of bond strength, a hybrid layer that is strong, stable, and has high durability is required. A hybrid layer is a layer formed from resin monomers that infiltrate demineralized intratubular, intertubular, and extra tubular collagen fibrils (3,4). Several factors that affect the quality of the hybrid layer, one of which is enzyme that involve in the demineralization of dentin collagen is Matrix metalloproteinase (MMP) on odontoblasts (1,3). MMP must be prevented for increasing the resin-dentin adhesion. The dentine resin attachment mechanism is a physical-mechanical attachment and a chemical reaction between the dentin bonding material and the collagen on the dentin surface. A stable bond can be achieved between the restorative material and the teeth (5). Dentin also contains water which can cause degradation of resin components, to increase resin-dentin adhesion, prevent water molecular retention are needed, thereby increasing collagen integrity, preventing resin degradation, and strengthening hybrid layer formation (6).

Epigallocatechin-3-gallate (EGCG) is one of the catechins with the highest percentage in green tea extract (49%), has a high affinity for metal ions which can inhibit the action of the enzyme (MMP) through the chelating process and increase the integrity and stability of collagen so it can increase the adhesion strength of the hybrid layer (2). Another research stated by Albuquerque (7) that the application of EGCG can increase the resistance of dentin-bonding attachments within 2 years. As a cross-linking agent, EGCG is also able to replace water molecules in the collagen bond chain by hydrogen bonding with the collagen peptide chain which can reduce collagen interaction with water so that collagen becomes more hydrophobic (8). Thus the monomer can better infiltrate the collagen fibrils and prevent water absorption to increase the monomer-dentin bond (9).

EGCG can also have a negative effect when mixed with adhesives at a certain concentration. Yu (10) stated that

the antioxidant effect of EGCG can change the degree of adhesive conversion. From the research results, it appears that the higher the concentration of EGCG used in the mixture, the lower the degree of conversion of the adhesive. This was confirmed by Du (2) who reported that giving 100-300 µg / mL of EGCG into the adhesive can cause EGCG to be trapped in the polymer linear chain after the irradiation process which causes the adhesive polymerization to be inadequate. This is because the anti-free radical properties of EGCG can interfere with the free radical polymerization process of the adhesive so that research is needed to find the right EGCG concentration.

Even though it has quite a lot of therapeutic effects, until now EGCG materials in the field of dentistry are still minimal. This literature study discusses the potential of EGCG as a cross-linking agent against dentine collagen and hybrid layer and chelating agent against MMPs.

DENTIN

Dentin is a layer of tooth structure underneath the enamel. This layer consists of 65% inorganic components in the form of hydroxyapatite crystals, 30% organic components in the form of collagen, and 5% water (11). The extracellular matrix of the dentin is made up of a complex three-dimensional network of collagen fibrils calcified by nanoapatite crystals. A central triple-helix area, a non-helical aminoterminal area (N-telopeptide), and a carboxy-terminal area make up the collagen chain (C-telopeptide). The length of the collagen fibrils looks to have a hollow of 15-20 nm, which the resin monomer will penetrate and polymerize under 150,000-fold magnification. The mechanical retention of dentin adhering to collagen is the result of this situation (3,12). Collagen chains in the dentin are the most stable collagen compared to collagen in the body system (13). This is due to intramolecular and intermolecular cross-links formed by covalent connections between the C terminal on one collagen molecule and the N terminal on the collagen molecule next to it. By linking the spaces between collagen molecules that are filled with water, hydrogen bonds help to stabilize the triple-helical chain (4). This crosslink plays a role in the acid etching process during the bonding procedure and prevents collagen denaturation so that a hybrid layer can be formed (3).

Elasticity, hardness, visco-elasticity, and fracture coefficient are all mechanical properties of teeth. When exposed to external forces, visco-elasticity is used to measure materials with viscous and elastic properties. The storage modulus and loss modulus are the measuring indices employed (11,14).

