A novel atraumatic self-bonding and self-healing dental composite to restore carious primary dentition

Hasan Jamal

Supervised by

Prof. Anne Young and Prof. Paul Ashley

University College London

The Eastman Dental Institute

Dental Doctorate in Paediatric Dentistry

2018 – 2021

Declaration

This report entitled “A novel atraumatic self-bonding and self-healing dental composite to restore carious primary dentition” was composed by me and is based on my own work. Where the work of the others has been used, it is fully acknowledged in the text and in captions to table illustrations. This report has not been submitted for any other qualification.

Hasan MohammedAli Jamal

21 / 07 / 21

Acknowledgments

There are several people and groups I would like to acknowledge and thank for their assistance and contributions towards the completion of this thesis, of which some are explicitly named here.

I want to first thank the ministry of higher education in Saudi Arabia and the Saudi Cultural Bureau in the United Kingdom, who offered me a generous scholarship, "*The Elite Scholarship Program*". Without their funding and financial support, this project would not have been possible.

I want to thank my project supervisors Prof. Anne Young and Prof. Paul Ashley, for their incredible support, assistance and guidance during the conduction of this project and beyond.

I want to thank the lab members who assisted and supported us throughout, with special thanks to Dr Nicky Morden, Dr Graham Palmer, Dr George Georgiou and Dr, Wendy Xia.

I would like to sincerely thank the Paediatric Dentistry programme directors and my mentors Dr Susan Parekh and Prof Paul Ashley, for their invaluable professional, and personal support throughout the past three years.

I would like to thank all my colleagues in the program, with special thanks to my batch colleagues Hussam Al-Siyabi and Violeta Kakiora, for making this journey worthwhile.

I would like to thank my wife Ghofran Radwan for her continued support, motivation and inspiration throughout.

I would like to dedicate this work to my parents and my sister, who have shown unconditional love and support, and I will be forever grateful to them.

Abstract

Background: Dental caries is considered a widespread global epidemic, affecting an alarming 60 to 90% of minors and adolescents worldwide. Current dental caries treatments pose various consequences on the healthcare system, children and parents. Although several adhesive restorative dental materials are available for dentists to utilise, such as dental composites or glass ionomers, they still demonstrate critical issues such as bulk shrinkage or adhesion failure. These will eventually lead to microleakage, recurrent caries, discolouration, and ultimately failure of the restoration. Moreover, most current materials require rigorous steps, which can be challenging in paediatric restorative dentistry. Thus, the need for a novel restorative option for paediatric patients is required. Here, we propose a novel restorative material with self-bonding and self-healing properties that can be applied in a short time. Two components that aided in this were polylysine (PLS), a food preservative, and mono-calcium phosphate monohydrate (MCPM).

Aims: This research aims to, first, test the ability of the final restorative formulation to form resin-tags on affected-dentine of primary teeth and to quantify the matrix metalloproteinase (MMP) activity at the demineralised dentine surface, in comparison to commercially available restorative materials (Z250, GIC Fuji IX and ACTIVA). **Methods:** Tests were performed to determine the ability of the final formulation to form resin tags and compared with the previously mentioned commercial fillings. Carious primary molars were obtained following ethical approval. The final formulation, along with commercially available restorations Renewal MI (Schottlander™), 3M ESPE Filtek Z250 with OptiBond Solo Plus adhesive, ACTIVA™ KIDS BioACTIVE compomer (PulpDent™) and GC Fuji IX, GIC, were applied on affected-carious dentine following

the manufacturer's instructions. Samples (n=5 per group) were cross-sectioned and visualised under CLSM to evaluate the resin-tags formation.

Moreover, tests were performed to quantify the ability of the final formulation in inhibiting MMP enzymes. 2mm thick sections of coronal dentine from sound human molars, obtained following ethical approval, were fully demineralised through immersion in 4M formic acid for 48 hours. Following the application of a green fluorescent probe (EnzCheck Collagenase Assay Kit) for 5 minutes, restorative materials were applied on one surface. Materials included Renewal MI (Schottlander^{TM}), 3M ESPE Filtek Z250 with OptiBond Solo Plus adhesive, ACTIVA^{TM} KIDS Bioactive compomer (PulpDent^{TM}) and GC Fuji IX, GIC according to the manufacturer's instructions. These commercial restorative materials were chosen, as they are frequently used in paediatric dentistry to restore carious primary teeth. Non-restored dentine was used as a control. Samples were stored in deionised water and incubated at 37^{o}C. Following 1 or 14 days, samples (n=5 per group) were sectioned, and the interface area imaged using Confocal Light Scanning Microscopy (CLSM). The percentage area of green fluorescence in sections 260*260 micron squared MMP activity was determined through Image J. **Results**: For the resin tags, the results demonstrated that the optimised formulation showed long resin-tags infiltration of >200µm long to carious-affected dentine when visualised under confocal light scanning microscopy (CLSM).

As for the MMP activity, the results showed that the final formulation had the least fluorescence initially (0.5%), which after 14 days almost totally disappeared. Z250 and ACTIVA's results were similar after incubation at day 1 (2.5%-2.0%) and day 14 (2.0% 1.8%) respectively. MMP activity of GIC (Fuji IX) was lower than Z250 and ACTIVA on day 1; however, it was higher on day 14, reaching 3.5%. The results

were used to support applications to MHRA for clinical trials and a notified body for CE mark.

Conclusion: In conclusion, the research achieved the aim of developing a novel composite restorative material that inhibits the degrading enzymes at the interface and provides good adhesive properties. This makes it a valuable option to restore decayed paediatric patient's teeth.

Project impact statement

According to the world health organisation, dental caries affects roughly 2.5 billion people globally, 37% of the world population (Cooper, 2018). The most common epidemiological measure of caries is referred to as DMFt; this is a measure of the number of teeth that are decayed (D), missing (M), or filled (F). In the UK, recent epidemiological investigations reviewed these parameters on three and five-year-olds. In 2013, the dmft in three-year-olds was 0.36 in all children and 3.07 in 12% with decayed teeth. Out of these teeth, 89% were unrestored. In 2017, the dmft in five-year-olds was 0.8 in all children and 3.4 in the 23.3% teeth with decay. About 78% of these decayed teeth were untreated (PHE, 2013; PHE, 2017b). The number of hospital admissions for tooth decay for children aged 5-9 has increased for the second consecutive year from 25,923, in 2016-2017, to 26,111 in 2017-2018 (Zhang et al., 2016). On a more regional scale, here at the Eastman Dental Hospital, referrals of children for dental extractions due to decay has more than doubled in the past six years.

Dental caries poses a significant public health issue in both high-income and low-income countries. It holds various consequences on the NHS, children and parents. These consequences range from high treatment expenses, children failing to attend school, hospitalization, pain that affects different lifestyle aspects and last but not least psychological concerns. It was estimated that 60,000 school days are being missed during hospital extractions alone done under general anaesthesia (GA), with an average of 3 days being missed due to dental-related problems. Furthermore, 41% of parents are also affected by missing work days accompanying their children (PHE, 2017a). Concerning expenses, a recent study showed that the NHS spends an

average of £3.4 billion per year on dental diseases. From 2015 to 2016, tooth extractions due to tooth decay in paediatric patients alone cost the NHS almost £60 million (White, 2017).

COVID-19 pandemic halted the provision of elective dental treatments. This was primarily because the majority of dental treatments are aerosol-generating, increasing the chances of cross-infection. Despite the threats presented by the pandemic, it also paved the way for employing minimally invasive treatments (MITs) and highlighted their potential in minimising or eliminating aerosols (Proffitt, 2020). These MITs include sealing a decayed tooth (using stainless steel crowns or filling materials) and applying specific solutions that arrest decay. These conservative treatments respect the integrity of and depend on the healing capabilities of the tissues. Hence, there is an increasing consensus that adopting minimally invasive biological approaches as dental treatments, where appropriate, is extremely important (Proffitt, 2020). Disasters can create opportunities, and this was one such case.

As stated earlier, most of the current restorative materials have various drawbacks, such as their rigorous steps, microleakage or their toxicity. Therefore, in order to overcome these issues, a novel restorative material is required. The material should be simple to use, atraumatic and preferably contains ideal properties to restore carious primary teeth of paediatric patients. Thus, this project, which is part of a large research group, aims to develop a novel composite material that can be easily applied, by a general dental practitioner (GDP), directly on carious-affected dentine without the need for drill or anaesthetic. This could then aid in reducing referrals by GDPs, reducing expenditure, and, most importantly, increasing treatment acceptance by our beloved paediatric patients.

COVID-19 impact statement

In April 2019, Eastman Dental Hospital (EDH) relocated to a new site from Gray's Inn Road to the new site at Huntley's Street. The laboratory was originally planned to move to the Royal Free Hospital (RFH) at Hammersmith. The laboratory at the old site continued to operate as the relocation was gradual. Nevertheless, around November 2019, all work at the old lab was seized, and I had to stop working on my experiments. As we were preparing to work in the new lab at the RFH, the COVID-19 pandemic started, and I did not have the chance to continue my experiments there.

As a result, this had a positive and negative outcome on both my thesis and my academic studies at the Eastman Dental Institute. As for our studies, the impact was primarily positive, as our program directors were resilient in adopting various online teaching methods from the start of the pandemic. The other positive impact to me personally is that I had spare time to attend various conferences and present my work. Moreover, I managed to research, write and publish few articles in several journals.

Regarding my thesis, few adaptations occurred. First, I could not continue certain parts of the experiments (experiments 1 and 2 in chapters 3 and 5). The planned parts of the experiments were added to the future remarks section in chapter 7. Secondly, a decision was made to conduct a systematic review. I have decided to conduct a systematic review along the lines of the experiment in chapter 4.

Table of contents

List of abbreviations

°C	Degree Celsius
2-HEMA	2-hydroxylmethyl methacrylate
ACR	Acrolein
AD	Affected dentine
AFM	Atomic force microscopes
ART	Atraumatic restorative treatments
Ba-ACP	Barium-glass filler amorphous calcium phosphate
BAC	Benzalkonium chloride
BAI	Baicalein
Bis-GMA	Bisphenol A-glycidyl methacrylate
BMP	Bone morphogenetic protein
BSP	Bone sialoprotein
Ca	Calcium
Ca-EDTA	Calcium ethylenediamine-tetra-acetic acid
CAD	Caries-affected dentine
CD	Circular dichroism
CEJ	Cemento-enamel junction
CHX	Chlorhexidine
Cl^-	Chloride
CLSM	Confocal light scanning microscopy
CO3	Carbonate
DCC	Dicyclohexylcarbodiimide
DEJ	Dentino-enamel junction

DGP	Dentine glycoprotein
DMA	Dopamine methacrylamide
DMFt	Decayed, missing, or filled, teeth
DMP-1	Dentine matrix protein-1
DMPT	N, N-dimethyl-p-toluidine
DPP	Dentine phosphoprotein
DSP	Dentine sialoprotein
ECM	Extracellular matrix
ED	Embryonic day
EDC	Carbodiimide
EDH	Eastman Dental Hospital
EDI	Eastman Dental Institute
EGCG	Epigallocatechin gallate
ERO	Eroded
F^-	Fluoride
$FeSO_4$	Ferrous sulfate
FGF	Fibroblast growth factor
FTIR	Fourier-transform infrared spectroscopy
GA	General anaesthesia
GD	Glutaraldehyde
GDP	General Dental Practitioner
GIC	Glass ionomer cement
Gly	Glycine
HA	Hydroxyapatite
HAP	Hydroxyapatite particles

HES	Hesperidin
HTS	Human Tissue Authority
ICCC	International Caries Consensus Collaboration
ICDAS	International Caries Detection and Assessment System
ID	Infected dentine
IEE	Inner enamel epithelium
IRAS	Integrated Research Approval System
IS	Inhalation sedation
kDa	Kilodalton
LA	Local anaesthesia
LC	Light cure
M	Molar
MAP	Mussel adhesive protein
MEPE	Matrix extracellular phosphoglycoprotein
MHRA	Medicine and Healthcare products Regulatory Agency
MID	Minimally invasive dentistry
MID	Minimal invasive dentistry
MIR	Minimally invasive restorations
MIT	Minimally invasive treatments/therapies
Mm	Millimetre
MMA	Methyl methacrylate
MMP	Matrix metalloproteinases
MVAS	Modified Venham Anxiety Scale
NACP	Nanoparticles of amorphous calcium phosphate
NAR	Naringin

NCP	Non-collagenous proteins
NHS	National Health Services
nm	Nanometer
NPG-GMA	N-(2-hydroxy-3-methacryloxypropyl)-N-phenylglycine
NTGGMA	Na-N-tolyglycine glycidyl methacrylate
OCT	Optical coherence tomography
ON	Osteonectin
OP	Osteopontin
PDL	Periodontal ligament
pH	Potential of hydrogen
PMDM	Pyromellitic Dimethacrylate
PMMA	Polymethylmethacrylate
PO4	Phosphate
PPGDMA	Poly (propylene glycol) dimethacrylate
PRO	Proanthocyanidin
Pro	Proline
PVPC	Polyvinylphosphonice
QAPEI	Quaternary ammonium polyethyleneimine
QUE	Quercetin
Res	Resveratrol
RMGIC	Resin modified glass ionomer cement
RUN	Rutin
SBS	Shear bond strength
SDF	Silver diamine fluoride
SEM	Scanning electron microscopy

SIBLING	Small integrin-binding ligand N-linked glycoprotein
SOP	Standard Operating procedure
TE	Tropoelastin
TEGDMA	Triethylene glycol dimethacrylate
Temp.	Temperature
UCL	University College London
UCLH	University College London Hospital
UDMA	Dimethacrylate
UK	United Kingdom
USFDA	United States Food and Drugs Administration
UVL	Ultraviolet light
Val	Valine
WSL	White spot lesions
X	Any codon
α	Alpha
β	Beta
ε	Epsilon
μm	Micrometre
μTBS	Microtensile bond strength

List of tables

List of figures

1. CHAPTER ONE: Introduction and literature review

1.1. Basic human tooth anatomy

Teeth serve several essential functions, such as masticatory function, speech, and aesthetics. Human teeth consist of different regions that vary in function, composition, and texture. Teeth can be divided into two portions, crown and root. The tooth crown, which is the visible portion of the tooth in the oral cavity, consists of both enamel, the outer layer of the crown, and dentine, the layer underneath the enamel, with an intermediate line that separates them known as the dentino-enamel junction (DEJ) (Nanci, 2018). The second portion, the dental root, is what aids the tooth to anchor to the jaws through the alveolar bone (Fig.1.1). It houses the nerves in a special casing referred to as the pulp chamber. Dental roots are covered with epithelial tissues known as the gingiva. The gingiva contains more specialized tissues called cementum which covers another essential ligament known as periodontal ligament (PDL). PDLs act as shock-absorbing springs deflecting loads encountered on teeth due to masticatory movements or impacts (Nanci, 2018).

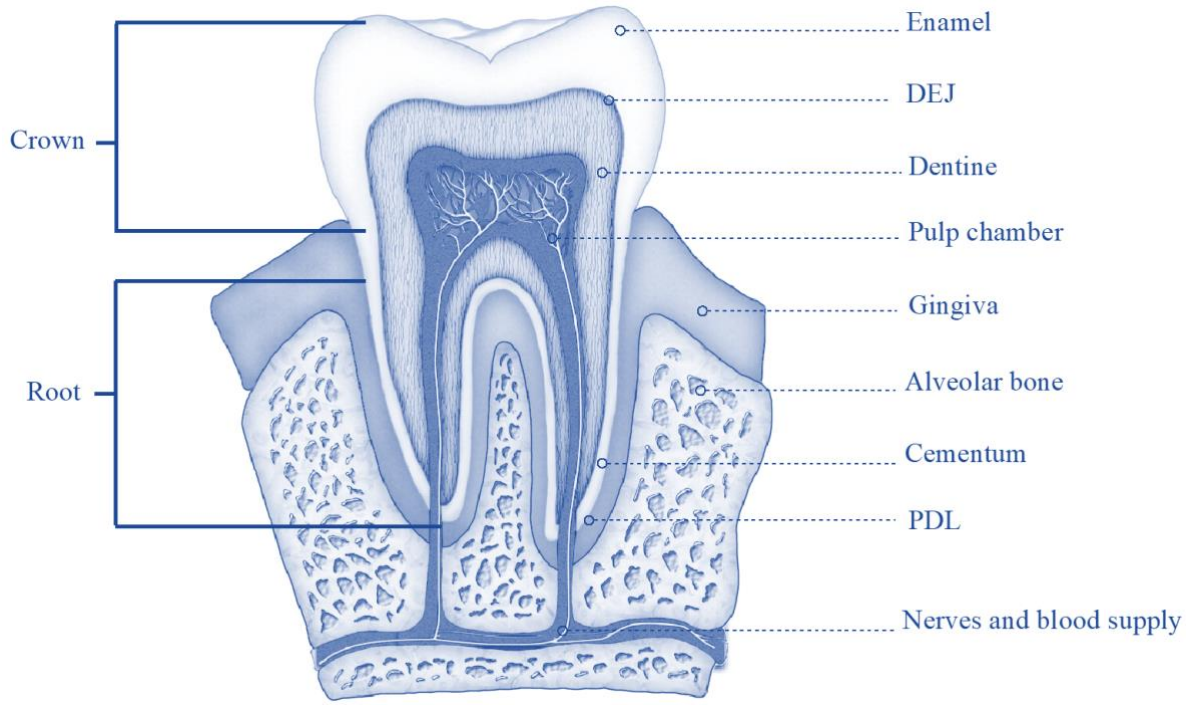

Figure 1.1 Human tooth anatomy. Schematic representation showing the different regions in a single human tooth. Image courtesy of *Amazonaws*.

1.2. Anatomical variations between permanent and primary teeth

Although both permanent and primary teeth share the same biological tissues and structures, they vary morphologically (Fig. 1.2). The crowns in permanent teeth are rectangular compared to a more bulbous crown in primary teeth. This, in return, provides a broad occlusal plan for permanent teeth and a narrower occlusal plan for the latter. In early erupted permanent incisors, mamelons, rounded protuberances on the incisal edge are usually seen. The crown of primary teeth is constricted cervically, unlike permanent teeth, in which constriction is less pronounced (Inada et al., 2012). In regard to the roots, in primary teeth, roots are shorter than permanent ones. Moreover, roots of primary molars are usually diverged and narrow with spindly root tips, unlike permanent roots, which usually have less divergence and are wider in diameter (Cleghorn et al., 2010). The enamel in primary teeth is generally thinner than permanent ones, 2-3mm and 1mm thick, accordingly. Enamel rods are oriented occlusally from the dentino-enamel junction (DEJ) in primary teeth and more gingivally oriented in permanent teeth. Regarding dentine, in primary teeth, dentine is overall thin and with less regular dentinal tubules. On the contrary, in permanent teeth, the dentine is thicker with more regularly formed dentinal tubules (Chowdhary and Reddy, 2010). Pulp horns in primary teeth are higher than permanent ones, with proportionally larger pulp chambers. Pulpal floor is more porous in primary teeth than denser and less porous pulpal floors in permanent teeth (Cleghorn et al., 2010). All variations mentioned above are essential in studying, diagnosing and managing these teeth.

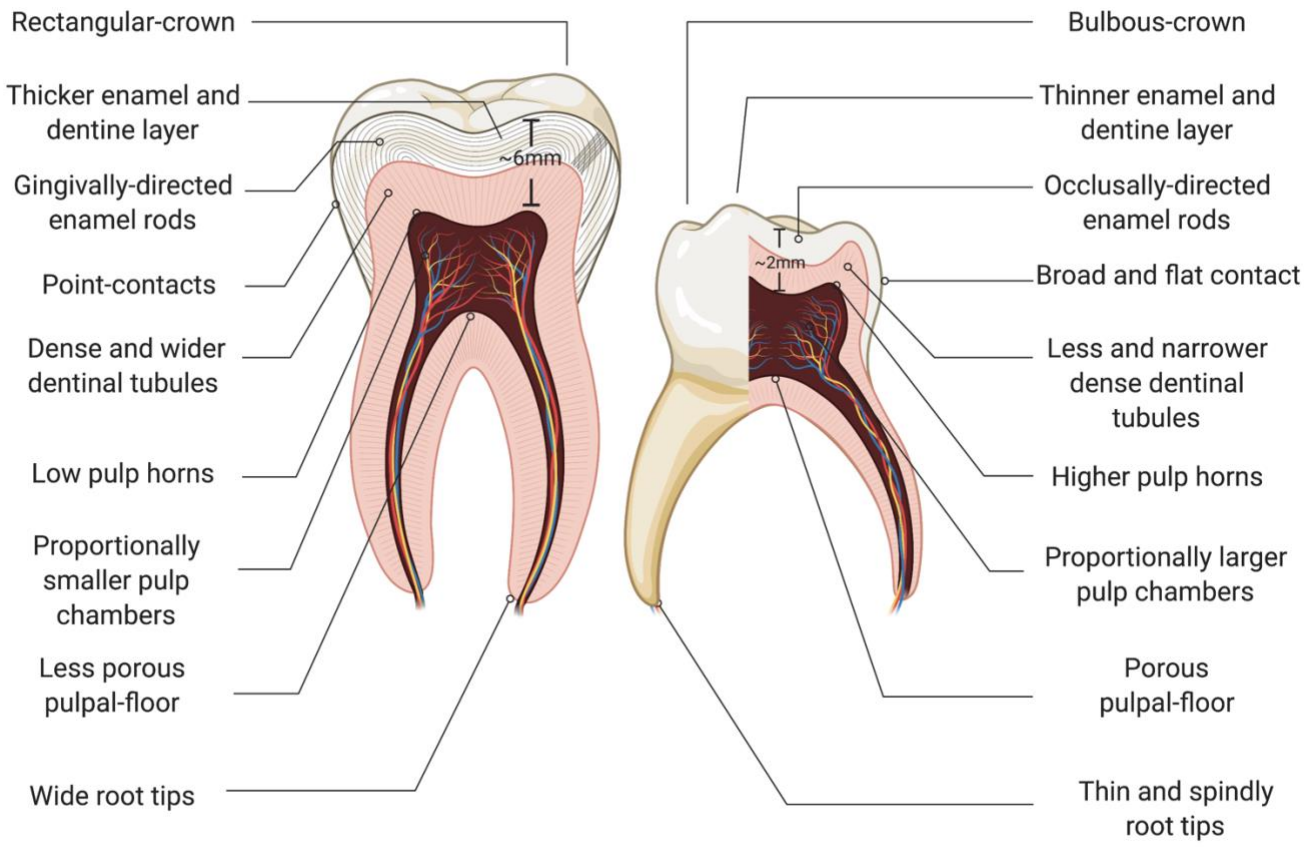

Figure 1.2 Schematic diagram showing anatomical variations between permanent and primary teeth (Created with Biorender.com).