MATRIX METALLOPROTEINASE (MMP)

The MMP enzyme is an enzyme that basically can degrade all components of the dentin extracellular matrix. Almost all MMPs are secreted as enzyme precursors, namely zymogens, in which cysteine propeptide binds to its sulfhydryl groups until the active zinc ion region as the fourth ligand undergoes "cysteine change". In vitro the change from a form to an active form can be achieved by proteolytic elimination of the propeptide, randomizing the cysteine-zinc interactions, or modifying the sulfhydryl groups, allowing the interaction between the zinc active region and the water molecule and contact with the active site. In many cases, the activation process occurs gradually including the autocatalytic process. In vitro proMMPs can be activated by various chemical compounds and reactions, including thiol-modified compounds, denaturation, chaotropic compounds, reactive oxygen, and heating. MMP-2, MMP-8, and MMP-9 can also be activated by acidic pH followed by neutralization (12).

HYBRID LAYER

The hybrid layer is the most vital part of the adhesive-based restoration. The quality of the hybrid layer determines the strength and durability of a restoration. The hybrid layer can be interpreted as a layer formed due to resin infiltration between collagen and hydroxyapatite fibers which functions as micromechanical retention of composite resin restorations to the dentin tissue. This layer consists of 50% collagen matrix and 50% resin. The hybrid layer serves to combine 2 different elements, namely hydrophilic dentin, and hydrophobic composite material, protecting the dentin surface from micro-leakage and increasing dentin resistance to acid. The ideal hybrid layer is characterized by the presence of a collagen network that is bonded and reinforced with polymers (3,15).

Hybrid Layer Degradation

The adhesive is now starting to use hydrophilic monomers such as Hydroxyethyl methacrylate (HEMA) as a hydrophobic monomer solvent to increase the wetting ability of the adhesive and prevent phase changes that occur when the diacrylate-based adhesive is applied to the dentin matrix which tends to be moist. Resin monomers which are hydrophilic in nature are very susceptible to hydrolysis due to the presence of ester bonds in the HEMA component. In addition, the increase in the HEMA component in the adhesive has been shown to increase water absorption in polymerized polymers, which causes a decrease in the mechanical properties of the hybrid layer.3 HEMA can provide good adhesions and is not easily degraded so that it can produce long-lasting restoration (16).

EPIGALLOCATHECHIN GALLATE (EGCG)

EGCG is a polyphenol that belongs to the catechin group, which able to inhibit the opening of sodium ion channels so that it has the potential to be anti-inflammatory. In addition, EGCG also has antioxidant properties that can reduce ROS by binding to ROS

(17,18). EGCG is composed of 2- phenylchromane framework which is substituted in chain number 3,5,7,31,41 with a hydroxyl group (Figure 1). During biosynthesis, if the B-ring comes from sinton gallic acid, the catechin will be substituted with the 51st position of the hydroxyl group, namely "gallo" catechins which will esterify with gallic acid to form "gallate". Levorotatory compounds (2R, 3R) are called "epi" while dextrorotatory compounds (2S, 3R) are called "catehchin" so that when combined, they will become epigallocatechin gallate (EGCG). EGCG compound has the chemical formula (2R, 3R) -5,7-dihydroxy-2-(3,4,5-trihydroxyphenly) -3,4-dihydro-2H-chromen-3-yl-3,4,5-trihydroxybenzoate (19) (Figure 2). Judging from its chemical structure, EGCG is said to have the most potent antioxidative properties.

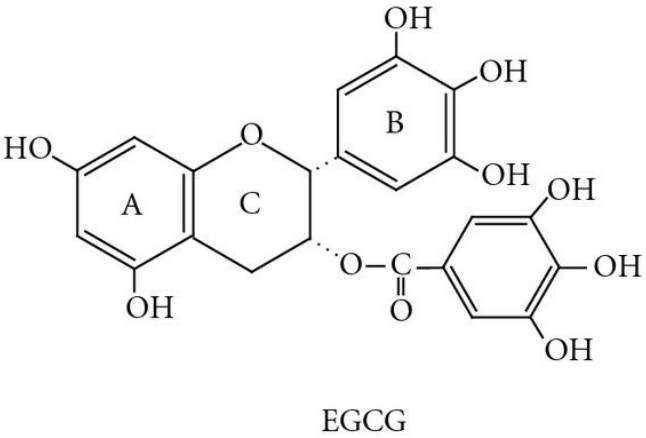
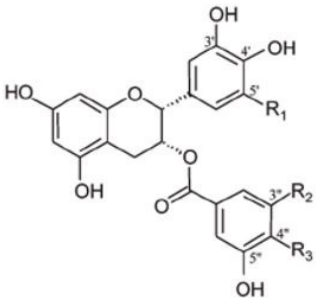