1.3. A glimpse into mammalian tooth development

All mammals share the same dental structures: enamel (crown), dentine (underlying tissue), and pulp chamber where the nerves supply reside. During embryonic development, dental organogenesis is managed via reciprocal interaction between both epithelial and mesenchymal-cell layers (Fig. 1.3).

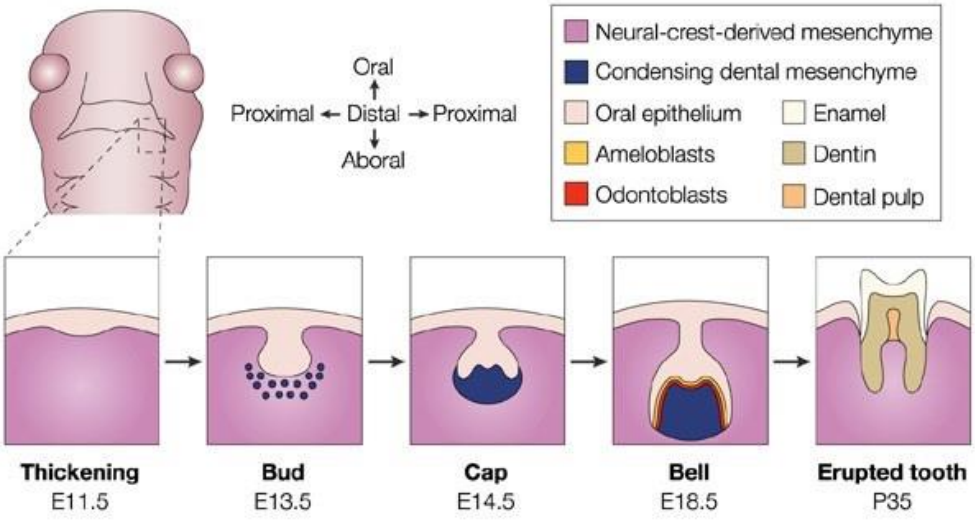

Figure 1.3 Schematic representation of tooth organogenesis stages. A schematic of an embryo head at ED 11.5 (dashed box) indicating the location at which lower molars will erupt. Bottom images are the stages of organogenesis. At E11.5 beginning of epithelial and mesenchymal cells, reciprocal interactions followed by tissue thickening (early bud stage and bud stage). At the bell stage, the ameloblasts and odontoblasts form in thicker layers at the site of interaction between the epithelium and mesenchyme. These layers generate the enamel and dentine of the fully-grown tooth (Tucker and Sharpe, 2004) **(Jamal, 2016b)**.

These developmental processes are orchestrated via transcription factors and signalling molecules. The dental lamina, the first cellular fold in organogenesis, starts to thicken (referred to as the lamina stage). This is followed by a thickening of the epithelial cells (Placode stage). In mice craniofacial development, sites of tooth formation are characterized at embryonic day (ED) 10-11 by expressing specific genes (such as Barx1, Lhx8, Msx1, and Msx2), and secretory molecules like fibroblast growth factors (FGFs) and bone morphogenetic proteins (BMPs) (Nakatomi et al., 2010). These signals induce a cascade of transcription factors expressions in the dental mesenchyme, condensing around the developing epithelial bud (bud stage). The signalling centre regulates dental development, referred to as enamel knot formed during ED13.5-14.5 (cap stage). At ED16-18, epithelial and mesenchymal cells differentiate into dental-tissue progenitor cells, such as ameloblasts, odontoblasts and dental follicle cells (bell stage). Ameloblasts generate the inorganic component

enamel, odontoblasts give rise to the dentine and pulp complexes, while dental follicle cells differentiate into periodontal tissues, cementum, and alveolar bone (Tucker and Sharpe, 2004; Jamal, 2016a).

1.4. Dental enamel

Dental enamel is the outermost layer that covers the anatomical crown, which is crowned to be the hardest calcified semipermeable tissue in the entire body (Nanci, 2018). The oldest teeth fossil discovered to date are 9.7 million years old (Lutz et al., 2017). The enamel substance of these teeth was still intact, which relates to its highly mineralized structure. Enamel is mainly composed of inorganic minerals of crystalline calcium phosphate by 96%, and the few remaining 4% are a mix of water and organic components (Fig.1.4) (Chiego, 2018).

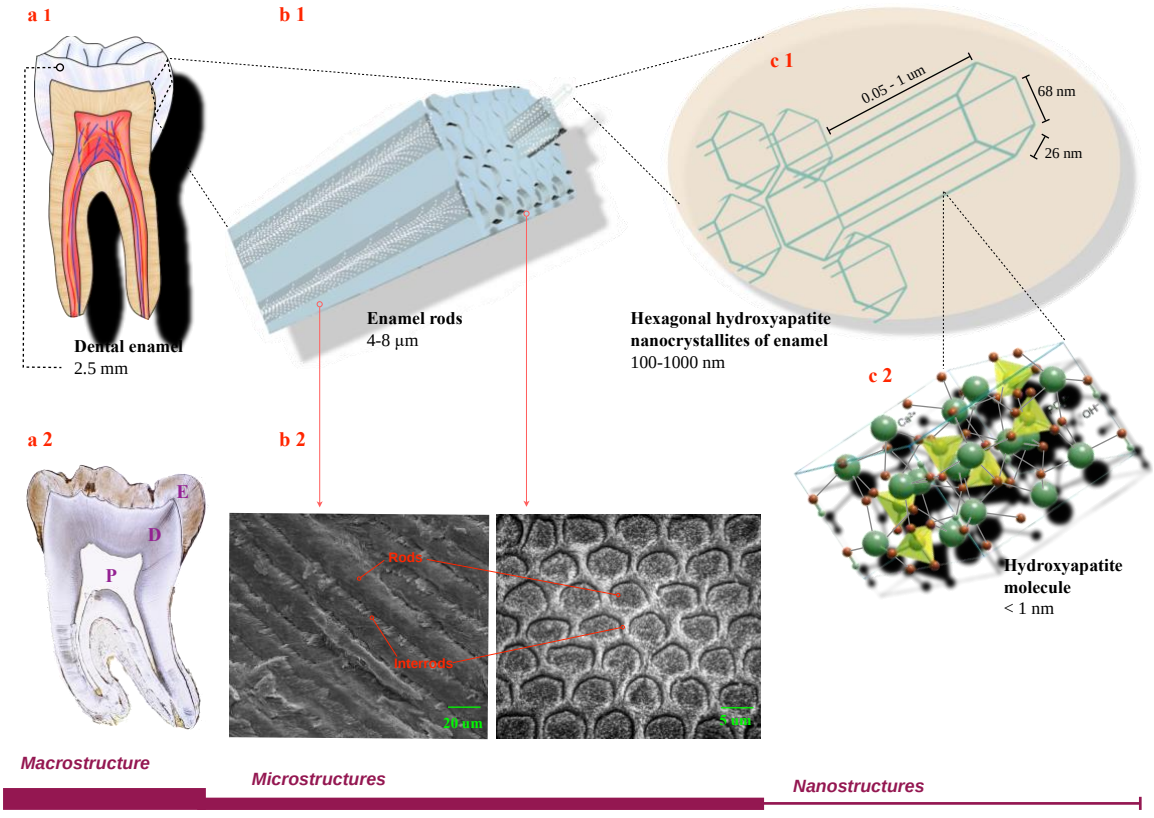

Figure 1.4 The hierarchical structure of dental enamel at different scales. a1, schematic representation of a human tooth, with dental enamel shown with the dotted line. a2, a ground section of a whole tooth were E for enamel, D for dentine and P for dental pulp *(Nanci, 2018)*. b1, a 3D schematic representation of enamel rods consisting of both heads and tails made up from packed crystals in white. b2, Two SEM images of enamel rods with their intermediate interrods shown in red dotted lines, at the scale of 20 microns (left-side view) and key-hole feature at the scale of 5 microns (right-top view). c1, Ergonomic representation showing the hexagonal morphology of hydroxyapatite nanocrystals. c2, a scheme of hydroxyapatite molecule cell unit (Hadzovic, 2018) (Created with Biorender.com).

Despite being deprived of almost all organic components, enamel has exceptional toughness and moderate bristliness. That relates to its distinct and well-organized hierarchical nanostructure (Fig. 1.4) (Chiego, 2018).

In mammalian enamel, the primordial structural units are called enamel rods (formerly known as enamel prisms); which are bundles of crystallites that have an inter-prismatic core, also referred to as enamel inter-rods. Ameloblasts are the cells responsible for the generation of enamel via the secretion of enamel organic matrices such as amelogenin, ameloblastin and enamelin, which later calcify via calcium hydroxyapatite (HA; Ca10[PO4]6[OH]2). After enamel formation, these cells tend to disappear; thus, unlike other biomineralized tissues such as bone and dentine, enamel lacks the regenerative capacity to generate new enamel tissues.

1.4.1. Hydroxyapatite: dental enamel building block

As formerly mentioned, the enamel is mainly composed of a mineralized substance, hydroxyapatite, Ca 10 (PO4) 6 (OH) 2. Enamel hydroxyapatite (HA) generates crystals with a hexagonal shape when viewed in cross-section, which is their crystal lattice comprised of PO4 tetrahedral balanced via Ca2+ ions (Fig.1.4 c2). The average dimensions of HA crystals are 68 nm wide, 26 nm thick and 0.05 – 1 μm long (Fig.1.4 c1). Although the length of the enamel HA crystals is still not precisely determined, several studies in the literature suggest that it can extend from the dentino-enamel junction (DEJ) to the outer surface of the enamel (Daculsi et al., 1984). Ca ions creates

diffusing channels allowing anions to be transported along the c-axis of the crystal apatite. For instance, hydroxyl-ions can diffuse through these channels and be then replaced via other ions such as fluoride (F-), chloride (Cl-) and carbonate (CO3 2-). The stoichiometric state of apatite crystals in teeth is inapplicable; instead, it is full of defects and primarily calcium deficient. Thus, the neutrality of electrons upon calcium depletion maintains the protonation of phosphate groups; the same applies to hydroxyl groups that are replaced with carbonate ions (Berkovitz et al., 2017).

1.4.2. Mechanical properties of dental enamel

As previously mentioned, enamel has a unique well-organized hierarchical structure; therefore, it has superior mechanical properties. Its mechanical properties range from hardness, stiffness, and elasticity to fracture toughness (Table 1.1). Although enamel has always been considered an isotropic material, meaning it has the same value of physical properties along with its structure, only recently, it was labelled as an anisotropic material. The term anisotropic relates to the variations in enamel physical property values in different areas in its structure. For instance, regarding anatomy, the enamel is thicker at the cusps and incisal edges when compared to the enamel at the cervical margins, which is relatively thin. Thus, when measuring its hardness, the hardness of enamel decreases from its surface towards the dentino-enamel junction (DEJ), the meeting point between enamel and dentine. Worth noting that the enamel solely can be brittle and liable to fracture easily. Nonetheless, the dentine underneath the enamel is much more resilient, compensating for enamel brittleness, allowing it to resist both shearing and impact forces (Nanci, 2018). Furthermore, when it comes to crack resistance, the outermost enamel layer has a higher resistance to crack than its inner layers (Berkovitz et al., 2017).

Table 1.1 Mechanical properties of enamel (Nanci, 2018)

Specific gravity	**2.9**
Hardness (Knoop no.)	**296**
Stiffness (Young's modulus)	**131 GN/m^2**
Compressive strength	**76 MN/m^2**
Tensile strength	**46 MN/m^2**

1.5. Dentine

Dentine is a porous bone-like tissue with a yellow hue forming the bulk of the tooth. Dentine is covered coronally by enamel and wrapped by cementum along the root surface (Nanci, 2018). As previously mentioned, dentine is developed by a process known as dentinogenesis, which begins prior to enamel formation via cells known as odontoblasts. These cells are aligned along the edge of the dentine, making up the periphery of the pulp. The very presence of these cells highly distinguishes dentine from enamel since, unlike enamel, odontoblasts aids in the repair and regenerative process of dentine (Song et al., 2017).

1.5.1. Types of dentine

Generally, dentine is divided into three types which are the primary, secondary and tertiary dentine. Firstly, the primary dentine, which is the most abundant dentine in the tooth. It is located near the dentinoenamel junction, between the enamel and the pulp chamber. The outermost layer closer to enamel is known as mantle dentine. Mantle dentine is a less mineralised tissue made of a loosely packed collagen fibril. Beneath it lies the circum-pulpal dentin, which is more mineralized and makes up most of the dentine layer. Secondly, is the secondary dentine which is formed after root formation is complete, generally after the tooth has erupted and is functional. Lastly is the tertiary

dentine, also known as reparative dentine or sclerotic dentin. This type of dentine is generated in response to an external stimulus either from a carious lesion, tooth wear or thermal stimuli (Nanci, 2018).

1.5.2. Hierarchical microstructure and composition of dentine

Dentine adopts a hierarchical structure comprised of multiple dentinal tubules propagating from the dentine-pulp complex to the entire surface area of dentine (Fig. 1.5). These dentinal tubules are enveloped by inter-tubular dentine and are ~2-4 μ in diameter (Wegst et al., 2015).

By weight, dentine is composed of 70% inorganic hydroxyapatite crystals, some organic matter, mainly collagen type-I (20%), and 10% water (Table 1.2). This mixture between soft (protein) and rigid (mineral) components of dentine is what gives it these unique mechanical properties (Launey et al., 2010).

Table 1.2 Dentine composition (Launey et al., 2010).

Dentine composition	**Apatite crystals** (minerals)	**Organic-matrix** (type-I collagen and non-collagenous proteins)	**Water** (Free and bound)
Percentage	70%	20%	10%

The inorganic part mainly consists of type-I collagen, includes hydroxyapatite (HAP) mineral nano-crystallites (~100 x 30 x 4 nm) and collagen fibrils. HAP is located either within collagen fibrils (intrafibrillar) or between them (extra-fibrils) (Nanci, 2018). On the other hand, the organic part mainly comprises non-collagenous proteins (NCPs), several enzymes, and proteoglycans (PGs). These proteins include small integrin-binding ligand N-linked glycoprotein (SIBLING) family, which contains dentine sialoprotein (DSP), dentine glycoprotein (DGP), dentine phosphoprotein (DPP), dentine matrix protein-1 (DMP-1), osteopontin (OP), osteonectin (ON), bone sialoprotein (BSP) and matrix extracellular phosphoglycoprotein (MEPE) (Orsini et al., 2009). Although NCPs are not abundant in dentine make-up, however, they are essential during biomineralization.

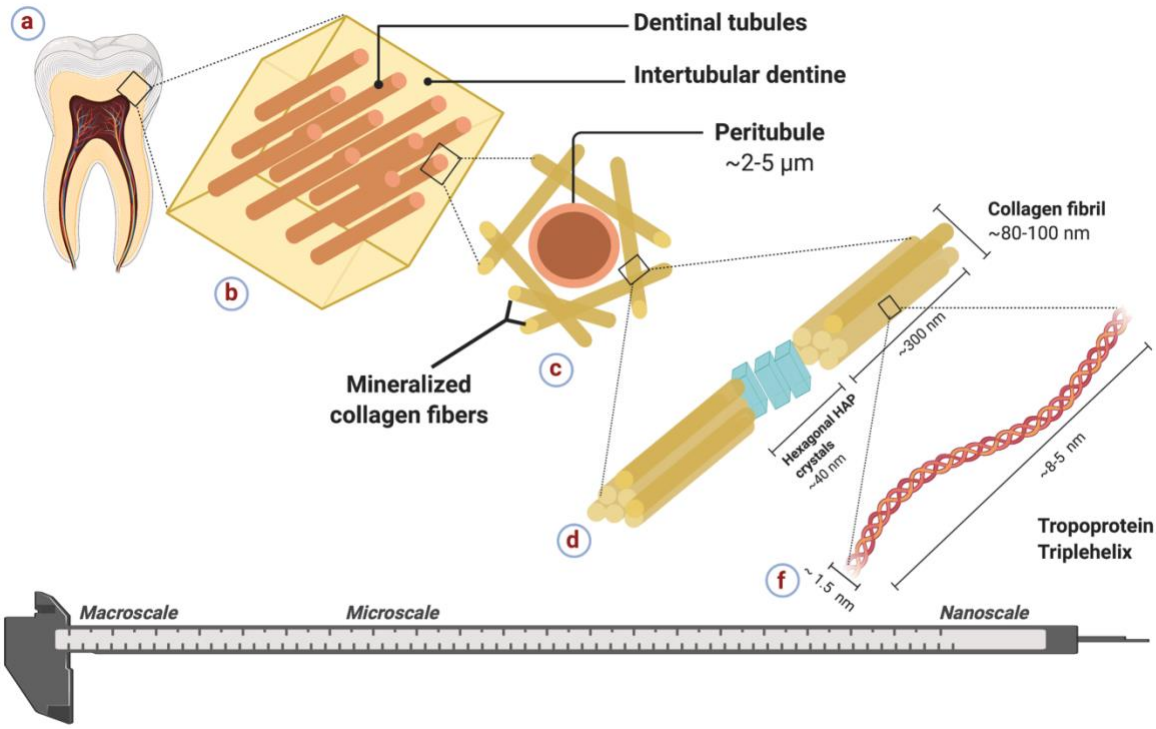

Figure 1.5 Schematic representation of the hierarchical structure of dentine. Schematic representation illustrating the dentine hierarchy, *a.* macroscale tooth with a dotted box of dentine. *b.* side-view both dentinal tubules and intertubular dentine. *c.* Peritubular dentine 2-5um in diameter, surrounded by mineralized collagen fibers. *d.* Collagen fibrils 80-100nm in diameter and around 200nm in length. Hexagonal hydroxyapatite crystals presented with the blue rectangles, and they are nearly 40nm in width. *f.* Triple helix protein with an average length of 8-5 nm to a width of around 1.5 nm. Modified from N. Biswas (Biswas et al., 2013) (Created with Biorender.com).

1.6. Dental caries

1.6.1. Introduction

Teeth surfaces can be affected by various factors such as abrasion, attrition or erosion. The most prominent is dental erosion, which results from acid attacks. Diets that contain acidic elements are one factor that causes the chemical dissolution of enamel. In dental caries (tooth decay), acids are secreted by a specific type of bacteria, namely streptococcus mutans. This consequently results in a progressive dissolution to the teeth' hard tissues, leaving it unsightly, weakened and with an impaired function.

1.6.2. Epidemiological factors of dental caries

The disease is considered a widespread epidemic globally, affecting an alarming 60 to 90% of minors and adolescents worldwide. According to the world health organization, it affects roughly 2.5 billion people globally, 37% of the world population (Cooper, 2018). The most common epidemiological measure of caries is referred to as DMFt; this is a measure of the number of teeth that are decayed (D), missing (M), or filled (F). In England, recent epidemiological studies investigated these parameters on 3 to 5-year olds. In 2013, the dmft in 3-year olds was 0.36 in all children and 3.07 in 12% with decayed teeth. 89% of these teeth were unrestored. In 2017, the dmft in 5-year olds was 0.8 in all children and 3.4 in the 23.3% teeth with decay. Around 78% of these decayed teeth were untreated (PHE, 2013), (PHE, 2017b).

1.6.3. Consequences of dental caries in children

Dental caries is still a significant public health issue in both high- and low-income countries. It holds various consequences ranging from high treatment expenses, failure to attend school, hospitalization, pain that affects different lifestyle aspects and last but

not least psychological aesthetic-related concerns. In the United Kingdom, 60,000 school days are being missed during hospital extractions alone, with an average of 3 days being missed due to dental-related problems. Furthermore, 41% of parents are also affected by missing work days while accompanying their children (PHE, 2017a). In relation to expenses, a recent study showed that the NHS spends an average of £3.4 billion per year on dental diseases. From 2015 to 2016, tooth extractions in paediatric patients alone cost the NHS almost £60 million, with the majority being from tooth decay (White, 2017).