Figure 1: EGCG



Substances	R_1	R_2	R_3
(−)-epigallocatechin-3-*O*-gallate (EGCG)	OH	OH	OH
(−)-epigallocatechin-3-*O*-(3-*O*-methyl)-gallate (EGCG-3Me)	OH	OCH_3	OH

Figure 2: The Chemical Structure of EGCG and EGCG-3Me

EGCG can close TLR4 which can increase the production of TNF-α and hydroxyl and gallate groups that will bind to free radical ions so that it can prevent oxidation reactions that can cause tissue damage (9,20). EGCG can also carry out an antioxidant activity, EGCG will bind free metal ions such as Fe2 + and Zn2 + ions and make them more stable so that the catalyzed reactions can be inhibited (1).

Demineralization in the caries process has been shown not only to be caused by contact with acids from bacteria but also due to the collagenolytic and gelatinolytic activity of the proteases present in the dentin organic matrix. The enzymes that play a role in this are MMP and cysteine cathepsin (21). Table I shows a summary of studies done on the efficacy of EGEC.

EGCG as Cross-Linking Agent Towards Hybrid Layer in Dentin Collagen

The technology regarding adhesive materials is still developing, one of the weaknesses is the decrease in the resin-dentin bond which is often associated with an unstable hybrid layer. On the other hand, dentin also has MMP proenzymes and cysteine cathepsin which play a role in the destruction of collagen fibrils in the hybrid layer.

Collagen fibrils' stability, tensile strength, and viscoelasticity depend on the intermolecular cross-links from a translation of collagen. This is indicated by an increase in the degree of cross-link which is accompanied by an increase in the elastic tension of collagen fibrils. Chu (9), reported that applying EGCG solution to the collagen surface resulted in more regular collagen fibrils with a larger diameter and smaller interfibrillar space. The higher the concentration used, the greater the diameter of the collagen fibrils formed.

The cross-linking between EGCG and collagen chains can also affect the permeability of the hybrid layer to water molecules. This is evidenced by the research of Sun (8), which shows that the application of 0.1% EGCG to dentin after acid etching can increase the contact angle of the dentine surface to water.

A different point was stated by Chu (9), who reported that administering EGCG 0.64% could reduce the contact angle of the dentin surface against water and allow maximum surface wetting. From the research method used, the two studies with different results used different methods. A dentin collagen membrane was immersed in EGCG solution for 1 hour at room temperature. Meanwhile, Sun (8), used pieces of dentin that had been polished and demineralized using acid etching and applied EGCG for 120 seconds. This difference in the material used allows for differences in research results even though they both use water droplets.

To determine the degree of attachment between the adhesive and dentin, most studies (n = 23) used a tensile bond strength test (TBS), and 4 journals evaluated nanoleakage analysis. Among the 17 studies that tested TBS with positive results, 10 of them use EGCG solution with varying concentrations on dentin before bonding application. According to Yu (10), EGCG can maintain adhesion stability through the inhibitory

Table I: Studies on the efficacy of EGCG

Tittle	Findings	Reference
Evaluation of epigallocatechin-3-gallate (EGCG) cross-linked collagen membranes and concerns on osteoblasts	Immersion of collagen membranes in 0.64%, 0.064%, and 0.0064% (w/v) EGCG solutions for 1 hour resulted in collagen that looks more compact, fibrils are more organized, increases fibril diameter, and narrows the space between fibrils. The higher the concentration of EGCG used, the more hydrophilic the dentinal collagen is. EGCG can also increase the modulus of elasticity and strength of collagen.	9
Epigallocatechin-3-gallate enhance dentin biomodification and bond stability of an etch-and-rinse adhesive sistem	Application of EGCG 0.1% for 120 seconds before bonding can increase Tensile Bond Strength (TBS) significantly compared to no treatment. EGCG can increase the contact angle by increasing the degree of hydrophobicity.	8
Effect of epigallocatechin-3- gallate solutions on bond durability at the adhesive interface in caries-affected dentin	Application 20µl of 0.02%, 0.2%, 0.5% EGCG for 60 seconds did not show a significant difference in FFB. Both the CHX and EGCG groups had lower TBS than the control. The application of 0.02% EGCG had the lowest degree of nanoleakage compared to other concentrations, CHX, and control although not significant.	6
Effect of adjunctive application of epigallocatechin-3-gallate and ethanol-wet bonding on adhesive-dentin bonds	Administration of 0.02% w/v EGCG for 60 seconds before bonding application showed higher TBS than other groups and control. Most fractures are adhesive failures. EGCG+ethanol also showed the lowest degree of nanoleakage compared to the other groups.	32
Functionalized epigallocatechin gallate copolymer inhibit dentin matrices degradation: Mechanical, solubilized telopeptide and proteomic assays	Application Adhesives containing 1%w/w EGCG showed the highest tensile strength compared to adhesives containing CHX and negative control. EGCG can also inhibit proteolytic enzymes (mmp and CT) (shown in soluble telopeptide analysis) and collagen biomodification.	22
Influence of dentin biomodification with epigallocatechin-3-gallate on the bond strength of self-etch adhesive: Twelve-month results	The use of 0.1 % EGCG for 60 seconds before bonding resulted in reduced microtensil bond strength on the first day compared to the control, but higher bond strength at 6 and 12 months. Adhesion failure was similarly higher in the EGCG group on day 1 compared to the control and CHX groups, but it increased after 6 and 12 months.	23
Effect of polymeric microparticles loaded with catechin on the physicochemical properties of an adhesive sistem	Addition of PLGA (poly(D-L lactide-coglycolide) Acid) containing EGCG in the form of microparticles into bonding as much as 0.5%, 1.0%, and 2.0% w/w manually and the description of the application of 0.1% and 1% EGCG solutions before the bonding application showed an increase in TBS after 12 months is characterized by decreased degradation of collagen fibrils	7
Epigallocatechin-3-gallate and epigallocatechin-3-O-(3-O-methyl)-gallate enhance the bonding stability of an etch-and-rinse adhesive to dentin	The addition of EGCG-Me 600 µg/mL to the adhesive applied to teeth that had been etched in 2 layers showed the highest TBS.	10
Effect of epigallocatechin gallate, green tea extract and chlorhexidine application on long-term bond strength of self-etch adhesive to dentin	There is no significant difference in tensile bond strength. Administration of 2% EGCG for 60 seconds did not increase the tensile bond strength. EGCG had the lowest bond strength compared to green tea extract, chlorhexidine, and control.	28
Epigallocatechin-3-gallate (EGCG) enhances the therapeutic activity of a dental adhesive	The application of a bonding agent that had been mixed with 200 g/ml EGCG on the etched teeth showed significantly higher TBS than the control and other concentrations. There was no significant difference in the degree of conversion in bonding after being given EGCG solution against the control.	2
Galloyl moieties enhance the dentin biomodification potential of plant-derived catechins	Dentin incubation in 0.65w/v percent EGCG solution for 1 hour resulted in a considerable increase in modulus of elasticity, a decrease in the degree of dentin biodegradation, a decrease in MMP-9 and CT-B.	24
Durability of resin on bleached dentin treated with antioxidant solutions or lasers	Application of 1mL 0.5% EGCG for 10m after bleaching before the filling procedure resulted in the highest shearbond strength compared to laser and control after 12 months.	25
Influence of protease inhibitors on the degradation of sound, sclerotic and caries-affected demineralized dentin	Before enzyme administration, dentin incubation in a 0.5% EGCG solution for 1 hour at 37°C had a beneficial effect on nanohardness and modulus of elasticity in the afflicted dentin. Tensile strength was likewise higher in the EGCG group than in the CHX group, but lower than in the control group.	26
Antioxidants and Collagen-Crosslinking: Benefit on Bond Strength and Clinical Applicability	The administration of primers that had been mixed with EGCG 100 M (wt./vol) for 20 seconds and dried for 5 seconds before bonding showed inferior shear bond strength and Weibull modulus performance compared to proantocyanin, hesperidin, and control, although no loss of shear bond strength was found.	27