1.6.4. Aetiology of dental caries

The initial step in caries development is the formation of plaque, a sticky substance that adheres to the tooth surface and acts as the host of bacteria, acid, food debris, and saliva. Plaque is mainly thought of as a biofilm, which will calcify if not cleaned by brushing, resulting in the formation of Tartar and Calculus. From a microbiology perspective, multiple species of bacteria cause caries, mainly the gram-positive carbohydrate-fermenting Streptococcus mutans (responsible for caries initiation) and lactobacillus acidophilus (responsible for caries progression) (Larsen and Fiehn, 2017). The presence of fermentable carbohydrates such as sucrose, glucose, fructose, along with the biofilm, aids the release of acidic substances, which causes demineralization of the carbonated hydroxyapatite crystal lattice of enamel, cementum and dentine (Featherstone, 2000);(Kidd and Fejerskov, 2004) (Fig. 1.6).

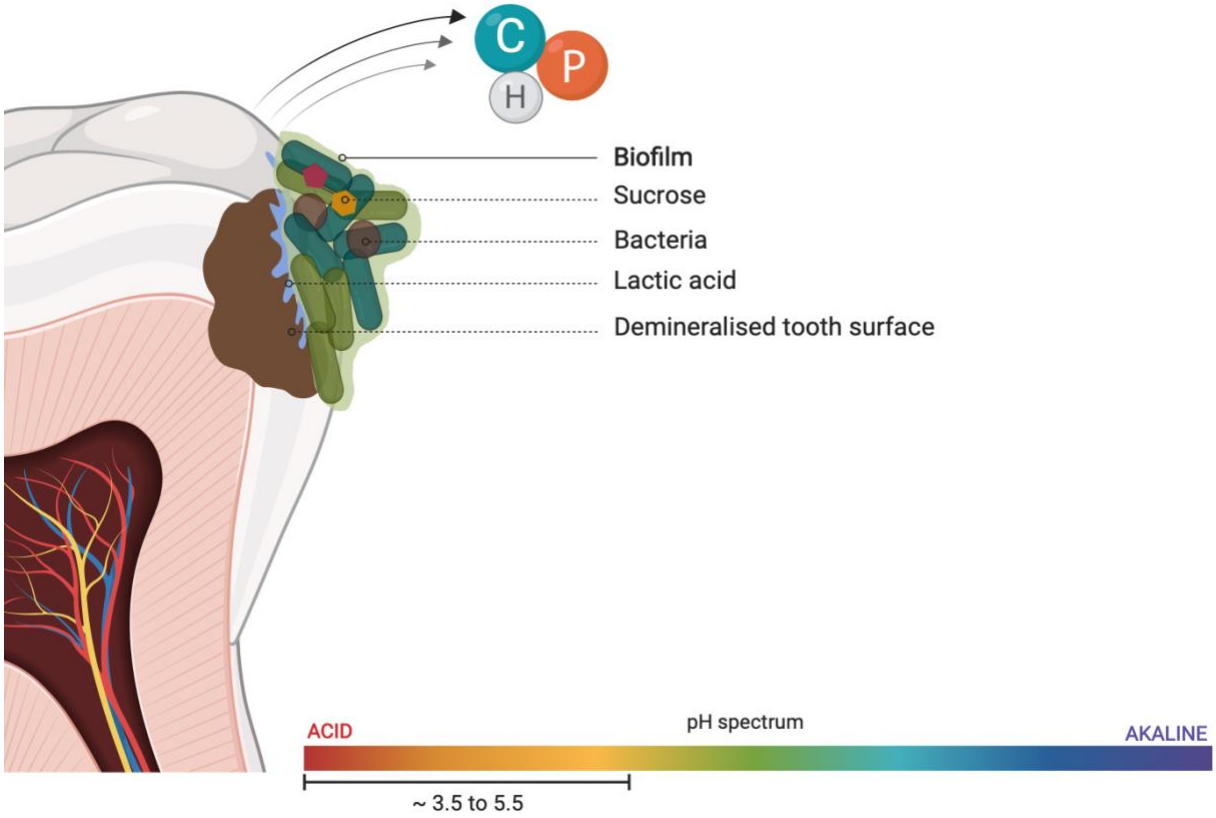

Figure 1.6 Schematic representation of dental biofilm (Created with Biorender.com).

Saliva can play a significant role in dental caries. It is a watery substance secreted via salivary glands with an average normal pH range of 6.2-7.6. Although it is composed of water by 99%, the rest of its composition (inorganic and organic substances), such as minerals (calcium and phosphate), enzymes and proteins, aid in performing essential functions. These functions vary from lubrication, taste, digestion to protection. Concerning teeth, saliva has a buffering capacity, protective function (via mucins aids in mastication, speech, and swallowing), re-mineralizing function (via ions such as Ca^{2+} and proteins), and an antibacterial role. Thus, when salivary secretions are impaired, the chances of caries increase.

1.6.5. Theories explaining the association of bacteria in dental caries development

At the end of the 19th century, the central concept regarding carious lesions was that they are initiated by microflora in all plaque. This hypothesis was termed 'The non-specific plaque hypothesis' by Walter J. Loesche in 1976, which was a continuum of the work done by Black (1884) and Miller (1890) (Loesche, 1976) (Black, 1884) (Miller, 1890). In the mid-70s, more advanced microscopic techniques paved the way for more accurate studies of specific species of bacteria. It was shown that the carious lesion is initiated by a few species of bacteria within the biofilm, mainly S. mutans and S. sobrinus, and by removing them, caries can be prevented. This hypothesis was then termed the 'Specific Plaque Hypothesis' by Walter J. Loesche in 1976 (Loesche, 1976). This hypothesis was later followed by the 'Ecological Plaque Hypothesis' proposed by Philip Marsh in 1994 (Marsh, 1994). The theory argued that the disease results from an imbalance in the total microflora due to ecological disturbances, which in turn causes aggregation of some oral pathogens. This hypothesis is currently the most agreed upon hypothesis.

1.6.6. Carious enamel

The initial to moderate stages of caries start to show on the outer layer of the tooth, the enamel. It typically is seen as a white spot referred to as white spot lesions (WSL). At this stage, the demineralised enamel cannot be detected by radiographs; however, it may be spotted clinically with proper illumination and when the enamel surface is dried for a specific time (Ekstrand et al., 2018). This stage is irreversible by the demineralisation/remineralisation effect of saliva and the use of fluoride. If the demineralisation continues, a localized enamel breakdown becomes apparent; however, dentine is still intact.

1.6.7. Carious dentine

The extensive stage is when the caries progress to dentine with distinct cavitation visible. Compared to enamel, dentine is less mineralised and contains high water content; therefore, the progression of caries is usually rapid. This stage is divided into three layers, soft, hard and translucent dentine layers. The soft caries is the infected part of dentine, where the collagen fibrils are completely demineralised and denatured and contains the highest bacterial count. Underneath this layer is the hard dentine, also referred to as the affected dentine. At this layer, most of the collagen fibrils are still variable, and generally, the layer contains fewer bacteria. This layer is then followed by a highly mineralised layer, the translucent layer where the dentine is repaired through the reparative mechanism of the pulp (Fejerskov and Kidd, 2009) (Fig. 1.7).

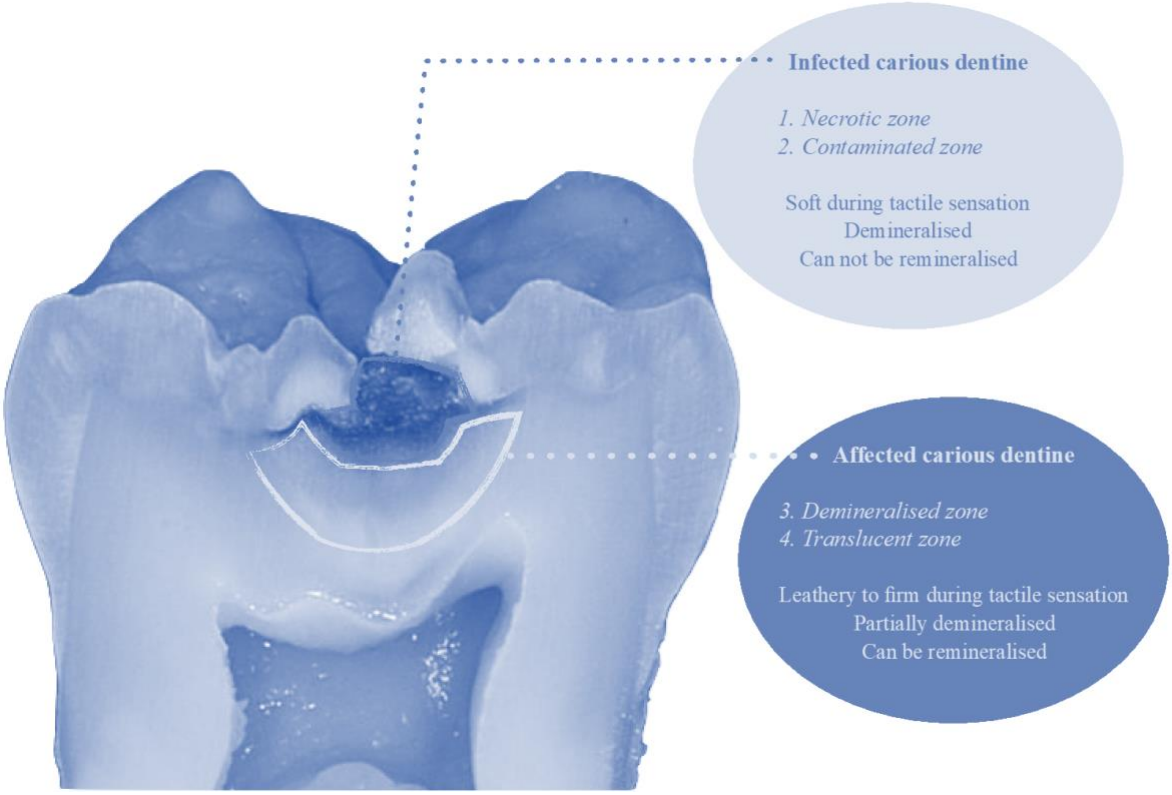

Figure 1.7 Carious dentine zones.

1.6.8. Enzymes in carious dentine

After dentine formation, some enzymes remain trapped in-situ within the dentine extracellular matrix (ECM). It was shown that in carious dentin, the cariogenic demineralization process re-exposes these enzymes and induce their activation (Tjaderhane et al., 1998). The demineralized collagen fibrils and the surrounding matrix becomes exposed to prolonged hydrolytic degradation via degradation enzymes such as collagenolytic enzymes, matrix metalloproteinases (MMPs) and cysteine cathepsins. The bacterial acids upregulate the MMP activation in deep carious lesions (Tjaderhane et al., 1998). MMP was shown to be, not only located within the dentine matrix, but also within the salivary contents, and it was shown to contribute to the degradation of dentinal ECM (Sulkala et al., 2001).

1.6.9. Significance of microleakage between restoration/tooth interface

Microleakage is a common undesired effect that occurs around dental restorative materials. It can be described as an undetectable passage of microorganisms, fluids, molecules in between the filling and the walls of the cavity (Radhika et al., 2010). This leak could eventually cause hypersensitivity to the restored tooth, discolouration and recurrent (secondary) caries underneath the restoration (Radhika et al., 2010). Many factors that can increase the chances of microleakage are restoration polymerisation shrinkage and the adaptation (bonding) of the filling to the tooth surface. Many composite restorations require light curing, which will alter the dimensions of the filling, causing it to shrink. This shrinkage will lead to a gap between the filling and the tooth surface, which leads to microleakage. The leading cause of this phenomenon was found to be related to the variations in thermal expansion coefficient between the filling and the tooth structure (Bullard et al., 1988). A number of different composite materials

were modified to reduce the microleakage side-effect and improve the durability of the material (Chesterman et al., 2017; Bakhsh et al., 2020; Guo et al., 2020).

1.7. Current restorative materials

Dental caries is usually treated operatively by drilling or excavating the carious lesion, followed by filling the cavity with filling materials. These materials include amalgam (silver-coloured restorations), composites or glass ionomers (tooth-coloured restorations).

1.7.1. Dental amalgam

Dental amalgam (silver-coloured restoration), by definition, is an alloy containing mercury. The alloys contain 50% mercury and varying concentrations of silver, zinc, tin and copper (Fredin, 1994). Although dental amalgam has a wide range of advantages such as high strength, high fracture toughness, good wear resistance and minimal micro-leakage, it still has critical disadvantages (Mjör, 1987). Dental amalgam does not chemically bond to dental tissues but relies only on mechanical retention (Black, 1895). Thus, it requires removing an extensive amount of sound tooth structures, making it an invasive option. Dental amalgam releases corrosive trace elements that aids in sealing the tooth-restoration interface; however, this was found to cause discolouration in the long run (Fathi and Mortazavi, 2004). Adding to that, amalgam was found to undergo bulk fracture, marginal ditching and overhanging, which all contribute to increases the chances of secondary caries (Letzel et al., 1989). Regarding aesthetics, the silver colour of amalgam stands out in the oral cavity, making it a non-favourable option by many patients.

Despite all the cons mentioned above of this material, it is still widely used by many clinicians worldwide. However, one main problem that cannot be neglected is the toxic concentration of released mercury, which was linked to many brain-, heart and lung diseases (Bjorklund et al., 2019; Farkhondeh et al., 2020; Warwick et al., 2019), along with environmental concerns (Araujo et al., 2019). In this regard, many efforts were made to reduce the use of amalgam. The last effort made was the signing and ratification of the 'Minamata Treaty on Mercury' that took place at the Minamata Convention in Geneva in 2013. The treaty was an international initiative to phase out mercury-containing materials, including dental amalgam, reducing the release of mercury into the environment (Kessler, 2013). As of November 2019, there are 128 signatories to the Convention and 115 ratifications. Moreover, since July 2018, amalgam was banned from restoring primary teeth (Sanderson, 2019).

1.7.2. Dental composites

Dental composites are usually the restoration of choice by many dentists due to their aesthetics and generally acceptable mechanical properties. They constitute two phases a dispersed phase (Filler silica), such as glass and a continuous phase (Matrix resin), such as bisphenol A-glycidyl methacrylate (Bis-GMA). Fillers primary function is to provide strength to the material and to minimize polymerisation shrinkage. Although these materials show a relatively good outcome, they still have numerous disadvantages. They are not dimensionally stable and thus undergo polymerization shrinkage leading to micro-leakage, recurrent caries and eventually material failure (Davidson and Feilzer, 1997).

1.7.2.1. Evolution of dental composites

Dental composite has gone through decades of constant modifications and alterations.

In the 20th century, the first aesthetic dental restorative material developed was silicate cement. Their prominent disadvantage was their rapid erosion due to their high solubility in oral fluids. This was then followed by materials that are only based on one monomer, namely methyl methacrylate (MMA), introducing “Acid-etching” for the first time in the early 50s (Buonocore, 1955) (Fig. 1.8). The main drawback is that methyl methacrylate is monofunctional, i.e. it has the ability only to be linked to a single molecule, which in return makes it impossible to be crosslinked. This leads to high polymerisation shrinkage after curing since all the cured monomers will be aggregated. To solve this issue, another type of monomer was introduced in the mid-’60s by Ralph Bowen, called a bifunctional monomer, otherwise known as a difunctional monomer (Bowen et al., 1968). The advantage of using this monomer type is that it can be linked to other monomer particles. The main two monomers from this development, which are still used today, are bisphenol A-glycidyl methacrylate (Bis-GMA), urethane dimethacrylate (UDMA) (Bowen et al., 1968). However, the density of these monomers mentioned above was relatively high and to decrease it, another monomer was added, Triethylene glycol dimethacrylate (TEGDMA). It is worth noting that TEGDMA is not a true monomer but rather an unfilled resin. Although the main reason for adding bifunctional monomers is to reduce polymerisation shrinkage, the shrinkage was still evident. Moreover, water sorption increased, and the overall strength was compromised due to the empty voids within the monomers. That led to the introduction of fillers in the early ’70s and its evolution to the present date.

As mentioned above, the filler’s primary function was to increase strength and eliminate polymerisation shrinkage. Currently, the primary type of filler is silica, which is subdivided into crystalline form (e.g. Crystobalite, Tridymite or Quartz) and non-crystalline form (glass form). Coupling agents were then introduced to bind the filler

and the resin together, known as 3-Methacryloxypropyl-trimerthoxy Silane, and it is still used till now. At this stage, dental composites were classified according to filler particle size as macro-filled, micro-filled, micro-hybrid and nano-filled.

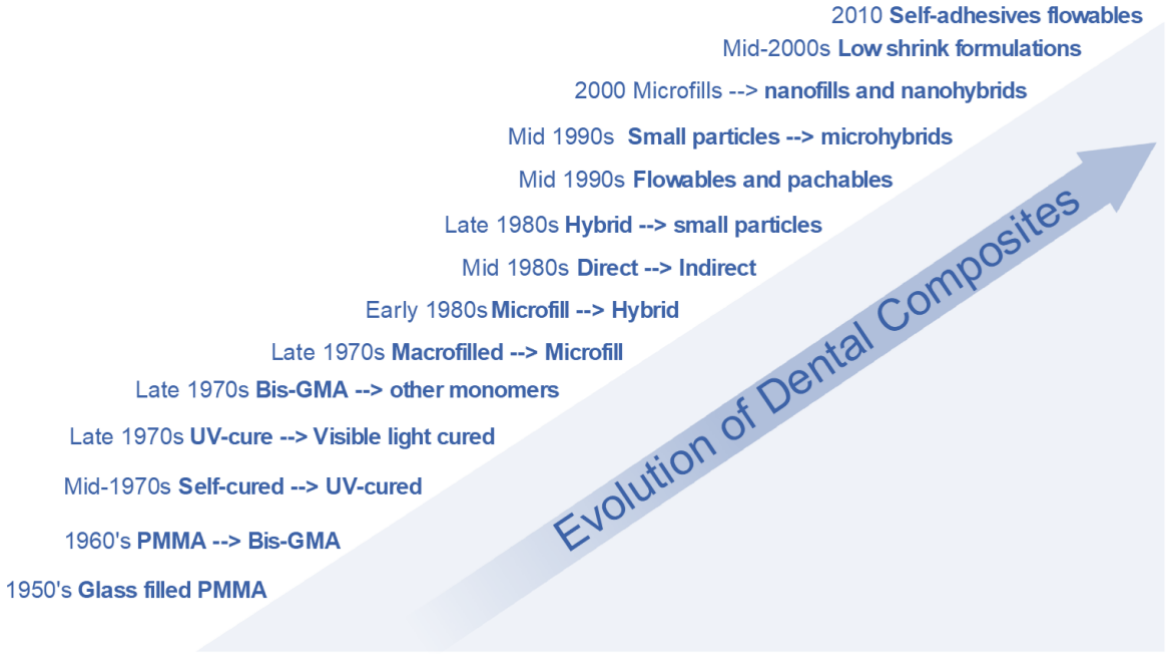

Figure 1.8 Evolution of dental composites.

1.7.2.2. Classification of resin-based dental composites according to type and particle size

There are numerous types of commercially available composites in the market classified according to their type. These include flowable, bulk-filled and self-adhesive composites. Each one is used depending on the clinical situation. Each type contains inorganic fillers, enhancing the final resin's properties (Habib et al., 2016). The resin can then be classified according to fillers particle size to micro-filled, macro-filled, micro-hybrid, nano-filled and bulk-filled.

Resins with macro-fillers were the first type to be introduced. They have a particle size range of 5 – 10 µm. They have excellent mechanical strength, however, low resistance to wear. Polishing these resins is rather tricky and eventually leaves a rough plaque-retentive surface (Bonsor and Pearson, 2012).

Resins with micro-fillers are made with fine silica particles with a size range of 0.4 – 0.5 µm. This, in return, made it easier to polish and provided a smoother surface that is less plaque retentive. Nonetheless, the fine silica particles compromised the mechanical properties of the final material. Thus, these resins is not indicated in areas with high bearing load (Bonsor and Pearson, 2012).

On the other hand, resins with hybrid filler particles are a further modification that aimed to possess the pros of both macro-and micro-filled resins providing the material with high mechanical properties and enhanced polishing ability (Bonsor and Pearson, 2012). Nonetheless, the material's dimensional stability was compromised, and these resins were shown to have high polymerisation shrinkage (Braga et al., 2005).

Further development of filler size continued, and nano-filled resins were produced. Resins with nanofiller are one with 20-70nm particle size range. They act as one unit and provides the final material with high mechanical properties, high resistance to wear and smooth surfaces after polishing. Although the particle size is at the nanoscale and provided the resin with the properties mentioned above, the volume of these fillers is high, which makes marginal cavity adaptability rather difficult (Bonsor and Pearson, 2012).

Recently, bulk-filled composite resins were introduced. Its composition contains both zirconia particles and non-agglomerated silica particles, and these are nanohybrid particles. The main advantage of these materials is that they can be bulk-filled, reducing the procedure time. This is due to the ability to light cure them as a bulk, 4-5mm thickness, rather than incremental curing. Despite that, they have low wear

resistance and low strength compared to conventional resin materials (Chesterman et al., 2017).

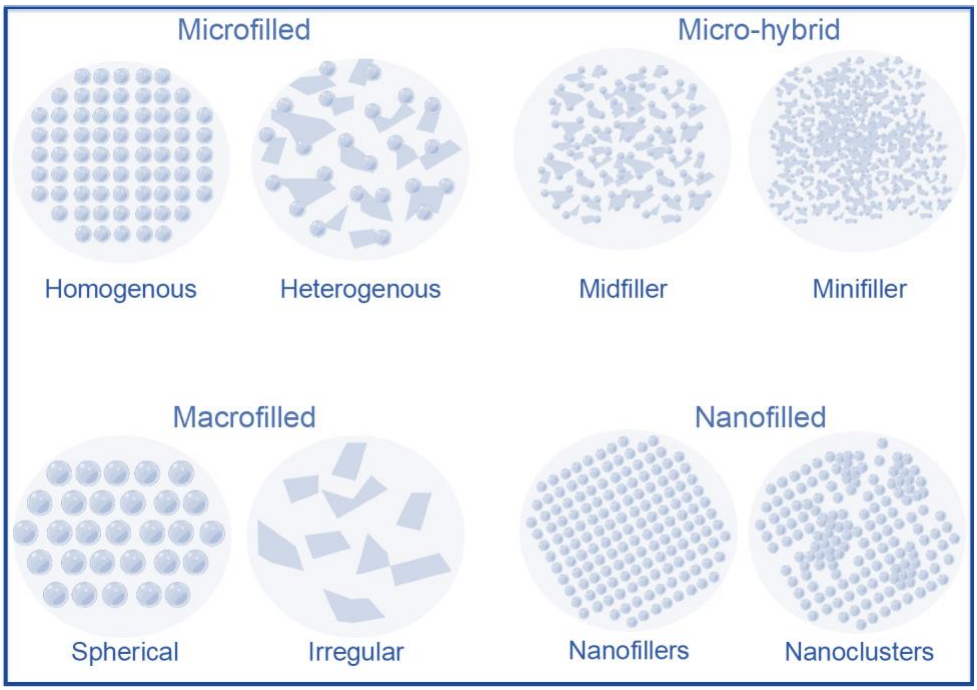

Figure 1.9 Classification of dental composites according to particle size.

1.7.3. Glass Ionomer Cement (GIC)

One of the main goals of a dental resin material is to adapt and seal the restored tooth structure. Glass ionomer cements were shown to achieve this due to their similarities in characteristics to the tooth structure. GIC is composed of two phases, powder phase, which is made of calcium, strontium aluminosilicate glass (base), and a liquid phase mainly composed of the water-soluble polymer (acid). GIC possesses various advantages, including good adhesion to dental tissues, fluoride ions release, biocompatibility, and a similar thermal expansion coefficient to tooth structures.

GIC was first introduced in 1962 with the development of zinc oxide and polyacrylic acid (Najeeb et al., 2016). Polyacrylic acid was shown to effectively bond to calcium and eventually form hydrogen bonds with collagen (Beech, 1973). They are intrinsically

made up of a cross-linked polyacid matrix, with fillers being glass. In resin-modified glass ionomer cements (RM-GICs), another improved version of GIC, the matrix contains a polymer network of resin materials, mainly 2-hydroxymethyl methacrylate (2-HEMA). RM-GICs were modified to acquire higher toughness and improved aesthetics compared to former GICs (Xu and Burgess, 2003). Nonetheless, RM-GICs still have drawback, like having low surface hardness and wear resistance (Rios et al., 2002). Moreover, RMGIC has another limitation which is water sorption, and that is due to the hydrophilicity of poly-HEMA (Kanchanavasita et al., 1997).

All in all, due to these restorative materials ease of use, they have been utilised widely in minimally invasive restorative treatments, which will be addressed later.

1.7.4. Bioactive Resin-Modified Glass Ionomer Cement

An American company, Pulpdent™, recently developed a novel resin-modified glass ionomer cement (RMGIC) called ACTIVA™, claiming that it has a bioactive property (Pulpdent). The company released children-version marketed as ACTIVA kids BioACTIVE™(Pulpdent). When released, it was branded as a self-adhesive cement. Nonetheless, after demonstrating unsatisfactory outcomes, the company currently recommends using an adhesive bonding system during application (Pulpdent, 2018; Benetti et al., 2019). One randomised controlled prospective clinical trial evaluated the claimed bioactivity of ACTIVA. In the study, 78 pairs of class-ll and 4 pairs of class-l cavities were filled with ACTIVA and nano-filled resin composite (CeramX; RC), according to the manufacturer's protocol. ACTIVA restored class-ll cavities, using phosphoric acid-etch pre-treatment without adhesive bonding, demonstrated substantial failure 1-year post-placement (van Dijken et al., 2019). Therefore, further

studies utilising bonding systems are required for ACTIVA to be considered a restorative option.

1.8. Adhesion principles of resin restorative dental materials

Dental adhesives facilitate the adhesion between the tooth structure and dental composite. Great effort and breakthroughs were made and are still going in adhesive dentistry to achieve a strong and durable bond between the restoration and both enamel and dentine (Fig.1.10). The fundamental mechanism of bonding to dental tissues is an exchange process that includes replacing dental tissue minerals by resin monomers. This mechanism is divided into two steps. First, dissolving calcium phosphate ions which in return creates micro-porosities in both enamel and dentine surfaces. Secondly, the infiltration of resin within these micro-porosities (Van Meerbeek et al., 2003). Once the material is set, the resin monomer becomes mechanically interlocked within the created micro-porosities (De Munck et al., 2005a). The interlocking mechanism was first introduced in the early 90s and was names “hybridization” or “hybrid layer formation’’ (Nakabayashi et al., 1991).

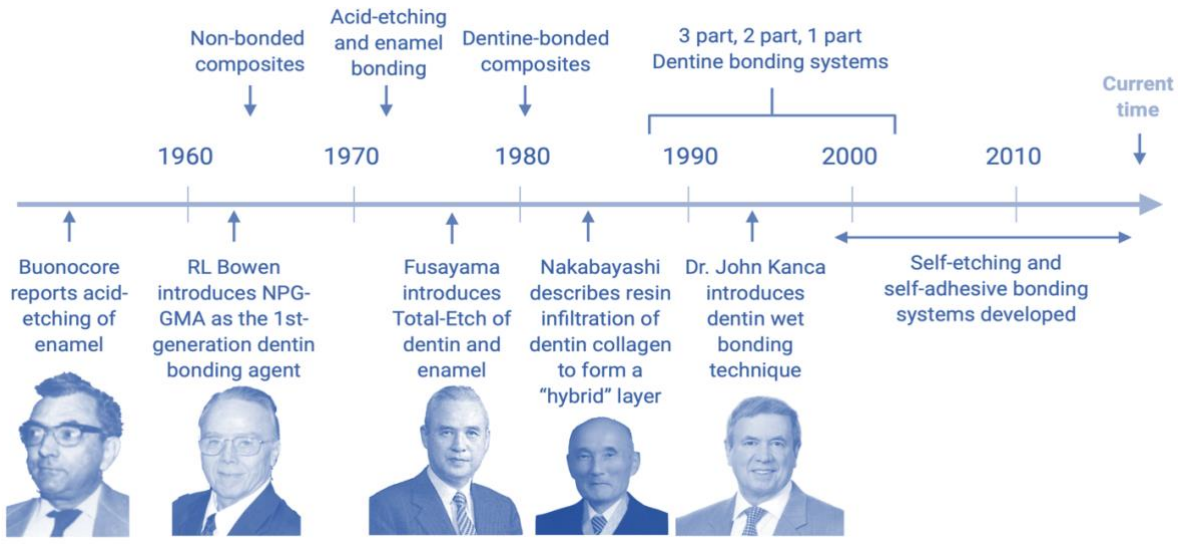

Figure 1.10 Timeline of significant breakthroughs in dental bonding technologies.

Adhesives can be divided into three categories according to their adhesion method. These are “etch & rinse”, “self-etch” adhesive systems.

1.8.1. Etch and rinse adhesive system

The first innovations in the bonding of dental composites originated more than 60 years ago by Buonocore, and it is considered the 1st generation of bonding systems (Buonocore, 1955). He suggested that resin could bond better into acid-etched dentine (Buonocore, 1955). In the 2nd generation, in the late 70s, the aim was to etch enamel only with phosphoric acid. A couple of brands were introduced at the time, such as Onebond™ (Kuraray) and Scotchbond™. However, since the etch was only applied on enamel and not dentine, the failure of the composite bond was relatively high.

There are basics to be comprehended when dealing with any bonding system. Following removing the decay via rotary devices, a loosely bound layer, referred to as “smear layer”, containing bacteria and other dental tissues remnants, blocks the micro-porosities (Pashley, 1992). This layer was shown to hinders the adhesion of the resin to the tooth structure (Ishioka and Caputo, 1989). In order to remove it, a highly acidic gel (pH<1) (e.g. phosphoric acid 37%) is applied. The acid gel etches the enamel by dissolving the hydroxyapatite molecules resulting in water-soluble mono-calcium phosphate. The enamel surface becomes rough, aiding in the hydrophobic composite adhesive infiltration. The main issue was that the composite (1st generation) was highly viscous at that time, which hindered the tooth structure infiltration. To mitigate that, another step was added, which is the use of a bonding agent, followed by adhesive resins application. In this generation, the adhesion steps were three (etch, bond, adhesive resin application). To reduce the steps, a two-step approach was introduced; primer and adhesive (De Munck et al., 2005a).

On the contrary, bonding to dentine is deemed more challenging. As previously mentioned, the composition of both enamel and dentine varies, with the enamel being highly inorganic (high hydroxyapatite crystals content) and dentine being highly organic (high collagen content) (Chiego, 2018; Launey et al., 2010).

When dentine is etched, it exposes a layer of collagen fibrils (2-4 microns) filled with water (hydrophilic). On the other hand, the composite resin is hydrophobic; thus, the adhesion tends to fail. To counter that, a primer was introduced, making it a 3-step process, and this was the 3rd generation of bonding systems. The primer has two ended polymers, one is hydrophilic, which bonds to the dentine, and the other is hydrophobic and bonds to the resin material.

Regarding the total-etch technique, which was the 4th generation adhesives, an issue of post-operative sensitivity was evident by patients. This was due to the use of 37% phosphoric acid, which was harsh on dentine. This, in return, resulted in the dissolution of the smear plugs, which exposed the dentinal tubules.

In the 5th generation, the alteration was to reduce the steps. The number of steps in this generation includes the phosphoric acid and 2nd, primer and bonding resin in one bottle as one step).

1.8.2. Self-etch adhesive systems

The self-etch adhesives were then introduced, and that was the 6th generation. In the 6th generation, the main difference was the elimination of phosphoric acid. They depend on acidic monomers to bond to tooth structures. The steps in this generation were two, a self-etching primer and an adhesive resin.

In early 2000, the one bottle self-etching systems were introduced, which was the 7th generation. In 2010, the 8th generation bonding system was introduced when the nano-filler composites were introduced. The average size of these fillers was 12nm and was shown to have a positive impact on the bonding due to increased penetration depth and the formation of the hybrid layer thickness.

1.9. Adhesion to carious dentine

Although it is clear that the first trials of Buonocore's to achieve bonding to enamel had a tremendous revolutionary effect in adhesive dentistry (Buonocore, 1955), in general bonding to enamel is more straightforward than that of dentine. The enamel, as previously mentioned, has a stable composition in nature. Contrarily, dentine is a dynamic tissue with regenerative capabilities.

Another challenge in the bonding ability of the materials can be the presence of caries in the cavity. As previously described, the dentine caries lesion has two zones, and an outer zone constitutes the "infected dentine (ID)" (soft dentine) and the inner zone which is the "affected dentine (AD)" (firm dentine). The ID has higher bacterial content and is highly demineralized. On the other hand, AD is partially demineralized. The dentinal tubules in AD contain mineral crystals, which could benefit the deeper etching of inter-tubular dentin; nonetheless, it can have a negative impact on the resin tag formation during bonding (Yoshiyama et al., 2002). Regardless of the type of adhesive system, caries-affected dentine (CAD) produces lower bond strength than healthy dentine.

The main factor in play is the enzymes produced in demineralized dentine, referred to as matrix metalloproteinases (MMP). These are a group of more than 25 secreted and

membrane-bound enzymes responsible for the degradation of pericellular substances. It was demonstrated by Pashley et al. that the MMP enzyme caused degradation of the unprotected and exposed collagen fibrils, which in return making it difficult for the resin to infiltrate dentinal tubules (Pashley et al., 2004).

1.10. Contemporary restorative approaches in paediatric dentistry

At one end of the spectrum is the traditional now obsolete restorative approaches where decayed tissue is removed entirely and, on the other end, there is the contemporary approach where decayed tissue is either selectively excavated or left in-situ. To overcome the limitations of the traditional restorative approaches, the majority of the focus nowadays has shifted towards more conservative and less invasive techniques (Schwendicke, 2017). Minimal intervention techniques have been shown to be advantageous in all dental fields, especially in paediatric dentistry. They require less procedural time and usually does not require using local anaesthesia (LA), both of which are vital factors when treating children, primarily those who are anxious or with special needs.

1.10.1. Minimal invasive dentistry (MID)

Minimal invasive dentistry (MID) is defined as conserving dental tissues' integrity, function, and aesthetics via hindering disease progression with minimal structural loss (Banerjee, 2015).

In 2017 at The International Caries Consensus Collaboration (ICCC), a number of recommendations regarding caries management were presented, focusing mainly on two points 1) Selective removal of carious tissue (infected dentine) and 2) stepwise

carious tissue removal (stage 1, removing soft dentine, stage 2, removal to firm dentine 6 to 12 months later).

1.10.2. Atraumatic restorative treatments (ART)

The atraumatic restorative techniques or treatments (ART) are conservative approaches in restoring decayed teeth and fit under the umbrella of minimal invasive dentistry (MID) approaches. It involves sealing fissures and pits that are susceptible to decay or, in other instances, excavating infected dentine using hand tools (e.g. spoon excavators) followed by filling the cavity with adhesive materials such as highly viscous-GIC (Frencken et al., 1996).

The ART concept originally started in Tanzania in the mid-'80s, where access to modern dental devices, electricity and water was unattainable (Frencken, 2009). Before ART, these carious teeth were used to be left unrestored in these deprived areas, which in return, end up being extracted. With ART, these teeth are restored conservatively with low cost and with minimal to no local anaesthesia (LA) utilization.

The method demonstrated high success rates and was shown to be highly acceptable by children. Ishan, K. et al. published a cross-sectional study to evaluate the anxiety levels among five-year-olds undergoing ART restorations. Teeth were restored with Fuji IX GIC, and the anxiety levels were measured via the Modified Venham Anxiety Scale (MVAS). They have shown that although the anxiety levels were higher pre-operatively, yet they dramatically decreased during the procedure (Ishan et al., 2017).

1.10.3. Sealing carious tissues

The seal is the deal; that is the prime concept of this approach which aims to seal the decayed tooth without drilling (with or without excavation). This technique was enormously praised in the literature due to its effectiveness. In a study done by Handelman et al., he reported that after sealing bacteria under restoration for two weeks, their number was radically reduced and continued to reduce after further monitoring (Handelman et al., 1976).

The idea of sealing carious tissues used to be thought of as an extreme measure; nonetheless, after contemplating and understanding the mode of action of caries, the approach became more evident. As previously mentioned, caries thrives at the biofilm, where it becomes pathogenically active at the tooth surface. Thus, the goal is to control this activity on both the tooth and the lesion surface, and this approach does precisely that (Schwendicke, 2017). A recent randomised controlled trial (RCT) study compared the effectiveness of 3 treatment procedures (Drilling, filling or prevention alone) in managing caries in children. The study included 1,144 children treated in 72 dental practices across the United Kingdom and was followed up for 3-years. The result showed no significant difference between all three treatment techniques in terms of episodes of dental pain or infection (Innes et al., 2020). Another recent Cochrane review compared conventional restorations, selective excavation, stepwise excavation, sealing caries with Hall crowns and non-restorative cavity control. The data included 27 RCTs with a total of 3350 participants (4195 teeth), with the majority of the participants are children, and the success rate was measured in 1 year to 2 years. The study included a network meta-analysis that demonstrated a significant probability of failure of conventional restorations compared to selective excavation and Hall crown

technique. Compared to conventional techniques, there were fewer failures with the Hall technique and selective excavation in primary teeth (Schwendicke et al., 2021).

1.10.4. Sealing caries with Hall crowns

The same group also put forward a new approach that was faced with high controversies at first but now tends to be widely used amongst paediatric dentists worldwide, known as Hall Technique. The technique is also based on sealing carious lesions without even excavating infected dentine. It was first introduced by Dr. Norna Hall, a general dental practitioner working in Scotland, who developed and used this technique for over 15 years. The technique was then published in the British Dental Journal in 2006 as a retrospective study. It was then picked up by Dafydd Evans group from the University of Dundee in 2011. The technique is simply achieved by cementing the preformed metal crown over the carious but asymptomatic primary molar, with no local anaesthesia, caries removal, or tooth preparation of any kind. The group conducted a 5-years randomised control trial using the Hall crown technique on primary molars. The techniques showed significantly outperformed the general dentist's standard restorations techniques in the long-term (Innes et al., 2011). Numerous global studies then followed, showing the effectiveness of the Hall crown technique over conventional techniques (Hussein et al., 2020; Elamin et al., 2019; Lloyd, 2020).

1.10.5. Sealing caries fissure or Glass Ionomer (GIC) sealants:

Fissure sealants and GIC have been recently used as an atraumatic restorative technique to seal carious tissues. Most of the fissure sealants used in the literature were used to seal initial (non-cavitated) caries lesions (Griffin et al., 2008). A meta-analysis analysed the progression of caries under fissure sealant on permanent

molars. They found a significant reduction in caries progression between sealed and non-sealed permanent molar with non-cavitated caries lesions (Oong et al., 2008). The bacterial count was also shown to be affected when sealing non-cavitated lesions with-resin based GIC sealants (de Assuncao et al., 2014).

Although minimal interventions are of prime importance, numerous efforts were made in improving the filling material's properties. Current advancements in the dental composite will be discussed in the next section.

1.11. Recent advancements in dental composite materials

1.11.1. Dental resin materials with antibacterial properties

As previously described, composite restorations fail with time due to the gap formation and bacterial stagnation, leading to recurrent (or secondary) caries. This type of failure account for around 50-70% of all dental fillings (Kirsch et al., 2016). To counteract this, various attempts were made to incorporate antibacterial agents within the composite lattice. They can be classified broadly according to antimicrobial filler nanostructures and antimicrobial polymeric/organic fillers, as shown in table 1. 3, (Makvandi et al., 2020). The most relevant antibacterial agents will be further described here.

Table 1.3 Different antibacterial agents.

Antimicrobial filler nanostructures	Antimicrobial polymeric/organic fillers
Silver nanoparticles	Quaternary ammonium polyethyleneimine nanoparticles
Zinc oxide	Chitosan nanoparticles
Graphene nanoparticles	Chlorhexidine (CHX) releasing fillers

Boron nitride	Antimicrobial peptide materials
Modified glass ionomer cement nanofillers	Poly-Lysine (PLS)
Nanodiamonds	Quaternary ammonium methacrylates (QAMs)

1.11.1.1. Silver (Ag) nanoparticles

Silver nanoparticles are considered to be the commonly used metallic nano-compounds due to their potent antibacterial effect (Siddiqi et al., 2018). They hold a broad bactericidal spectrum, affecting both Gram-negative and Gram-positive bacteria. Moreover, various studies have incorporated Ag within the composition of dental composite. Not only that the introduction of Ag nanoparticles provided the composite with antibacterial properties, but studies have shown that it improved its mechanical properties with a significant margin (Fatemeh et al., 2017). Nonetheless, the main drawback of silver nanoparticles is nanotoxicity, this related to the free silver ions release. Another disadvantage of incorporating silver nanoparticles are the corrosive products which may lead to discoloration of the filling material (Park et al., 2010).

1.11.1.2. Graphene nanoparticles

Graphene, a two-dimensional layer exfoliated from graphite, comprises one layer of hexagonally arranged carbon atoms few nanometers thick. The structure of graphene ranges widely from nanotubes, nanoflakes, nanoparticles and nanosheets. Due to this unique configuration, graphene was shown to be 200 times stronger than stainless steel, which provides excellent mechanical properties (Malik et al., 2018). It was also shown that it has an antibacterial property gaining it from the nano-honeycomb structure of the carbon atoms they adopt (Xu et al., 2011).

Many studies have incorporated low molecular weight concentrations of graphene, which demonstrated an improvement in both mechanical and antibacterial properties (Malik et al., 2018). One main disadvantage of graphene is that it is not yet evident in the literature whether or not its cytotoxic, and this raises numerous flags, especially with graphene oxide (Liao et al., 2011). Furthermore, graphene was shown to leave a black colour on the final resin restoration, which ends up providing a restoration with low aesthetics (Nizami et al., 2020).

1.11.1.3. Boron nitride nanoparticles

Boron nitride nanoparticles are nanocrystals that have properties almost identical to graphene; thus, they are usually referred to as the “white graphite”. Although they are almost identical, boron nitride is considered being better. They are transparent, which makes them a better alternative to graphene in terms of aesthetics (Bohns et al., 2019), and they have been shown to have no cytotoxic effect on cell culture (Kivanc et al., 2018). Boron nitride has gained more attention lately by various material scientists, and it was used in resin-based materials to assess its antibacterial, chemical and mechanical properties. Degrazia et al. group incorporated boron nitride nanotubes into the resin-based adhesive with up to 0.15% wt. Their results demonstrated substantial improvements in both mechanical and chemical properties or the restoration (Degrazia et al., 2017).

1.11.1.4. Quaternary ammonium polyethyleneimine (QAPEI) nanoparticles

Besides inorganic nanoparticles, organic polymers have been synthesized to provide antibacterial effects; QAPEI is a great example showing effectiveness against both Gram-positive and Gram-negative bacteria (Yudovin-Farber et al., 2010; Garg et al.,

2019). For instance, one study aimed to compare the antibacterial effect of QAPEI addition. A 1wt% of QAPEI were incorporated with commercially available dental composite material, and one left without QAPEI. The material with 1wt% QAPEI showed a six-fold reduction of both *S. mutans* and *Actinomyces viscous* growth compared to no inhibition to the controlled group (Pietrokovski et al., 2016).