activity of MMPs. Meanwhile, Maria Fonesca (14), EGCG has hydrophobic properties through aromatic groups and hydrophilic through polar hydroxyl groups. The hydrophobic group can induce Van der Waals bonds with hydrophobic molecules in the resin. While the hydrophilic group hydrogen bonds with proteins in the collagen chain. Gerhardt (28), reported that the administration of EGCG solution with a concentration of 2% before the bonding application showed lower TBS rates than controls both immediately after application and 6 months later. Gerhardt added that these results could be due to the formation of a precipitate in the EGCG solution.

Application of EGCG gel containing 400 µM for 5 minutes 5 days before the filling procedure showed the same Tensile Bond Strength (TBS) as the control in both normal and erotion dentin (29). The addition of PLGA (poly (D-L lactide-coglycolide) Acid) containing EGCG in the form of microparticles into bonding as much as 0.5%, 1.0%, and 2.0% w/w manually and the application of 0.1% EGCG solution and 1% before the bonding showed an increase in TBS after 12 months, which was marked by a decrease in the degradation of collagen fibrils (7). Addition of 200 g/mL EGCG solution before bonding application using the etch and rinse method had the highest TBS when compared to

mixing the bonding material with EGCG solution with the same concentration, pretreatment with CHX and control. Flexural strength of bonding materials that have been mixed with EGCG is lower than bonding materials without EGCG (30).

Yang (31), reported that giving EGCG in ethanol and water solvents with a concentration of 0.02% showed a lower degree of nanolekakage than the 0.1% concentration, this research is in line with another research that stated EGCG with the smallest concentration (0.0065%) can inhibit protease activity but its effectiveness drops significantly when it reaches a certain concentration. This raises the theory that the EGCG used in the treatment is highly dependent on the concentration used. Regarding this, Du (2), explained that giving EGCG in high concentrations can reduce the degree of adhesive conversion, because, during the polymerization period, EGCG trapped in the adhesive can interfere with the formation of polymer linear chains.

EGCG as a cross-linking agent and the wetting ability of the bonding material caused by the EGCG solvent, which is water. The use of different solvents can also cause differences in test results, Chemical bonding using acetone solvent can produce a stronger bond between the bonding material and dentin collagen when compared to using ethanol solvent (15). A study conducted by Soetojo (32), compared HEMA water solvent expresses less MDA and has good biocompatibility when compared to HEMA with ethanol or acetone solvent. Water solvents are considered to provide a wetting effect that can act as plasticizers of dentin collagen fibrils and keep collagen from collapsing. Water molecules in collagen make the diffusion of the bonding material with ethanol and acetone solvents into collagen fibrils easier, but the water molecules will also diffuse into the solvent, so the concentration of the adhesive solvent and the concentration of BisGMA decreases which causes the infiltration of BisGMA to be inadequate (33). Yang (31), used ethanol solvent in his EGCG solution. The use of ethanol as a solvent to replace water can increase the TBS of most hydrophobic resins such as BisGMA / TEGDMA by making the total ethanol-matrix cohesive force approaching the ethanol-BisGMA / TEGDMA solution so that the collagen matrix will immediately form interpeptid hydrogen bonds. This evidence showed a high number of TBS and nanoleakage of the EGCG-ethanol group compared to the EGCG-water group. EGCG is able to increase the stability of the hybrid layer, which can be proven by the amount of soluble type I collagen telopeptide, which is lower than the control (22).

EGCG as Chelating Agent against MMPs' Expression

The use of MMP inhibitors is thought to increase the bond strength of the adhesive to dentin. EGCG also functions as a chelating agent, EGCG interacts with MMP by exploiting the nature of catechins which have a high affinity for metal ions. Yang (23) states that the Zn ion which is the chemical structure of MMP can be bound by EGCG so that MMP is no longer recognized by the collagen matrix so that collagen degradation can be inhibited. But Cheng (34) has a different theory that stated EGCG inhibits the gelatinolytic activity of MMP by binding EGCG with the catalytic area or the area close to the catalytic area where gelatinolytic activity is occurring.

Based on journal search results, most studies (n = 15) regarding the application of EGCG to dentin collagen did show positive results both on TBS, nanoleakage, and the morphology of collagen fibrils. However, there is one randomized clinical trial that showed negative results. Costa (35), reported that administering 0.1% EGCG solution for 60 seconds before application of the bonding to the cervical lesion restoration procedure without caries (NCCL) did not show any difference in terms of retention, margin adaptation, secondary caries, and post-restoration sensitivity after 24 months with or without the procedure. This result explains that although EGCG does not have a positive effect on the restoration results, EGCG also does not have a negative effect that can worsen the restoration. Therefore, further evaluation is needed regarding concentrations, solvents, and application methods that can be used to increase the resin-dentin adhesion in the hybrid layer.

Vidal (24) reported a degree of MMP-9 inhibition that exceeded positive controls after incubation in an EGCG solution. MMP is basically secreted as an inactive proenzyme, but will be active when the pH of the environment drops. This will lead to degradation of the extracellular matrix in both biological and pathological processes. This process usually occurs in affected dentin, where cariogenic bacteria still produce lactic acid which can activate MMP (28). Administration of EGCG-nanohydroxyapatite solution in 100mg/mL distilled water for 30 seconds before bonding application showed significantly higher TBS than the control, either with or without the etching procedure. The EGCG-nanohydroxyapatite group also showed significantly lower nanoleakage rates than the control group. The EGCG group also showed a significant decrease in gelatinolytic activity due to MMPs compared to the control group (36).

CONCLUSION

The application of EGCG can have a positive effect on the morphology of dentinal collagen fibrils, inhibit MMPs activity in dentinal collagen, decrease the hydrophilic properties of dentinal collagen fibrils, increase the tensile bond strength (TBS) of the hybrid layer and reduce the nanoleakage of the hybrid layer in dentin collagen.

ACKNOWLEDGEMENT

The authors would like to thank the staff of Endodontic Division of Faculty of Dental Medicine, Universitas Airlangga. The authors declare no potential conflicts of interest with respect to the authorship and publication of this article.