1.11.1.5. Chlorhexidine (CHX)

Chlorhexidine, a frequently prescribed antiplaque component, also has a broad antibacterial spectrum affecting Gram-negative and Gram-positive bacteria. They were found to cause damage to the cellular membrane of the bacterial wall (Boaro et al., 2019). Various studies attempted to assess CHX's effect when mixed within the dental composite matrix. The main issue with CHX is its failure to be immobilized in the polymer lattice, which was shown to affect both the physical and mechanical properties of the resulting dental composite (Chaudhari et al., 2020). Another two main drawbacks of CHX addition is their cytotoxicity and potential discoloration of both the filling and the tooth surface (Addy et al., 1979; Babich et al., 1995; Liu et al., 2018).

1.11.1.6. Poly-Lysine (PLS)

Poly-Lysine (PLS) or ε-Poly-L-lysine (εPLS) is a homo-poly-amino acid characterized by the amide bond between the α-carboxyl and ε-amino groups of L-lysine. It has a broad antibacterial spectrum (Gram-positive and Gram-negative bacteria) and maintained its stability at different acidic conditions and varying temperatures (Hyldgaard et al., 2014). The antibacterial activity of ε-PL was first discovered in Japan around four decades ago, and in the 80's it was approved to be used as a food preservative (Chheda and Vernekar, 2015; Hiraki et al., 2003; Shirling and Gottlieb,

1966). It was then approved by the United States Food and Drugs Administration (USFDA) (US Food and Drug Administration, 2004).

1.11.2. Dental composite materials with re-mineralising properties

As discussed earlier, demineralization is the process of removing mineral ions from the hydroxyapatite crystals of dental hard tissues. The process of restoring these lost minerals back to the hydroxyapatite crystal is referred to as remineralization. The following chemical reaction occurs when hydroxyapatite is in contact with saliva (Dawes, 2003):

$$Ca_{10}(PO_4)_6(OH)_2 \rightleftarrows 10Ca^{2+} + 6PO_4^{3-} + 2OH-$$

The above reaction is a balance between remineralisation (precipitation) and demineralisation (dissolution). Numerous new dental composite materials were developed to promote and regulate tooth remineralisation (Skrtic et al., 2000; Dickens et al., 2003; Langhorst et al., 2009; Xu et al., 2007; Xu et al., 2010; Marovic et al., 2014). These restorations rely on incorporating different components, mainly calcium (Ca) and phosphate (P). Some of these newly developed materials will be discussed here.

1.11.2.1. Dental resins containing calcium phosphate (CaP) filler particles

Dental resins containing calcium phosphate are developed with the aim of remineralising demineralised tooth surfaces. They usually contain calcium phosphate particles with a size range of 1 μm to 55 μm. Some in-vitro studies have shown that the release of calcium and phosphate from these resin materials promoted remineralisation at the interface in dental lesions (Skrtic et al., 2000; Dickens et al., 2003). Another study demonstrated the ability of such restorations to remineralised sound dentine and caries-affected dentine, which was proposed to be used in

minimally invasive restorations (MIR). The main drawback observed in this material was the low mechanical properties observed (Skrtic et al., 2000; Dickens et al., 2003).

1.11.2.2. Barium-glass filler amorphous calcium phosphate (Ba-ACP) containing composites

To counter the low mechanical properties of CaP resins, other studies have introduced barium-glass filler into an amorphous calcium phosphate to improve the filling's mechanical property (Marovic et al., 2014). They have demonstrated an improvement in the load-bearing qualities of the material with enhancement in both flexural strength and elastic modulus (Marovic et al., 2014). A recent study also demonstrated the improvement of flexural strength and modulus of materials containing Ba-ACP, with no adverse effect on ion-release (Marovic et al., 2016).

1.11.2.3. Nanoparticles of amorphous calcium phosphate (NACP) composites

Emerging studies are introducing nanoparticles of amorphous calcium phosphate (NACP) to the resin lattice. The main advantage of incorporating NACP in composite resin is that it has a greater surface area to volume ratio, or around 18 m^2/g, than conventional calcium phosphate particle size of 0.5 m^2/g (Cheng et al., 2015). In return, this was shown to enhance the remineralisation capability of the resin material. However, more studies are still required prior to commercialising such materials (Cheng et al., 2015).

The main disadvantage of resins containing calcium phosphate is the duration at which CaP is released, which usually lasts for a few weeks, then diminishes (Weir et al., 2012). Hence, efforts are being made to develop resin materials that are "rechargeable",; meaning that the materials act as a hub for Ca and P ions that can be exchanged indefinitely. One recent study used different resin matrices to test the ability to recharge dental composites (Zhang et al., 2016). These included bisphenol A

glycidyl dimethacrylate (BisGMA) and 48riethylene glycol dimethacrylate (TEGDMA) (BT group), pyromellitic glycerol dimethacrylate (PMGDM) and ethoxylated bisphenol A dimethacrylate (EBPADMA) (PE group) and BisGMA, TEGDMA, and Bis[2-(methacryloyloxy)ethyl] phosphate (BisMEP) (BTM group). Each composite lattice contained 20% NACP and 50% glass particles. Ca and P ion release, and Ca and P ion recharge and re-release was then tested. The PE group showed the highest CaP ion rechargeability, followed by the BTM group then the BT group, with the BT group being the lowest. For every recharge cycle, the ion re-release reached comparable high levels, demonstrating that the ion re-release did not decrease with increasing the number of recharge cycles (Zhang et al., 2016).

1.12. Novel material formulation at UCL Eastman

Two novel agents were added to a conventional composite filler of the new experimental formulation: Poly-Lysine (PLS) and Monocalcium phosphate monohydrate (MCPM). The incorporation of PLS in dental composite resin was first developed at University College London (UCL), Eastman Dental Institute (EDH), Biomaterials and Tissue Engineering department, and was patent protected (WO2015015212A1). The formulation has been licensed to Schottlander™ dental company, which registered a trademark name for the optimized formulation calling it '*Renewal MI*™'. More information on manufacturing, trademarking, and external testing will be discussed in chapter 6.

Both PLS and MCP were mixed with adhesion and etching promoting monomer (4META) and dissolved in a low shrinkage liquid dimethacrylate phase. The monomers utilised in the liquid phase were UDMA (bulk-monomer) and a novel low shrinkage

diluent monomer (Polypropylene glycol dimethacrylate, PPGDMA) with CQ as an initiator.

As PLS was shown to act as an antibacterial molecule, the purpose of adding it to the lattice is to kill residual bacteria at the substrate/restoration interface. The antibacterial effect was demonstrated in our lab before (Lygidakis et al., 2020; Yaghmoor et al., 2020). In regard to the MCP addition, the aim is to initiate the remineralisation process, i.e. to remineralise the demineralised collagen fibrils. This was also investigated previously in our lab (Panpisut et al., 2016). These processes will compensate for any micro-gap formation and close the interfacial gap, providing improved surface adhesion. Moreover, this will significantly reduce the procedural steps and make the material a minimally invasive material suitable for treating children's teeth.

Previous results that led to the development of these formulations have used many different compositions. The formulations were, however, always prepared by mixing two phases; liquid and powder. The powder phase contained glass fillers (powder and fibres), antibacterial agent (either Chlorhexidine or PolyLysine up to 10%wt) and remineralising agent (MCP or TCP or both). The liquid phase previously contained different combinations of:

- UDMA,
- TEGDMA or PPGDMA (diluent),
- 4META, HEMA or Pyromellitic Dimethacrylate (PMDM) (adhesion-promoting monomer),
- CQ (initiator),
- and N, N-dimethyl-p-toluidine (DMPT) or Na-N-tolyglycine glycidyl methacrylate (NTGGMA) (activator).

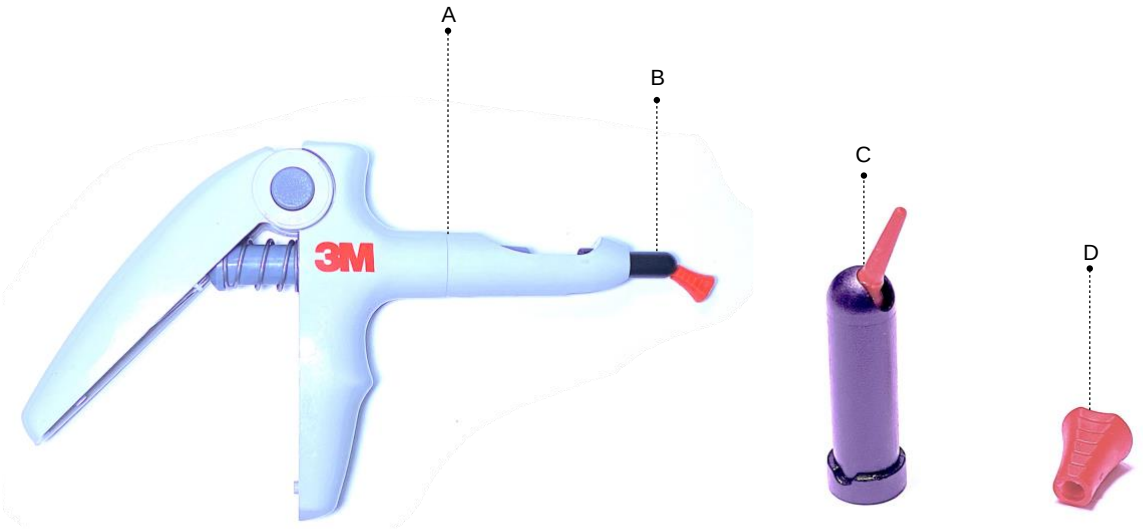

Figure 1.11 Renewal MI **composite dispensing gun and compules.** A, The dispensing (applicator) gun. B, The packaging compules. C, The adjustable angulation tip. D, The compules cap.

A trademark was registered for the novel optimised formulation by Schottlander™ company (ISO 13485), calling it '*Renewal MI*™'. The optimised formulation will be referred to as 'Renewal MI' from this point forward.

1.13. Project aims

The broad aim of the later in-vitro experiments was to formulate a novel dental composite material ideal for the use in children. The specific aims are to:

- Test the ability of the experimental formulation to form resin-tags to carious primary teeth **(Chapter 3).**
- Assess the ability of the experimental formulation to inhibit matrix metalloproteinase (MMP) activity **(Chapter 4).**
- Test the ability of the experimental formulation to limit microleakage at the restoration-substrate interface **(Chapter 5).**

- Conduct a systematic review to collect and analyse available evidence on the influence of different MMP inhibitors, as dentine surface pretreatment, on the immediate and long-term bond strength of direct coronal composite restorations **(Chapter 6).**

2. CHAPTER TWO: Overview of materials and methods

2.1. Introduction

This chapter summarises the materials and methods used in the experiments of this study. In this chapter the following will be described:

- teeth collection and the ethical approval obtained
- teeth storage medium and its preparation
- teeth preparation
- the chemical used in the optimized formulation
- the commercial filling materials used
- the armamentarium used for the restorative work
- the imaging and equipment used for visualizing the samples

The details of the methods of materials in this chapter include those frequently utilised in each experiment. Any variation from these methods will be discussed further in each chapter.

2.2. Materials and methods

2.2.1. Teeth collection

All human teeth collected (primary and permanent) were obtained following the ethical approval by the University College London (UCL) Eastman Dental Institute's (EDI) Standard Operating Procedure (SOP) Biobank Human Tissue Authority (HTA), with project licence number ***12277***.

2.2.2. Teeth storage

All human teeth were used within 6 months of their extraction date. After extraction, teeth were cleaned and disinfected by storing in Chloramine T 1% for 2 days. All samples were then stored in deionised water (dH_2O) and kept in the fridge until they are used. When any of the samples are used or prepared, they are then left at room temperature.

2.2.3. Teeth preparation:

For resin-tag experiments, soft infected-carious on primary molars was excavated with a spoon excavator. Cavities were them with the optimised formulation and the commercial filling materials, sectioned and scanned. Composition of the optamised formulation and the restorations and chemical used for the commercial materials are shown in table 3.1 and table 3.2. The armamentarium for the restorative work is shown in figure 3.1. As for the MMP experiment, collected sound human teeth were sectioned to get a 2mm thick dentine discs. The Green fluorescent solution kit used are shown in figure 3.2 and the experiment is discussed further in chapter 5. These discs were immersed in an acid solution, restored, sectioned and then visualised. Teeth were sectioned using Accutom-5 by Struers^{TM} device.

Table 3.1 Chemical used in the optimized formulation 'Renewal MI'.

Composition/role	Company and Lot #
Urethane dimethacrylate UDMA: Basic monomer	DMG 100112/97406
Poly(propylene glycol) dimethacrylate PPGDMA 400: Diluent monomer	Polysciences 626208
4-methacryloyloxyethy trimellitate anhydride (4META): Adhesive monomer	Polysciences 697058
Camphorquinone (CQ): Initiator	DMG 100134/90339
Glass particles 7micron: Filler	DMG 020684/680326
Glass particles 0.7micron: Filler	DMG 02110/688344
Fumed silica: Filler	Aerosil OX50, Evonik industries, Germany 153022145
Monocalcium phosphate monohydrate (MCP): Remineralising agent	Himed, NY, USA. MCP-369925
PolyLysine (PLS): Antibacterial agent	Handary, Belgium. Epolyly, 020120160203

Table 3.2 Commercial restorations and conditioning materials

Commercial materials	Description	Manufacture	Composition
Filtek Z250	Aesthetic, light-cured, radiopaque composite	3M ESPE ™	BIS-GMA and a blend of UDMA and Bis-GMA. The particle size distribution is 0.01µm to 3.5µm with an average particle size of 0.6µm a combination of silica filler, zirconia filler, and zirconia/silica cluster filler
ACTIVA Kids	Bioactive restorative material (RMGI)	Pulpdent ™	Blend of diurethane and other methacrylates with modified polyacrylic acid, Silica and Sodium fluoride
GIC Fuji IX	Fluoride releasing glass ionomer	GC ™	- Powder: liquid ratio 3.6/1 - Liquid: Distilled water 50%, Polyacrylic acid 40%, Polycarboxylic acid - Powder: Alumino-fluoro-silicate glass, Polyacrylic acid powder
OptiBond^{M} Solo^{TM} Plus	Adhesive agent	Kerr^{TM}	Ethanol; ethyl alcohol, 2-hydroxyethyl methacrylate, Alkali fluorosilicate
Super etch	Acid-etchant gel	SDI ™	37% Phosphoric acid
Ortho Gel Conditioner	Conditioner (primer)	GC ™	Polyacrylic acid 20%

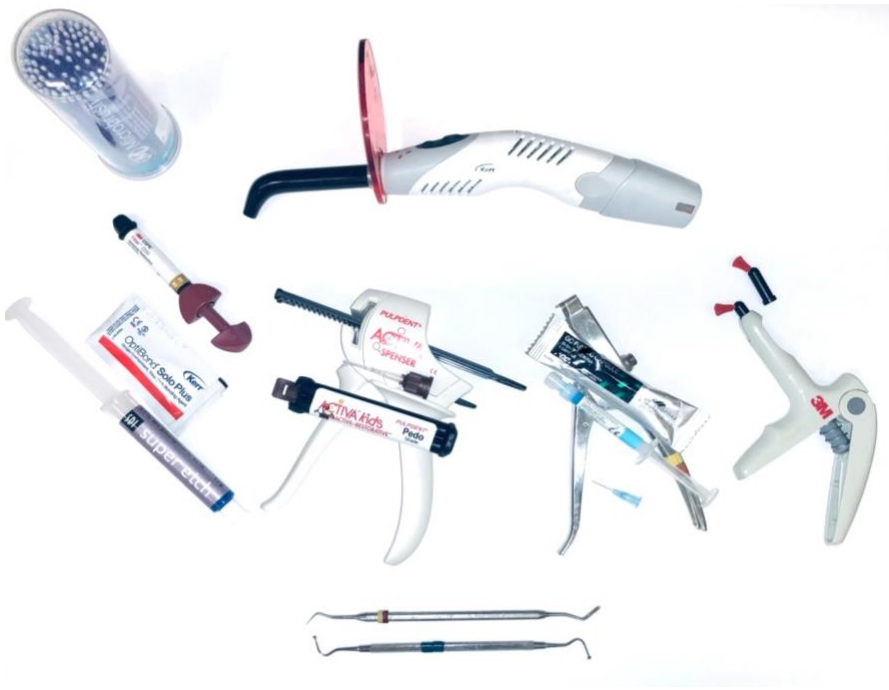

Figure 3.1 Dental armamentarium used. Microbrush pack.DeMITM Light-cure by KerrTM. Optimised formulation composite in black complues attached to 3M gun-dispenser. GIC Fuji packet by GCTM with GIC applicator and conditioning gel (primer) syringe and needle tip. ACTIVA Kids BioACTIVETM restorative syringe with re-fill opaque white shade by PulpDentTM. Filtek Z250 composite by 3M ESPETM. OptiBondTM SoloPlusTM bond agent pack by KerrTM. Acid-etch 37% phosphoric acid super etchTM syringe by SDITM. Metal flat plastic octagonal instrument LustraTM and medium-sized metallic octagonal spoon excavator LustraTM, by DentsplyTM.

Figure 3.2 Green fluorescent solution kit from Invitrogen™ by Thermo Fisher Scientific™.DQ™Collagen type 1 fluorescein conjugate (blue-cap tube). Collagenase from *Clostridium histolyticum* (MMP-1) (yellow-cap tube). 1,10-Phenanthroline monohydrate is a general metalloproteinase inhibitor (red-cap tube). 10C Reaction buffer 2mM (white bottle).

2.2.4. Imaging

Two imaging systems used in this research. The first confocal light scanning microscopy (CLSM) by BioRad Radiance2100, Zeiss™, Welwyn Garden City, Herts, UK. The second was optical coherence tomography (OCT) VivoSight™ OCT scanner (Michelson Diagnostics, Kent, United Kingdom). All were used with their designated software for documentation of results.

3. CHAPTER THREE: Resin tag formation on carious dentine

3.1. Abstract

Aims: The aim of this part of the study is to test the ability of the final formulation (Renewal MI) to form resin-tags on affected-dentine of primary teeth and comparing it with commercially available restorative materials.

Materials and methods: Carious primary molars were obtained following ethical approval. The final formulation, along with commercially available restorations Renewal MI (Schottlander^{TM}), 3M ESPE Filtek Z250 with OptiBond Solo Plus adhesive, ACTIVA^{TM} KIDS BioACTIVE compomer (PulpDent^{TM}) and GC Fuji IX, GIC, were applied on affected-carious dentine following the manufacturer's instructions. Samples (n=5 per material and time point) were cross-sectioned and visualised under CLSM to evaluate the resin-tags formation.

Results: In comparison to all the commercial materials, only Renewal MI formed resin-tags within caries affected dentine.

Conclusion: Although the final formulation is applied via a single step, yet it exhibited high adaptability with no gap formation. Moreover, when compared to other commercial materials, it showed a high penetrative ability to affected-carious dentine (>200 µm long).

3.2. Introduction

As discussed in chapter one, adhesive dentistry has come a long way in improving and enhancing the efficiency and effectiveness of dental restorative materials. However, the simplicity of the application is yet to be achieved. The main issues with current resin materials lie in components within their composition. For instance, many

adhesives contain HEMA or Bis-GMA. Their main drawbacks are cytotoxicity, shrinkage and hydrophobicity. This eventually leads to phase separation and failure of adhesion at the interface.

The aim of the optimised formulation is mainly to improve adhesion and reduce application time for it to be suitable in restoring primary teeth. As previously mentioned, the optimised formulation contains 4META (acidic monomer) and PLS and MCP (hydrophilic components), both of which should aid in bonding without the use of acid-etch and bond.

In this chapter, the adhesion will be tested via evaluating the resin-tags formation at the interface between the restoration (the optimised formulation and commercial filling materials) and carious-affected dentine. The ability of the optimised formulation to form resin-tags to carious dentine will prove that it will be a more straightforward and efficient material to be used in restoring children's teeth.

3.3. Hypothesis

The null hypothesis is that: there are no differences between the proposed experimental formulation and the commercially available restorations (Z250, ACTIVA, GIC Fuji IX with and without primer) in terms of forming resin-tags in primary carious teeth.

3.4. Aims and objectives

The tests in this chapter aimed to evaluate the ability of the optimised formulations to adhere to tooth structure by assessing their ability to form tags within carious dentine.

3.5. Materials and methods

3.5.1. Tags formation

Carious primary molars collected were obtained following the ethical approval by UCL EDI SOP (Biobank HTA licence number 12277). Teeth were excavated using a small spoon excavator to excavate all soft dentine (infected dentine), leaving only light brown/yellow affected-dentine. All molars were then restored with the following materials: Renewal MI (Schottlander^{TM}), 3M ESPE Filtek Z250 with OptiBond Solo Plus adhesive, ACTIVA^{TM} KIDS BioACTIVE compomer (PulpDent^{TM}) and GC Fuji IX, GIC according to the manufacturer's instructions. These commercial restorative materials were chosen, as they are frequently used in paediatric dentistry to restore carious primary teeth. All samples (n = 5 per material and time point) were then sectioned to expose the interface between the filling materials and dentine. Rhodamine B dye was then applied to the interface and left to soak for 2 minutes. Excess dye was then rinsed with a copious amount of deionised for 1 minute. When samples are not being scanned, they are stored in deionised water at room temperature. Samples were then visualised under confocal light scanning microscopy (CLSM) using an oil objective lens (X60), under red fluorescent light. The laser microscope settings for Rhodamine B dye were 568 nm excitation and emission through a 600 – 630 nm filter. The Z motor was used to scan different levels at the interface, and the saved scans were mounted as Z project with the aid of the ImageJ software package. Three-dimensional projects were also created to aid visualisation (Fig. 4.1).

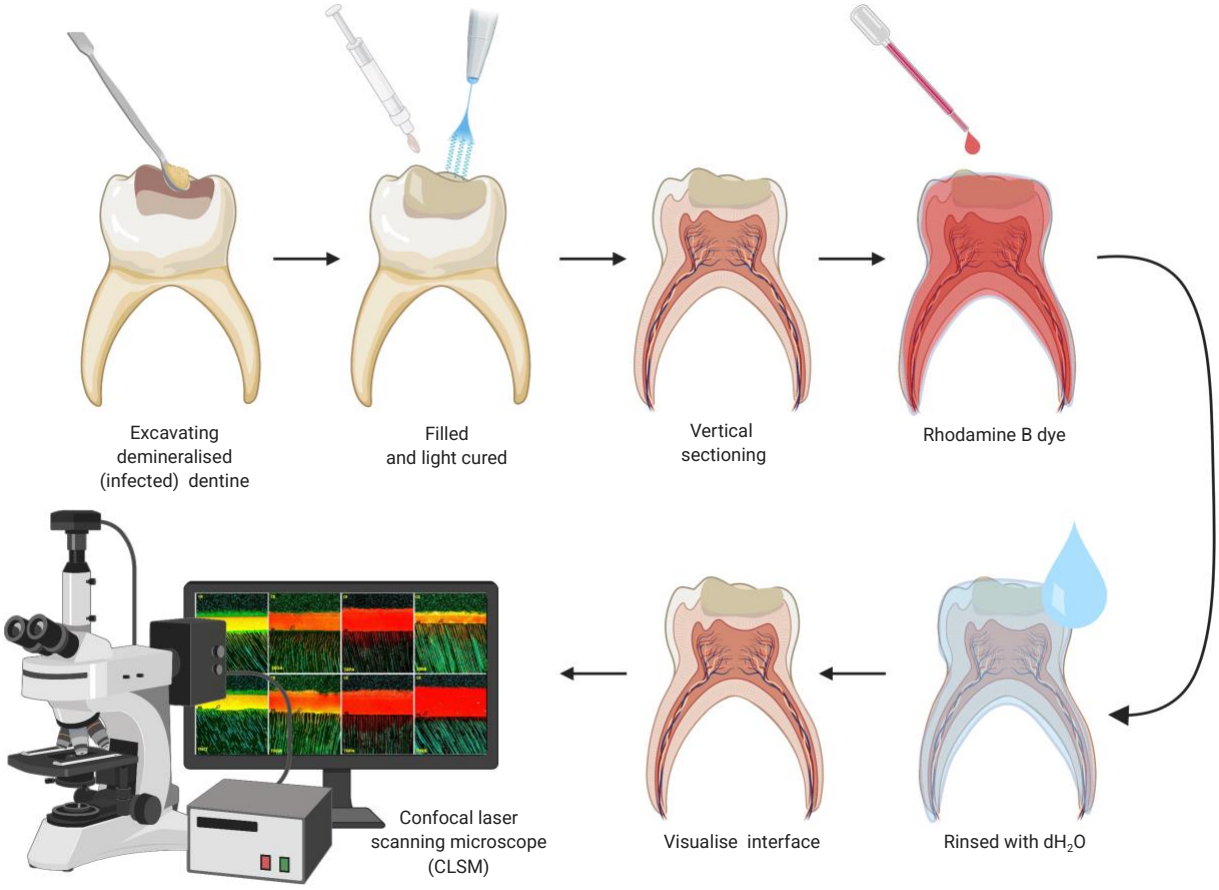

Figure 4.1 Schematic representation of resin tags experiment protocol steps.

3.6. Results

3.6.1. Resin-tags formation with caries-affected dentine

CLSM was utilized to evaluate the ability of the restorations to infiltrate dentinal tubules and form resin-tags on carious-affected dentine of primary molars.

Restorative materials used are Renewal MI, Z250 with bond (OptiBond solo), ACTIVA, GIC Fuji IX and GIC Fuji IX (with primer) as per the manufacturer's instructions. Higher penetrative ability to affected-dentine was demonstrated by Renewal MI in comparison to all other commercial materials. Under CLSM, Renewal MI demonstrated resin-tags

that are >200μm in length. All commercial materials (Z250 with bond, ACTIVA and GIC Fuji IX) showed no evidence of resin tags at the interface (Figure 4.2). GIC Fuji IX (with primer) showed some evidance resin-tag infiltrating dentinal tubules, however it was not consistent in all surfaces.

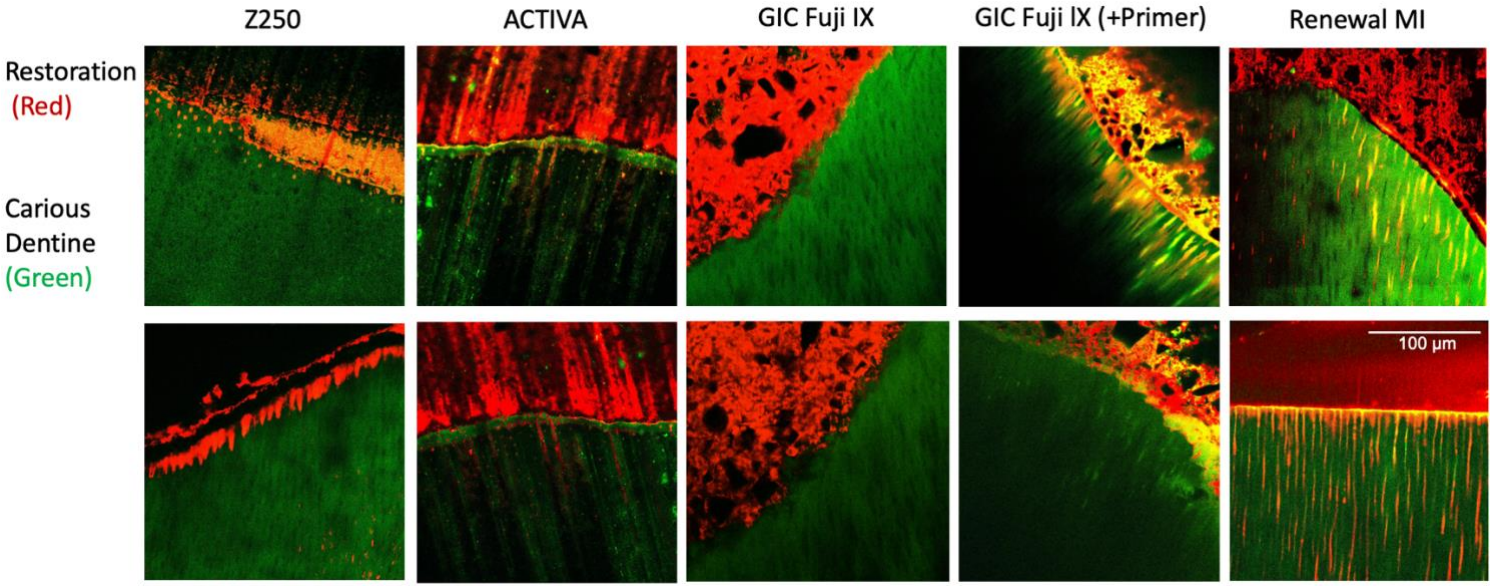

Figure 4.2 Representative confocal scanning light microscopic (CLSM) images of resin tags formation.

3.7. Discussion

Current restorative materials require numerous surface treatment steps on the substrate prior to its application. These pre-surface treatments include the application of acid-etch, primers and adhesive bonding systems. As discussed earlier, the smear layer created on the substrate surface occluded the dentinal tubules. Acid-etch, usually 37% phosphoric acid gel, is used to remove the smear layer and creates micro-porosities allowing for better adhesion. The problem with acid-etch on dentine, is that it exposes the dentinal tubules and demineralize its collagen fibrils. Unlike sound dentine, carious-affected dentine is partially demineralized and the use of acid-etch causes more demineralization.

Moreover, the smear layer of carious-affected dentine is different from that of sound dentine, as collagen is more disorganized with trapped minerals (Spencer et al., 2001). This was shown to be challenging to remove and was shown to hinder adhesion when etch-and-rinse adhesives were used (Oliveira et al., 2003). As mentioned earlier, most primers and adhesive systems contain HEMA or Bis-GMA. The main issues with these components are that they are hydrophobic, which hinders adhesion by causing phase separation, failure of dentinal tubules infiltration, water sorption and eventually causes recurrent caries and bond failure.

As shown in the results, the optimized formulation demonstrated the ability to infiltrate into dentinal tubules of carious-affected dentine. On the contrary, all commercial filling materials (Z250, ACTIVA, GIC Fuji lX with and without primer) failed to penetrate carious-affected dentine layer. The optimized formulation not only penetrated the dentinal tubules, but also formed resin-tags that are >200μm in length. This is related to the presence of PLS and MCP in the formulation lattice. Both components are hydrophilic, aiding in the infiltration of resin tags through the dentinal tubules. Furthermore, this might be facilitated via interaction between the negatively charged non-collagenous proteins within the dentinal tubules (mainly phosphophoryn and chondroitin sulphate) and positively charged PLS, aiding in the attraction of PLS into the tubules.

The ability of an adhesive resin material to infiltrate and adhere to carious-affected dentine is of prime importance, especially in the field of paediatric dentistry. The optimized formulation showed to do precisely that with a single-step application. This will reduce the procedure time as it will not require the use of any of the rigorous steps used in current commercial restorative materials and will be children friendly.

3.8. Conclusion

The proposed experimental formulation showed differences in terms of forming resin-tags in primary carious teeth in comparison to the commercially available restorations.

4. CHAPTER FOUR: Inhibition of matrix metalloproteinase (MMP) activity

4.1. Abstract

Aim: This study quantifies matrix metalloproteinase (MMP) activity at the surface of demineralised dentine following sealing by the optimised formulation (Renewal MI) composite versus commercially available restorative materials.

Methods: 2mm thick sections of coronal dentine from sound human molars, obtained following ethical approval, were fully demineralised through immersion in 4M formic acid for 48 hours. Following the application of a green fluorescent probe (EnzCheck Collagenase Assay Kit) for 5 minutes, restorative materials were applied on one surface. Materials included Renewal MI (Schottlander^{TM}), 3M ESPE Filtek Z250 with OptiBond Solo Plus adhesive, ACTIVA^{TM} KIDS Bioactive compomer (PulpDent^{TM}) and GC Fuji IX, GIC according to the manufacturer's instructions. These commercial restorative materials were chosen, as they are frequently used in paediatric dentistry to restore carious primary teeth. Non-restored dentine was used as a control. Samples were stored in deionised water and incubated at 37^{o}C. Following 1 or 14 days, samples (n=5 per material and time point) were sectioned, and the interface area imaged using Confocal Light Scanning Microscopy (CLSM). The percentage area of green fluorescence in sections 260*260 micron squared MMP activity was determined through Image J.

Results: Example CLSM images are shown in Figure 1, and the percentage areas that are green. Renewal MI restoration had the least fluorescence initially (0.5%), which after 14 days almost totally disappeared. Z250 and ACTIVA's results were similar after incubation at day 1 (2.5%-2.0%) and day 14 (2.0% 1.8%) respectively. MMP activity of GIC (Fuji lX) was lower than Z250 and ACTIVA on day 1; however, it was higher on day 14, reaching 3.5%.

Conclusion: The optimised formulation demonstrated a substantial reduction of MMP activity at the surface of demineralised dentine when compared to other commercial filling materials.

4.2. Introduction

Since the development of adhesive restorative materials around six decades ago, a plethora of advancements followed to improve them (Buonocore, 1955; Van Meerbeek et al., 2020). Despite these advancements, it is well documented that adhesive restorations lose their bond strength with time, leading to their inevitable failure (De Munck et al., 2005a; Mjor et al., 2000). Adhesive restorations rely on the bond between them and the tooth substrate. The interface between them, the hybrid layer, is crucial in determining the longevity and the stability of the bond (Breschi et al., 2018; Nakabayashi et al., 1991). In dentine, collagen fibrils, mainly made of collagen type-I, are the key to establishing a strong bond. The main reason behind bond failure to dentine has mainly been attributed to the hybrid layer degradation and the deterioration of the dentine collagen fibrils (Frassetto et al., 2016).

Recently, the focus was aimed towards endogenous enzymes present within the dentine extracellular matrix (ECM), and their effect on bond stability. Among these enzymes are matrix metalloproteinases (MMPs), a well-studied endogenous enzyme. MMPs are a group of calcium- and zinc-dependent host-derived enzymes (Visse and Nagase, 2003). They are divided into six subgroups: collagenases (MMP-1 and MMP-8), stromelysins (MMP-3, MMP-10, MMP-11 and MMP-20), gelatinases or type-IV collagenases (MMP-2 and MMP-9), matrilysin (MMP-7), metalloelastase (MMP-12) and membrane-type metalloproteinases (MMP-14, MMP-15, MMP-16 and MMP-17) (Nagase et al., 2006).

To date, four types of MMP were identified within the dentine extracellular matrix. These are MMP-2, -8, -9 and -20, with MMP-2 and MMP-9 being the most abundant (Sulkala et al., 2007; Mazzoni et al., 2007). During odontogenesis, these enzymes are secreted via the odontoblasts are remains silenced and inactive within the dentine extracellular matrix (Tjaderhane et al., 2001). They become active when they are in an acidic environment, either by biological acids produced by caries (Chaussain-Miller et al., 2006), or by introduced acids during acid etching (Apolonio et al., 2017; DeVito-Moraes et al., 2016). When activated, they start degrading the unprotected and exposed collagen fibrils within the dentine (Pashley et al., 2004), which is why inhibiting the MMP could be useful in the preservation of the hybrid layer and, eventually, the bond stability.

Several types of MMP inhibitors were studied, such as benzalkonium chloride (Sabatini and Pashley, 2015; Sabatini and Patel, 2013), Chlorhexidine (Sabatini and Patel, 2013; Breschi et al., 2010b; Kim et al., 2010; Lenzi et al., 2012), ethylenediaminetetracetic acid (EDTA) during adhesive application (Osorio et al., 2005), galardin (Breschi et al., 2010a), Doxycycline (de Carvalho et al., 2020), the active component of green tea (catechins) and green tea content epigallocatechin gallate (EGCG) content (Barbosa et al., 2011; Czech et al., 2019), polyamidoamine dendrimers (PAMAM) (Wu et al., 2019) and zinc methacrylate (Henn et al., 2012) to name a few.

4.3. Hypothesis

The null hypothesis states that:

The optimised formulation cannot inhibit the MMP activity at the interface (material/tooth substrate), when compared to other commercial filling materials.

4.4. Aims and objectives

This study quantifies MMP activity at the surface of demineralised dentine following sealing by the optimised formulation versus commercially available restorative materials.

4.5. Materials and methods

4.5.1. Inhibiting MMP activity using demineralised dentine discs

2 mm thick sections of coronal dentine from sound human primary molars, obtained following ethical approval by UCL EDI SOP (Biobank HTA licence number 12277), were fully demineralised through 4M formic acid immersion for 48hrs. 20µL of the green fluorescent probe (EnzCheck Collagenase Assay Kit) applied on one surface of the dentine discs and blot dried and allowed to soak for 5 minutes. The preparation of the green fluorescent probe is discussed later. Restorative materials, 1mm thick, were applied on the same surface where the probe is applied. Restorative materials included the optimised formulation Renewal MI (Schottlander™), 3M ESPE Filtek Z250, ACTIVA™ KIDS BioACTIVE (Pulpdent™) compomer (+OptiBond Solo Plus™ adhesive) and GIC Fuji IX, according to the manufacturer's instructions. Non-restored dentine was used as a control. Samples were stored in deionised water and incubated at 37^{o}C. Following 1 or 14 days, samples (n=5) were sectioned, and the interface area was imaged using Confocal Light Scanning Microscopy (CLSM). The percentage area of green fluorescence in sections 260x260µm² MMP activity was determined through ImageJ (Fig. 5.1).

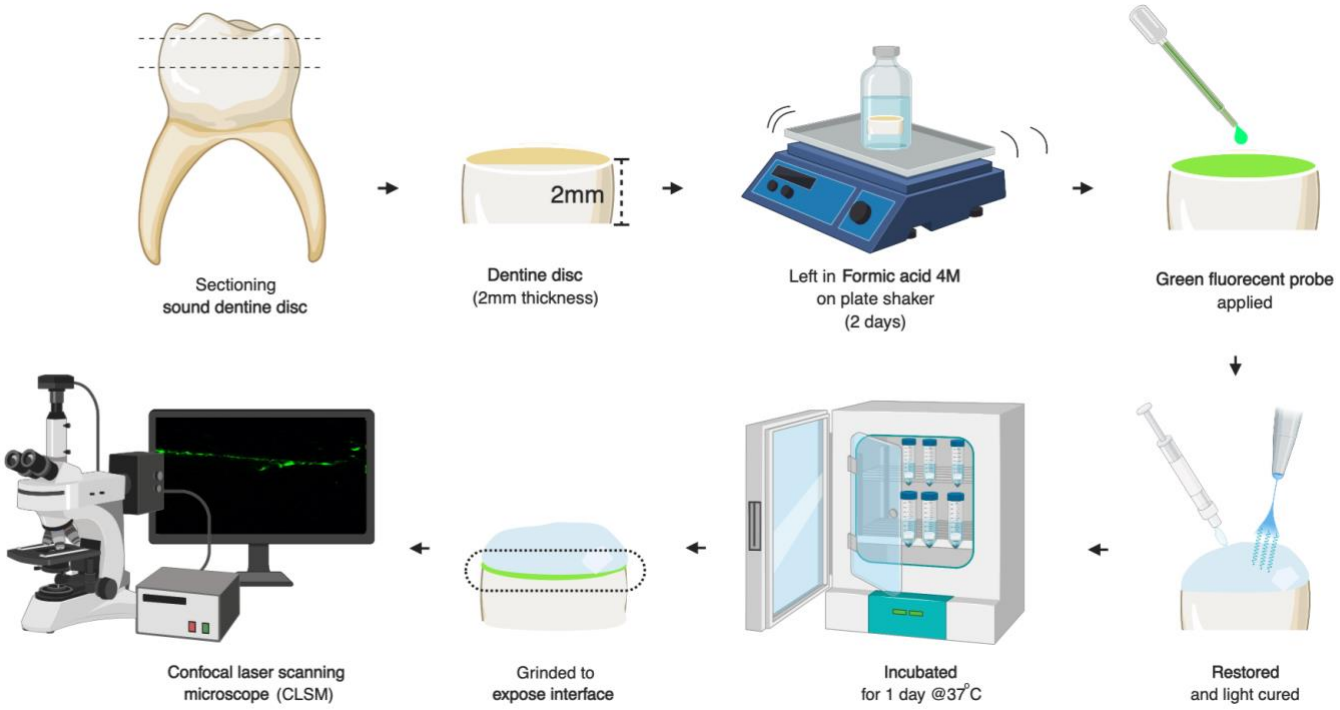

Figure 5.1 Schematic representation of the MMP activity and remineralisation experimental protocol steps.

4.5.2. Preparation of green fluorescence probe solutions

Following a Molecular Probe protocol (EnzCheck Gelatinase/Collagenase Assay Kit, Molecular Probe (figure 3.2)), solutions where prepared as follows:

1. Gelatin substrate from pigskin: This substrate is conjugated with fluorescein, which releases fluorescence when broken down. The vial contents (1mg) were dissolved in 1mL of deionised water (concentration is 1mg/mL) and agitated in an ultrasonic water bath for 5 minutes to facilitate mixing.
2. Collagen substrate: It is a highly quenched type I collagen that is heavily labelled with fluorescein and has a more complicated structure in comparison to gelatin. When broken down by enzyme activity, it releases fluorescent peptides. The solution was prepared as with the Gelatin substrate.

3. Reaction Buffer (10X): contains 50mL 0f 0.5M Tris-HCL, 1.5M NaCl, 50mM of $CaCl_2$ and 2mM of sodium azide, prepared to a pH of 7.6. It was diluted by a factor of 10 by adding 2mL of the stock solution to 18mL of deionised water (1X reaction buffer).

4. Collagenase from *Clostridium histolyticum* (MMP-1): collagenase is more selective for collagen type-I (which is the abundant organic component of dentine). The vial contains 0.5mL (1000 units per mL). The stock solution was diluted to 0.2 unit per mL by adding 0.2µL of it to 1000µL of 1X reaction buffer.

5. 1,10-Phenanthroline monohydrate is a general metalloproteinase inhibitor. Two concentrations were prepared by adding 9.9mg to 25µL of ethanol then 10mM solution was prepared by adding 10µL of this solution to 2mL of 1X reaction buffer. This solution was then serially diluted to two concentrations (0.2mM and 1mM).

6. In addition, different concentrations of PLS were prepared (0.1, 1, 10, 100, 250, 500, 750, 1000mM).

4.6. Results

4.6.1. Matrix metalloproteinase (MMP) activity at the interface between demineralised dentine discs and different restorative materials

Representative CLSM images are shown in Figure 5.2, and the percentage areas is represented in Figure 2.3. Renewal MI restoration had the least fluorescence at day 1 (0.5%). After 14 days, the MMP activity decreased to 0.1%. Z250 and ACTIVA's results were both similar after incubation at day 1 (2.5%-2.0%). After 14 days of incubation, slight inhibition to the MMP activity was observed (2.0% 1.8%). On the other end, MMP activity of GIC (Fuji lX) was lower than Z250 and ACTIVA on day 1; nevertheless, it increased on day 14 reaching 3.5%.