REFERENCES

1. Münchow EA, Bottino MC. Recent advances in adhesive bonding: The role of biomolecules, nanocompounds, and bonding strategies in enhancing resin bonding to dental substrates. Current oral health reports. 2017;4(3):215-27.
2. Du X, Huang X, Huang C, Wang Y, Zhang Y. Epigallocatechin-3-gallate (EGCG) enhances the therapeutic activity of a dental adhesive. Journal of dentistry. 2012;40(6):485-92.
3. Breschi L, Maravic T, Cunha SR, Comba A, Cadenaro M, Tjɒderhane L, Pashley DH, Tay FR, Mazzoni A. Dentin bonding systems: from dentin collagen structure to bond preservation and clinical applications. Dental Materials. 2018;34(1):78-96.
4. Tezvergil-Mutluay A, Pashley D, Mutluay MM. Long-term durability of dental adhesives. Current Oral Health Reports. 2015;2(4):174-81.
5. Soetojo A, Purnamasari D, Lunardhi CG, Widjiastuti I. Cytotoxicity test of 4-methacryloxyethyl trimellitic anhydride–based dentine bonding material using acetone solution in dental pulp fibroblast. Journal International of Oral Health. 2019:191-6.
6. Fialho MP, Hass V, Nogueira RP, Franɜa FM, Turssi CP, Basting RT, Amaral FL. Effect of epigallocatechin-3-gallate solutions on bond durability at the adhesive interface in caries-affected dentin. Journal of the mechanical behavior of biomedical materials. 2019;91:398-405.
7. Albuquerque NL, Neri JR, Lemos MV, Yamauti M, de Sousa FF, Santiago SL. Effect of polymeric microparticles loaded with catechin on the physicochemical properties of an adhesive system. Operative dentistry. 2019;44(4):E202-11.
8. Sun Q, Gu L, Quan J, Yu X, Huang Z, Wang R, Mai S. Epigallocatechin-3-gallate enhance dentin biomodification and bond stability of an etch-and-rinse adhesive system. International Journal of Adhesion and Adhesives. 2018;80:115-21.
9. Chu C, Deng J, Xiang L, Wu Y, Wei X, Qu Y, Man Y. Evaluation of epigallocatechin-3-gallate (EGCG) cross-linked collagen membranes and concerns on osteoblasts. Materials Science and Engineering: C. 2016;67:386-94.
10. Yu HH, Zhang L, Yu F, Li F, Liu ZY, Chen JH. Epigallocatechin-3-gallate and Epigallocatechin-3-O-(3-O-methyl)-gallate Enhance the Bonding Stability of an Etch-and-Rinse Adhesive to Dentin. Materials. 2017;10(2):183.
11. Garg N, Garg A. Textbook of Endodontics. 3rd ed. New Delhi: Jaypee Brothers Medical Publishers;2014.
12. Zirta UA, Juanita AG, Nurrohman H. The role of matrix metalloproteinases in dentin caries. J Indones Dent Assoc. 2009;58(2):25-31.
13. Pashley D, Tay F. Mechanical stability of resin–dentine bonds. InDental Biomaterials. Woodhead Publishing;2008.
14. Fonseca BM, Barcellos DC, Silva TM, Borges AL, Cavalcanti BD, Prakki A, Oliveira HP, Gonɜalves SE. Mechanical-physicochemical properties and biocompatibility of catechin-incorporated adhesive resins. Journal of Applied Oral Science. 2019;27.
15. Zubaidah N, Effendy R, Soetojo A, Estiyaningsih T, Tanzil MI, Khotimah K. Difference of Chemical Bonds Between UDMA Bonding Agents with Ethanol Solvent and Acetone Solvent on Dentin Collagen. Pesquisa Brasileira em Odontopediatria e Clнnica Integrada. 2021;21.
16. Saraswati W, Widjiastuti I, Rukmo M, Wahjuningrum DA. The expression of HMGB1 in Dentin Pulp Complex Induced by Resin Monomer HEMA.2017.
17. Ismiyatin K, Wahluyo S, Purwanto B, Adioro Soetojo N, Rahayu RP, Mukono IS. Topical epigallocatechin-3-gallate hydrogels regulated inflammation and pain. Journal of International Dental and Medical Research. 2018;12(1):54-60.
18. Ismiyatin K, Adioro Soetojo N, Wahluyo S, Mukono IS. Therapeutic efficacy of topical epigallocatechin-gallate as a new therapeutic strategy for inhibition of pain conduction on rat models with acute pulpal inflammation. 2017.
19. Legeay S, Rodier M, Fillon L, Faure S, Clere N. Epigallocatechin gallate: a review of its beneficial properties to prevent metabolic syndrome. Nutrients. 2015;7(7):5443-68.
20. Ismiyatin K, Wahluyo S, Purwanto DA, Rahayu RP, Adioro Soetojo N, Mukono IS. Effect of topical epigallocatechin-gallate on lipopolysaccharide-induced pulpal inflammation in rat models. Iranian Endodontic Journal. 2011;13(4):528-33.
21. Van Doren SR. Matrix metalloproteinase interactions with collagen and elastin. Matrix Biology. 2015;44:224-31.
22. Prakki A, Xiong Y, Bortolatto J, Gonɜalves LL, Bafail A, Anderson G, Stavroullakis AT. Functionalized epigallocatechin gallate copolymer inhibit dentin matrices degradation: Mechanical, solubilized telopeptide and proteomic assays. Dental Materials. 2018;34(11):1625-33.
23. Neri JR, Yamauti M, da Silveira FD, Mendonɜa JS, de Carvalho RM, Santiago SL. Influence of dentin biomodification with epigallocatechin-3-gallate on the bond strength of self-etch adhesive: twelve-month results. International Journal of Adhesion and Adhesives. 2016;71:81-6.
24. Vidal CM, Aguiar TR, Phansalkar R, McAlpine