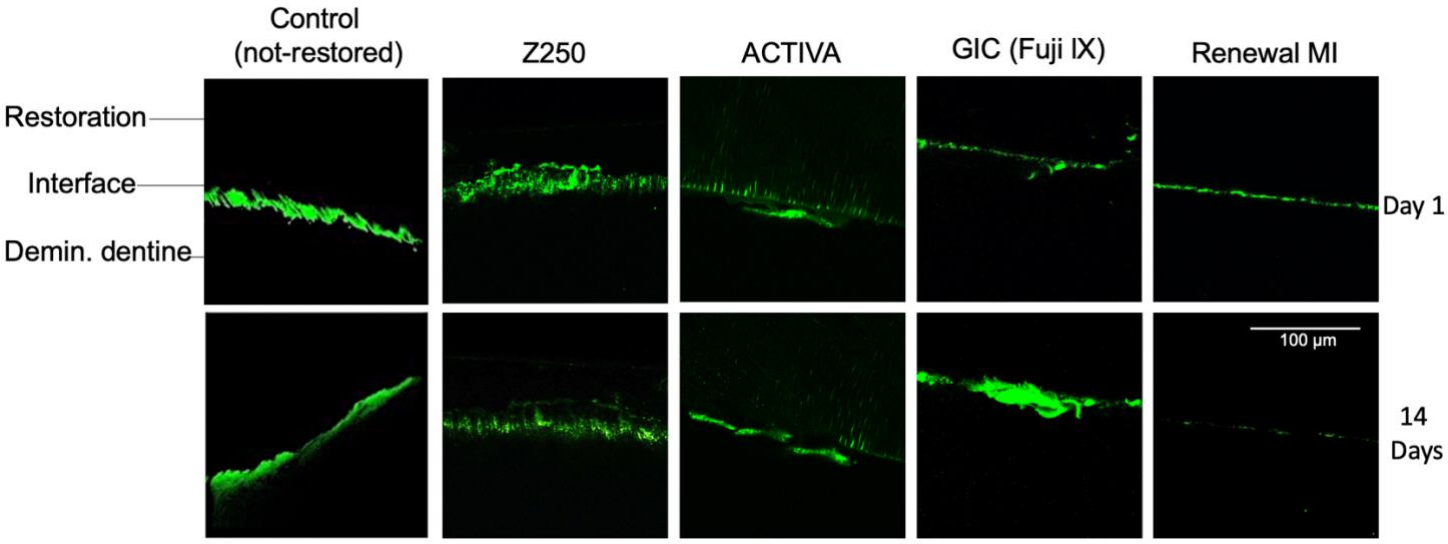

Figure 5.2 Confocal Light Scanning Microscopy (CLSM) images of the matrix metalloproteinase enzyme (MMP) activity. Each image show restoration on the top (black), MMP at the interface in the middle (green) and demineralized dentine disc on the bottom (black). All samples showed MMP activity at day 1 (top row) with varying degrees. Renewal MI, on the other hand, showed a substantial reduction of MMP activity on day 14 (bottom row) in comparison to the rest of the commercial restorative materials.

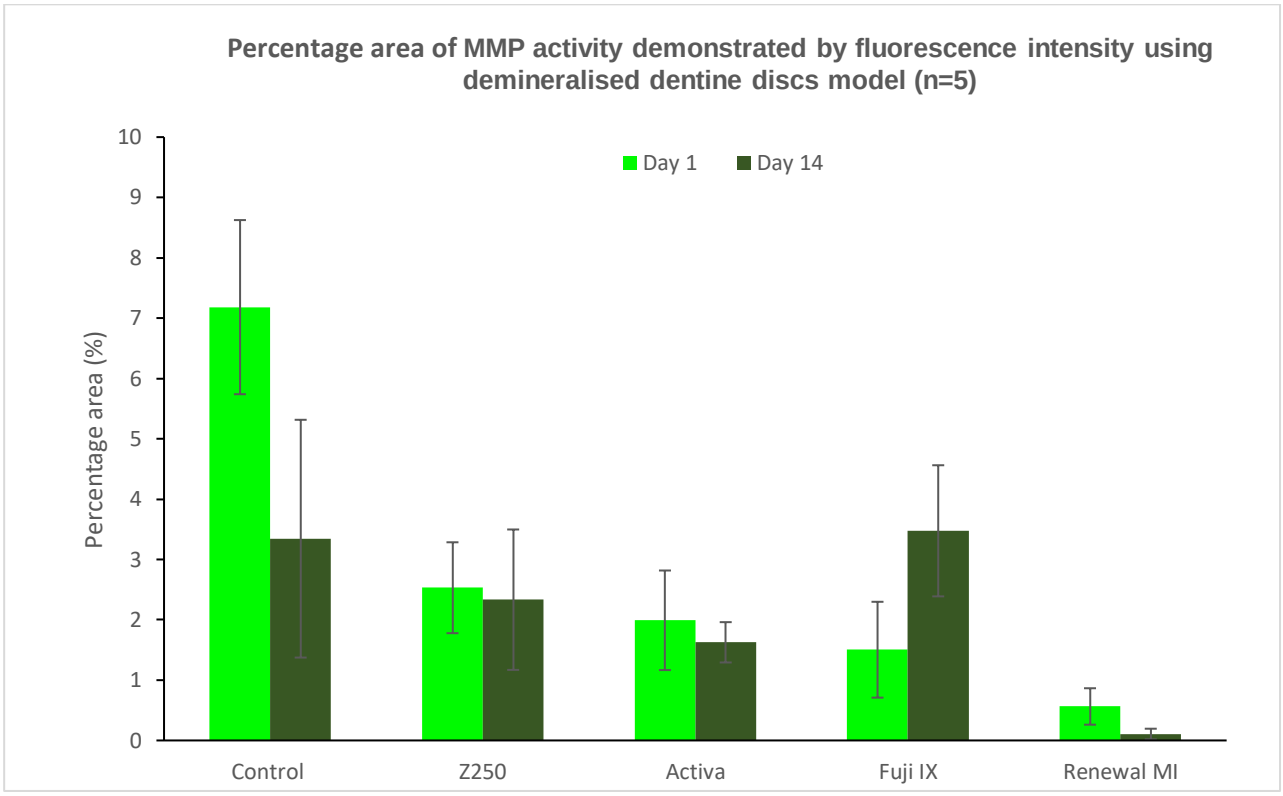

Figure 5.3 Bar chart demonstrating the MMP activity. Renewal MI restoration had the least fluorescence initially (0.5%), which after 14 days almost totally disappeared. Z250 and ACTIVA's results were similar after incubation at day 1 (2.5%-2.0%) and day 14 (2.0% 1.8%) respectively. MMP activity of GIC (Fuji IX) was lower than Z250 and ACTIVA on day 1; however, it was higher at day 14, reaching 3.5%.

4.7. Discussion

4.7.1. MMP activity inhibition

The main reason behind bond failure to dentine has mainly been attributed to the degradation of the hybrid layer and the deterioration of the dentine collagen fibrils (Frassetto et al., 2016). To date, four types of MMP were identified within the dentine extracellular matrix. These are MMP-2, -8, -9 and -20, with MMP-2 and MMP-9 being the most abundant (Sulkala et al., 2007; Mazzoni et al., 2007). During odontogenesis, these enzymes are secreted via the odontoblasts are remains silenced and inactive within the dentine extracellular matrix (Tjaderhane et al., 2001). As mentioned earlier, they become active when they are in an acidic environment, either by biological acids produced by caries (Chaussain-Miller et al., 2006), or by introduced acids during acid etching (Apolonio et al., 2017; DeVito-Moraes et al., 2016). When activated, they start degrading the unprotected and exposed collagen fibrils within the dentine (Pashley et al., 2004), which is why inhibiting the MMP could be useful in the preservation of the hybrid layer and, ultimately, the bond stability. As previously stated, MMP can be inhibited in different ways, such as the use of MMP inhibitors, achieving a hermetic seal by the restoration or by the use of remineralising agents (Jun et al., 2018).

In the optimised formulation, three key elements seem to play a part in inhibiting MMP activities, PLS, MCP and the seal achieved by the restoration. As demonstrated from the confocal images, the optimised formulation inhibited MMP at day 1, which was followed by a reduction of MMP after 14 days of incubation. This could be related to both the PLS and the MCP action. PLS was aiding in providing a hermetic seal via forming long resin-tags as shown in the previous chapter, and MCP in remineralising

the demineralised tissues. Therefore, the combined action of both PLS and MCP would be the main components in inhibiting MMP activity.

On the contrary, the other commercial filling materials showed a meagre reduction of MMP activity. Z250 and ACTIVA's results were somewhat similar, with high MMP activity initially at day 1 followed by a slight reduction after 14 days of incubation. It is worth noting that ACTIVA restorations were repeated a couple of times as they were detached from the dentine disc during application. ACTIVA was initially branded as a self-adhesive RMGIC; nonetheless, after demonstrating unsatisfactory outcomes, the manufacturer now recommends the use of an adhesive prior to its application (Pulpdent, 2018). Although an adhesive was applied, yet they still detached from the dentine discs in numerous attempts.

As described earlier, MMP becomes activated with acids (acid-etch or bacterial acids), followed by degradation of the collagen matrix. It was demonstrated that MMP has the ability to degrade all extracellular matrix at a slow but prolonged pace; this can also explain why some restorative materials fail with time. Therefore, although the seal is crucial for any restorative material, the incorporation of remineralising components is as important. Hence, the optimized formulation was shown to provide both properties, sealability and remineralising ability, which both shown to inhibited the MMP activity.

4.8. Conclusion

The optimised formulation inhibited the MMP activity at the interface (material/tooth substrate) with time in comparison to other commercial restorative materials.

5. CHAPTER FIVE: Optical quantification of microgaps and microleakage of the optimised formulation

5.1 Abstract

Aim: The aim of this experiment was to quantify the microgaps formation and microleakage, at the interface, between the optimised formulation and the commercially available restorative materials.

Methods: Sound permanent and primary teeth (n= 5) were collected following the ethical guidelines. All teeth were prepared with Class V cavities and restored with the optimised formulation Renewal MI (Schottlander^{TM}), and the commercial fillings 3M ESPE Filtek Z250 with OptiBond Solo Plus adhesive, ACTIVA^{TM} KIDS Bioactive compomer (PulpDent^{TM}) and GC Fuji IX, GIC materials as per instructions. Then they were painted with varnish (0.5 from the restoration margins). All samples were then stored in 100% humidity for 1 day. They were then immersed in contrast agent solution for 1 day. All samples were then retrieved the next day and immersed in a developing solution under fluorescent light for 8 hours to enhance the contrast. Finally, all samples were visualised under OCT and analysed. Image analysis (Figure A.2) was done by importing raw OCT data to ImageJ software. A median filter was applied (2 px radius), and images were converted to 8-bit grayscale, followed by image binarization.

Results: experiment was not continued (refer to COVID-19 impact statement)

Conclusion: experiment was not continued (refer to COVID-19 impact statement)

5.2 Introduction

The gold standard for assessing microleakage of dental filling materials is usually performed via the use of methylene blue dye. Although this is the gold standard, yet it still has some limitations. The first limitation is sectioning the samples. After restoring the substrate, teeth must be sectioned, then dyed with the methylene blue dye before being imaged. The problem is that the tissue loses its biological integrity after sectioning it, which affects the results and might give false positive microleakage and microgaps results.

The second problem is the inability to assess microgaps/microleakage at different time scales. Once the tooth is sectioned and the dye is applied, the microleakage cannot be assessed for progression. By doing so, this will only provide a snapshot in time of the microleakage. Furthermore, qualitative results can only be gained from such experiments. Although a numerical grading system is used to detect microleakage, these numbers are only a rough estimate and will not provide accurate quantifications.

To bypass these limitations, Optical Coherence Tomography (OCT) scanner was utilised, instead of a methylene blue-dye test, to assess microleakage and microgaps. As mentioned before, OCT is a delicate device that ophthalmologists usually utilise to assess the cornea's health and other investigations. This experiment aimed to incorporate this technique in testing for microleakage, as it was shown to be effective by various labs worldwide (Bakhsh et al., 2011; Turkistani et al., 2018; Alshahni et al., 2019; Haak et al., 2019). Because of its conservativeness, there is no requirement to sections the samples; consequently, the biological integrity will be intact. Hence the results would be more accurate. Adding to that, the fact that there is no need to section and dye the tooth means that the samples can be examined at different time points.

Additionally, and more importantly, one will get both qualitative and quantitative results for such a technique.

5.2 Hypothesis

The null hypothesis is:

The optimised formulation does not reduce microleakage

5.3 Aims and objectives

This experiment aimed to quantify the microgaps formation and microleakage, at the interface, between the optimised formulation, the commercially available restorative materials, and dentine.

5.4 Materials and methods

Figure. A.1 shows a schematic representation of the steps used for this experiment. Sound permanent and primary teeth were collected (n = 5) following the ethical guidelines. All teeth were prepared with Class V cavities (with a small round diamond bur) and restored with the optimised formulation Renewal MI (Schottlander^{TM}), and the commercial filling materials 3M ESPE Filtek Z250 with OptiBond Solo Plus adhesive, ACTIVA^{TM} KIDS Bioactive compomer (PulpDent^{TM}) and GC Fuji IX, GIC materials as per instructions. Then they were painted with varnish (0.5 from the restoration margins). All samples were then stored in 100% humidity for 1 day. They were then immersed in contrast agent solution for 1 day. All samples were then retrieved the next day and immersed in a developing solution under fluorescent light for 8 hours to enhance the contrast. Finally, all samples were visualised under OCT and analysed.

Image analysis (Figure A.2) was done by importing raw OCT data to ImageJ software. A median filter was applied (2 px radius), and images were converted to 8-bit grayscale, followed by image binarization. The area of interest (cavity floor) was located on the binarized image, cropped, and gap percentage was calculated as:

$$Gap\ Percentage = \frac{Gap\ width}{Total\ floor\ width} \times 100$$

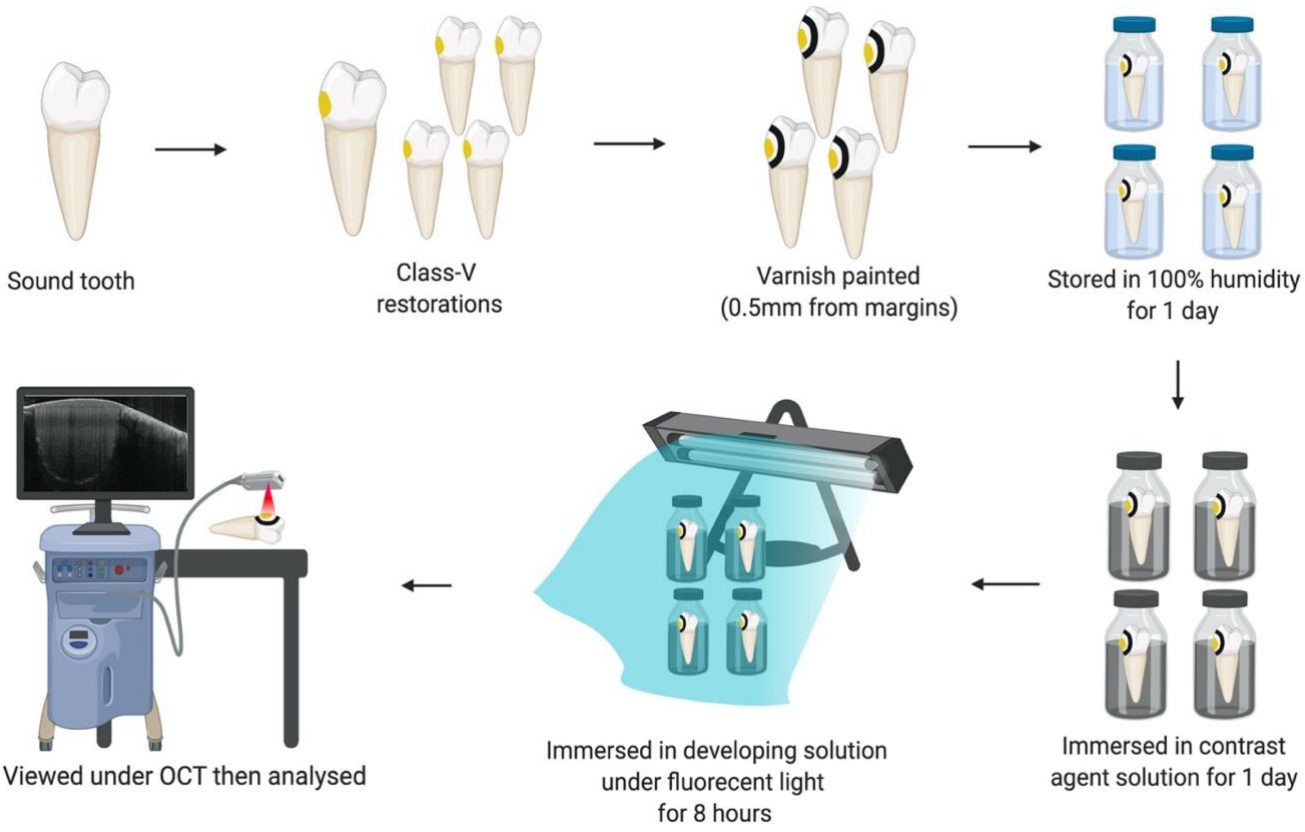

Table A.1 Schematic representation of the microleakage/microgaps experiment.

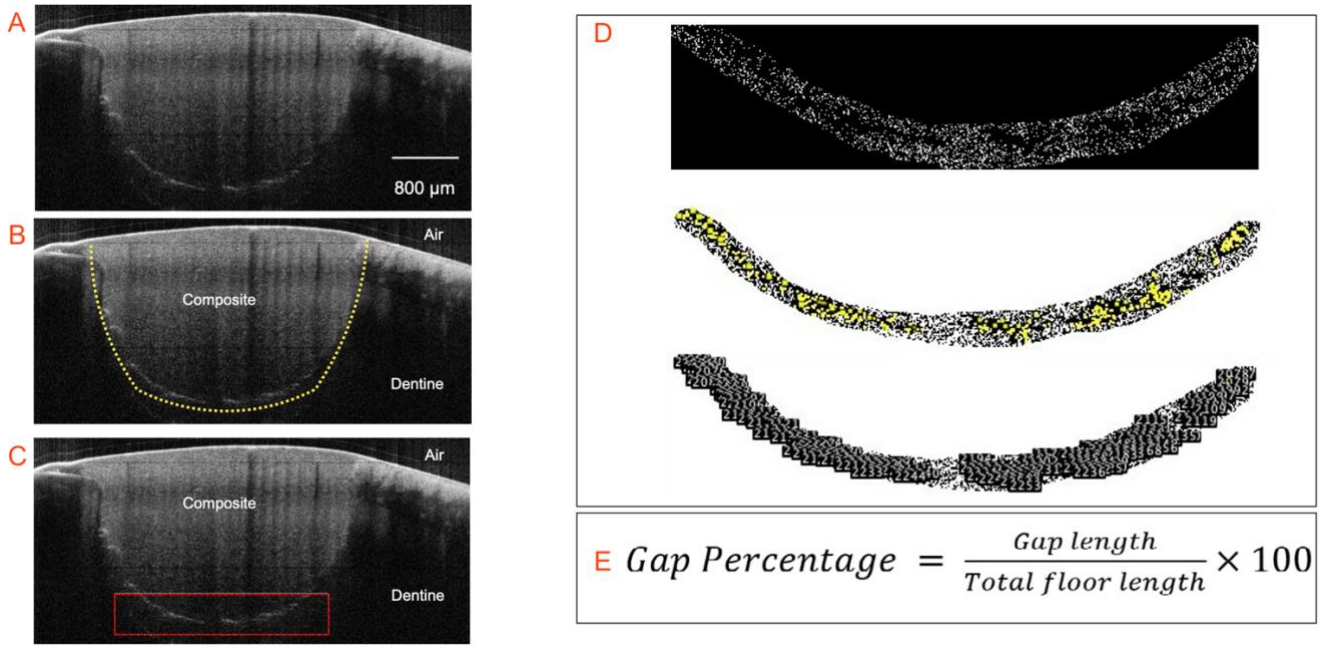

Figure A.2 Representative OCT images to assess microleakage / microgaps. A, Showing dental resin filling Class V cavity. B, Outline (yellow-dashed lines) of the restorative material. C, Gap floor (dashed red box). D, Sections of the interface, followed by binarization analysis. E, Gap percentage equation used to calculate microleakage.