JB, Napolitano JG, Chen SN, Araьjo LS, Pauli GF, Bedran-Russo A. Galloyl moieties enhance the dentin biomodification potential of plant-derived catechins. Acta biomaterialia. 2014;10(7):3288-94.
25. Souza-Gabriel AE, Sousa-Neto MD, Scatolin RS, Corona SA. Durability of resin on bleached dentin treated with antioxidant solutions or lasers. Journal of the mechanical behavior of biomedical materials. 2020;104:103647.
26. Oliveira-Reis B, Maluly-Proni AT, Fagundes TC, Vasconcelos G, Bresciani E, Prakki A, Dos Santos PH. Influence of protease inhibitors on the degradation of sound, sclerotic and caries-affected demineralized dentin. Journal of the mechanical behavior of biomedical materials. 2019;97:1-6.
27. Beck F, Ilie N. Antioxidants and Collagen-Crosslinking: Benefit on Bond Strength and Clinical Applicability. Materials. 2020;13(23):5483.
28. Gerhardt KM, Oliveira CA, Franza FM, Basting RT, Turssi CP, Amaral FL. Effect of epigallocatechin gallate, green tea extract and chlorhexidine application on long-term bond strength of self-etch adhesive to dentin. International Journal of Adhesion and Adhesives. 2016;71:23-7.
29. Landmayer K, Liberatti GA, Farias-Neto AM, Wang L, Honyrio HM, Francisconi-dos-Rios LF. Could applying gels containing chlorhexidine, epigallocatechin-3-gallate, or proanthocyanidin to control tooth wear progression improve bond strength to eroded dentin?. The Journal of Prosthetic Dentistry. 2020;124(6):798-e1.
30. Czech R, Oliveira CA, Franza FM, Basting RT, Turssi CP, Amaral FL. Incorporation of EGCG into an etch-and-rinse adhesive system: mechanical properties and bond strength to caries affected dentin. Journal of Adhesion Science and Technology. 2019;33(22):2430-42.
31. Yang H, Guo J, Deng D, Chen Z, Huang C. Effect of adjunctive application of epigallocatechin-3-gallate and ethanol–wet bonding on adhesive–dentin bonds. Journal of dentistry. 2016;44:44-9.
32. Soetojo A, Cahyadi KE, Prasetyo EA. Malondialdehyde expressions on pulp odontoblast cells after application of 2-hydroxyethyl methacrylate mixed with water, ethanol, and acetone solvents. Saudi Endodontic Journal. 2019;9(2):96.
33. Prasetyo AI, Kunarti S, Soetojo A, Prasetyo EA. Chemical bond strength difference between 4-meta bonding agents with ethanol and acetone solvent on type i collagen. Journal of International Dental and Medical Research. 2018;11(2):191-6.
34. Cheng XW, Kuzuya M, Kanda S, Maeda K, Sasaki T, Wang QL, Tamaya-Mori N, Shibata T, Iguchi A. Epigallocatechin-3-gallate binding to MMP-2 inhibits gelatinolytic activity without influencing the attachment to extracellular matrix proteins but enhances MMP-2 binding to TIMP-2. Archives of Biochemistry and Biophysics. 2003;415(1):126-32.
35. Costa CA, Albuquerque NL, Mendonza JS, Loguercio AD, Saboia VP, Santiago SL. Catechin-based dentin pretreatment and the clinical performance of a universal adhesive: a two-year randomized clinical trial. Operative dentistry. 2020;45(5):473-83.
36. Vickers NJ. Animal communication: when i'm calling you, will you answer too?. Current biology. 2017;27(14):R713-5.