6. CHAPTER SIX: The effect of MMP inhibitors as dentine pretreatment on the bond strength of direct coronal composite restorations: a systematic review of in-vitro studies

6.1. Abstract

This systematic review aims to collect and analyse available evidence on the influence of different MMP inhibitors, as dentine surface pretreatment, on the immediate and long-term bond strength of direct coronal composite restorations. A systematic literature search was conducted through three databases, MIDLINE Ovid (1964 to 2020), Embase Ovid (1980 to 2020), and Google Scholar. Out of a total of 891 studies, 62 studies were included in this systematic review. For the risk of bias assessment, a quality assessment tool was adapted from a previous study (Montagner et al., 2014a) and was independently utilised by two reviewers (HJ and RY). The tool evaluated the bias in terms of sample randomisation, quality of the samples used, duration of dentine pretreatment, the use of materials according to the manufacturer's instructions, storage medium, interface surface area, restorative and bond test performed by the same operator, sample size calculation and blinding of the operator during bond strength testing. Findings were summarised narratively, using text and tables. For example, findings were summarised according to, type of MMP inhibitor used, duration of MMP inhibitor used as dentine pretreatment, substrate type (caries-free or caries-affected), type of bonding agent, type of ageing solution, period of ageing, type of bond strength test, and lastly bond strength results. There were 31 different types of MMP inhibitors were used in the included studies. In terms of the type of bond strength test used, microtensilse bond strength test was used in all included studies, except five studies that used microshear bond strength tests. Regardless of the ageing effect, 12 MMP inhibitors showed no favourable trend in increasing or decreasing the bond strength. On the other hand, 19 MMP inhibitors showed a slight favourable trend towards increasing the bond strength. Regarding the pretreatment duration, regardless

of both the ageing period and the MMP inhibitor type, a slight favourable trend in bond strength can be observed in the durations between 30s through 60s to 120s. No apparent trend was observed when the inhibitors were applied for less than 30s or more 120s. Regardless of both the inhibitor application duration and whether the inhibitor was used or not, the majority of the studies, 44 studies, showed a decrease in bond strength with ageing. Calculations were made in correspondence to the relevant control group. No statistical analysis for the obtained data were conducted. Additionally, the study that incorporated MMP inhibitors in the adhesive systems were not included. Nevertheless, another systematic review currently under process by the same authors is currently looking at this. Another limitation to be considered is that this review only included in-vitro studies. The data provided in this systematic review may aid clinicians in predicting the outcome for their restoration's bond strength and give them support based on evidence.

6.2. Background

Since the development of adhesive restorative materials around six decades ago, a plethora of advancements followed to improve them (Buonocore, 1955; Van Meerbeek et al., 2020). Nevertheless, despite these advancements, it is well documented that adhesive restorations lose their bond strength with time, leading to their inevitable failure (De Munck et al., 2005a; Mjor et al., 2000).

Adhesive restorations rely on the bond between them and the tooth substrate. The interface between them, the hybrid layer, is crucial in determining the bond's longevity and stability (Breschi et al., 2018; Nakabayashi et al., 1991). In dentine, collagen fibrils, mainly made of collagen type-I, are the key to establishing a strong bond. The main reason behind bond failure to dentine has mainly been attributed to the degradation of the hybrid layer as a consequence of dentine collagen fibrils deterioration (Frassetto et al., 2016).

The focus was recently on endogenous enzymes present within the dentine extracellular matrix (ECM) and their effect on bond stability. Among these enzymes are matrix metalloproteinases (MMPs), a well-studied endogenous enzyme. MMPs are a group of calcium- and zinc-dependent host-derived enzymes (Visse and Nagase, 2003). They are divided into six subgroups: collagenases (MMP-1 and MMP-8), stromelysins (MMP-3, MMP-10, MMP-11 and MMP-20), gelatinases or type-IV collagenases (MMP-2 and MMP-9), matrilysin (MMP-7), metalloelastase (MMP-12) and membrane-type metalloproteinases (MMP-14, MMP-15, MMP-16 and MMP-17) (Nagase et al., 2006).

To date, four types of MMP were identified within the dentine extracellular matrix. These are MMP-2, -8, -9 and -20, with MMP-2 and MMP-9 being the most abundant

(Sulkala et al., 2007; Mazzoni et al., 2007). These enzymes are secreted via the odontoblasts during odontogenesis and remain silenced and inactive within the dentine extracellular matrix (Tjaderhane et al., 2001). However, they become active when subjected to an acidic environment, either by biological acids produced by caries (Chaussain-Miller et al., 2006) or by acids introduced during acid etching (Apolonio et al., 2017; DeVito-Moraes et al., 2016). When activated, they start degrading the unprotected and exposed collagen fibrils within the dentine (Pashley et al., 2004), which is why inhibiting the MMP could be beneficial in preserving the hybrid layer and, subsequently, the bond stability.

Several types of MMP inhibitors (synthetic or natural) were shown to inhibit MMP enzymes, such as benzalkonium chloride (Sabatini and Pashley, 2015; Sabatini and Patel, 2013), chlorhexidine (Sabatini and Patel, 2013; Breschi et al., 2010b; Kim et al., 2010; Lenzi et al., 2012), galardin (Breschi et al., 2010a), green tea extracts (Barbosa et al., 2011; Czech et al., 2019) and zinc (Henn et al., 2012) from naming a few. The application mode of the MMPs inhibitors can be categorised into either dentine surface pretreatment or via incorporation into the adhesive system. However, the later mode will be assessed in another systematic review. Importantly, this systematic review aimed to collect and analyse available evidence on the influence of different MMP inhibitors, as dentine surface pretreatment, on the immediate and long-term bond.

6.3. Materials and methods

6.3.1. Protocol registration

This systematic review protocol was reported in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analyses Protocols (PRISMA-P)

Statement (Moher et al., 2015). Noteworthy, it is a good practice to register the systematic review's protocol in the International Prospective Register of Systematic Reviews (PROSPERO) which is produced by the Centre for Reviews and Dissemination (CRD). Moreover, it is a database for ongoing or published systematic reviews on human or animal studies. This systematic review protocol, however, could not be registered, as the current systematic review aims to collect and analyse *in-vitro* studies only.

6.3.2. Eligibility criteria

The systematic review was developed according to the criteria outlined below:

Population: This systematic review included all studies that examined extracted teeth, human teeth, caries-free dentine, healthy dentine, sound dentine, carious affected dentine or affected dentin.

Interventions: This systematic review included all studies examining MMP inhibitors as dentine surface pretreatment prior to direct coronal composite restoration placement. Therefore, studies that used luting cements and glass ionomer cements were excluded.

Comparator(s)/control(s): The comparators were teeth without intervention (i.e., without the addition of MMP inhibitor). Studies that included no comparator were excluded.

Outcome: The main outcome was bond strength or bond stability in the micro-scale (micro-tensile and micro-shear tests). Studies that tested the bond strength at the macro-scale were excluded. Included studies must age the samples for more than

24hrs in water or artificial saliva. Thus, studies with ageing up to 24hrs only, and/or studies that used ageing solutions other than water or artificial saliva were excluded.

6.3.3. Search strategy

6.3.3.1. Types of searched studies

The reviewers searched for published, peer-reviewed quantitative in-vitro studies which present the results quantitively and numerically in the English language. Thus, studies that reported the results in graphs or figures only, were excluded. Non-peer reviewed studies, conference posters, letters, thesis, reviews and editorials were excluded.

6.3.3.2. Period of reviews (timing) and databases

A systematic literature search was conducted in three databases:

1. **Ovid MEDLINE** (1946 to Feb 2021): This is the National Library of Medicine's premier database that contains bibliographic citations and author abstracts from more than 4,600 biomedical journals published in the United States and abroad. It considers one of two primary ways to access MEDLINE (the other being PubMed).

2. **EMBASE** (1974 to Feb 2021): This is a biomedical and pharmacological bibliographic database of published literature designed to support information managers and pharmacovigilance in complying with the regulatory requirements of a licensed drug.

3. **Google Scholar** (to Feb 2021): Google Scholar is a freely accessible database that has the full text of scholarly literature across an array of fields.

Table 2.1 shows the search strategy keywords used. The references of each included paper were assessed for additional eligible studies. PROSPERO was also searched for ongoing or recently completed systematic reviews.

Table 2.1 Search strategy.

Search keywords and the strategy used on MEDLINE®, EMBASE and Google Scholar.

	MEDLINE (Ovid)	EMBASE
P	1. Extracted human teeth.mp. / OR Human teeth.mp. 2. Sound dentine.mp. / OR healthy dentine.mp. 3. Carious affected dentine.mp. / OR Caries affected dentine.mp. / OR affected dentine.mp. 4. dentine$.mp. 5. 1 OR 2 OR 3 OR 4	1. Extracted human teeth.mp. / OR Human teeth.mp. 2. Sound dentine.mp. / OR healthy dentine.mp. 3. Carious affected dentine.mp. / OR Caries affected dentine.mp. / OR affected dentine.mp. 4. dentine$.mp. 5. 1 OR 2 OR 3 OR 4
I	6. Matrix metalloproteinase inhibitors/ OR MMP inhibitors.mp.	6. Matrix metalloproteinase inhibitors/ OR MMP inhibitors.mp.
C	7. No matrix metalloproteinase inhibitors/ OR No MMP inhibitors	7. No matrix metalloproteinase inhibitors/ OR No MMP inhibitors
O	8. Bond strength/ OR Bond stability 8. Bond strength/ OR Bond stability	8. Bond strength/ OR Bond stability Bond strength/ OR Bond stability
Combined	1 OR 2 OR 3 OR 4 AND 6 AND 7 AND 8)	1 OR 2 OR 3 OR 4 AND 6 AND 7 AND 8)

6.3.3.3. Google scholar search strategy

“Extracted human teeth” OR “human teeth” OR “Sound dentine” OR “healthy dentine” OR “affected dentine” OR “Carious affected dentine” OR “Caries affected dentine” OR “Dentine” AND “Matrix metalloproteinase inhibitors” OR “MMP inhibitors” AND “Bond strength” OR “Bond stability”

6.3.4. Data selection and collection processes

Full texts of all eligible studies were uploaded to reference management software (EndNote X9.3.1™). Reviewers (HJ and RY) screened the titles and abstracts of the selected studies at that stage. Studies that met the inclusion criteria were read fully.

Duplicate publications were excluded by the same reference management software. Two evaluators (HJ and RY) independently screened each full-text paper based on the eligibility criteria. In case of discrepancies for the eligibility of the study to be included or excluded, a further evaluator was involved (HA or PA). At this stage, reasons for exclusion were recognised and categorised.

A data extraction form was created to help the reviewers during data extraction. This included authors' names and year of publication, type of MMP inhibitor used, duration of MMP inhibitor used as dentine pretreatment, substrate condition, type of bonding agent, type of ageing solution, period of ageing, type of bond strength test and lastly, bond strength results. Two reviewers (HJ and RY) were independently involved during the data collection stage. An experienced third reviewer (PA) independently extracted data on 10% of studies to check the consistency of the process. Conflicts of opinion was resolved through consensus by consulting a further reviewer (HA or AY).

6.3.5. Risks of bias and quality assessment

Assessment of study quality is the process of assessing a study's trustworthiness. It allows developing reliably and efficiently study with robustness recommendations for future studies. The quality assessment tool was adapted from a previous study (Montagner et al., 2014b) and was independently utilised by two reviewers (HJ and

RY). The tool evaluated the bias in terms of sample randomisation, quality of the samples used, duration of dentine pretreatment, the use of materials according to the manufacturer's instructions, storage medium, interface surface area, restorative and bond test performed by a single operator, sample size calculation (power analysis) and blinding of the operator during bond strength testing. Under each component of the tool, the letter 'Y' (yes) was added if the author reported the component and 'N' (no) if it was not reported. The grading judgement of "low", "medium", or "high" for the study was based on the total number of 'Y' following grading system: one to five (high), six or seven (medium) and eight or nine (low).

6.3.6. Data synthesis

Findings were summarised narratively, using text and tables. For example, findings were summarised according to, type of MMP inhibitor used, duration of MMP inhibitor used as dentine pretreatment, substrate condition (caries-free or caries-affected), type of bonding agent, type of ageing solution, period of ageing, type of bond strength test, and lastly bond strength results in relation to type of inhibitor, ageing period, and the duration of the MMP inhibitor used.

6.4. Results

6.4.1. Study selection

Figure 2.1 shows a flowchart summarising the selection process according to the PRISMA statement (Page et al., 2021). As stated in the materials and methods section, three databases were utilised for the search MEDLINE, EMBASE and Google Scholar. During the initial search of all the databases, 891 potentially eligible studies were retrieved. After the removal of the duplicates, 757 studies remained. Of these, after

reviewing the titles, 193 studies remained. 163 studies remained after reviewing the abstracts. Following the full-text reading, 62 studies remained and were included in the study.

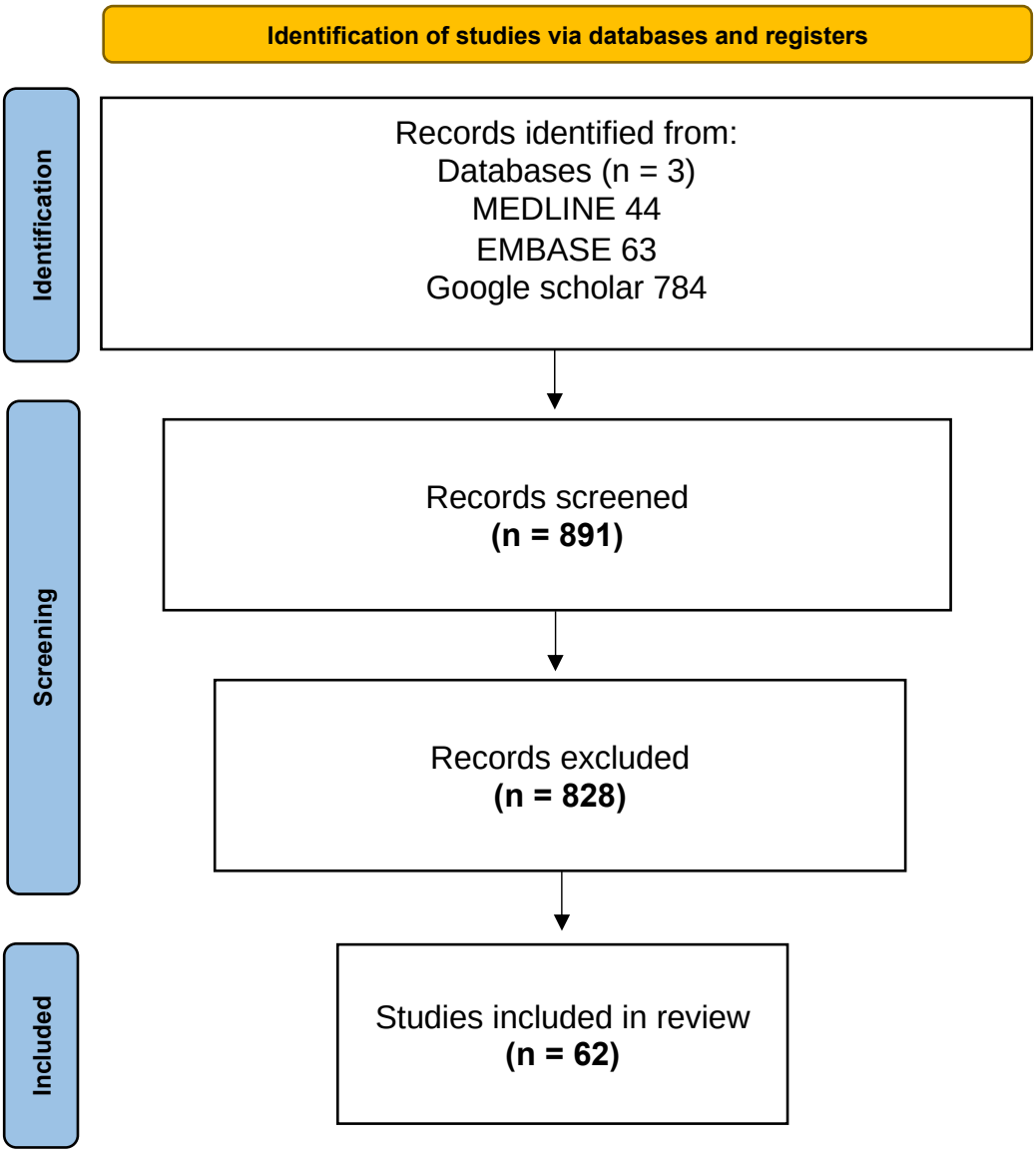

Figure 2.1 PRISMA 2020 flowchart diagram.

6.4.2. Study characteristics

The data obtained from the included publications are listed in Table 2.2. The 62 included in vitro studies were published between the years 2009 and 2020.

There were 31 different types of MMP inhibitors were used in the included studies. Regarding the MMP inhibitor's origin, 14 synthetically-derived MMP inhibitors were used in 50 of the included studies, while 17 naturally-derived MMP inhibitors were used in 28 of the included studies.

According to the synthetically-derived MMP inhibitors used, the majority of the studies have used chlorhexidine (CHX), adding to 37 studies (Balloni et al., 2016; Bravo et al., 2017; Bueno et al., 2015; de Araújo Costaa et al., 2019; Fernandes et al., 2020; Fialho et al., 2019; Gerhardt et al., 2016; Campos et al., 2019; Giacomini et al., 2020; Grandizoli, 2018; Karrabi and Danesh Kazemi, 2016; Lenzi et al., 2014; Loguercio et al., 2009b; Loguercio et al., 2016a; Ou et al., 2018a; Perote et al., 2015; Porto, 2018; Ruksaphon K, 2017; Sacramento et al., 2012; Sadeghi, 2017; Santiago et al., 2013; Shen et al., 2020; Kazemi-Yazdi et al., 2020; Da Silva et al., 2015; Zheng et al., 2015; Sadek et al., 2010; Stanislawczuk et al., 2009; Giacomini et al., 2017; Sabatini et al., 2014; Carvalho et al., 2016; Loguercio et al., 2016b; Mobarak, 2011; Manso et al., 2014; Breschi et al., 2010b; Montagner et al., 2015; Kalagi et al., 2020; Tekce et al., 2016). Four studies have used carbodiimide (EDC) (Mazzoni et al., 2013; Mazzoni et al., 2018; Paulose and Fawzy, 2018; Venigalla et al., 2016). Moreover, there were two studies for each of the following, glutaraldehyde (GD) (Hass et al., 2016; Li et al., 2018), benzalkonium chloride (BAC) (Xu et al., 2020; Sabatini et al., 2014), and galardin (Zheng et al., 2015; Breschi et al., 2010a). Also one study for each of the following N,N'-dicyclohexylcarbodiimide (DCC) (Comba et al., 2020), dopamine

methacrylamide (DMA) (Li et al., 2020), GM6001 (Fang et al., 2017), Acrolein (ACR) (Maravic et al., 2018), MMP8-I (Ou et al., 2018a), Ferrous sulfate ($FeSO_4$) (Zheng et al., 2015), silver diamine fluoride (SDF) (Firouzmandi et al., 2020b), E-64 (Giacomini et al., 2017), and polyvinylphosphonice acid (PVPC) (Xu et al., 2020).

As for the naturally-derived MMP inhibitors, most of the studies have used epigallocatechin gallate (EGCG), adding to 8 studies (Czech et al., 2019; de Araújo Costaa et al., 2019; Maha A. El Baz, 2018; Fernandes et al., 2020; Fialho et al., 2019; Gerhardt et al., 2016; Santiago et al., 2013; Carvalho et al., 2016). There were four studies for each of the following proanthocyanidin (PRO) (Davila-Sanchez et al., 2020; Hass et al., 2016; Venigalla et al., 2016; Xu et al., 2020) and riboflavin (Cova et al., 2011; Hass et al., 2016; Kasraei S., 2017; Venigalla et al., 2016). Three studies have used rosmarinic acid (Prasansuttiporn et al., 2020; Prasansuttiporn et al., 2017; Ruksaphon K, 2017) and two studies for each of the following; green tea (GT) (Gerhardt et al., 2016; Zheng et al., 2015), chitosan (Baena et al., 2020; Mohamed et al., 2020), quercetin (Davila-Sanchez et al., 2020; Porto, 2018), Morus alba (MA) leaves (Mosallam et al., 2018; Mosallam et al., 2019), Morus nigra (MN) leaves (Mosallam et al., 2018; Mosallam et al., 2019). Only one study for each of the following; hesperidin (HES) (Davila-Sanchez et al., 2020), rutin (RUN) (Davila-Sanchez et al., 2020), naringin (NAR) (Davila-Sanchez et al., 2020), mussel adhesive protein (MAP) (Fang et al., 2017), baicalein (BAI) (Li et al., 2018), caffeic acid (Pedrosa et al., 2018), propolis extracts (Perote et al., 2015), and resveratrol (Res) (Porto, 2018).

In terms of the type of bond strength test used, microtensilse bond strength test was used in all included studies, except five studies that used microshear bond strength

tests (Maha A. El Baz, 2018; Sacramento et al., 2012; Da Silva et al., 2015; Firouzmandi et al., 2020b; Mobarak, 2011).

As for the substrate, most of the studies have used caries-free dentine substrate, adding to 52 studies. Twelve studies used caries-affected dentine, two studies used eroded dentine, and only one study used dentine without mentioning its condition. It is worth mentioning that all studies used permanent teeth except one study, which used primary teeth (Lenzi et al., 2014).

As for storage medium, the majority of the studies have used distilled water (39 studies) and artificial saliva was used in 21 studies. Only one study has used both artificial and deionized water. Additionally, one study used both distilled and deionized water.

In regard to, the duration of MMP inhibitor application, the majority of the studies applied it for 60s in 45 studies. This was followed by 30s in six studies, 120s in four studies, 5s in three studies, and 15s in two studies. As for the 20s and 180s, they were used in one study each. Additionally, only one study did not report the application duration.

As for the ageing periods, they were ranged between 24hrs and 5 years, in addition, various thermocycling ageing period were used. The majority of studies aged the samples for 24hrs as an immediate ageing period, adding to 60 studies. As for long-term ageing, 30 studies aged the samples for 6 months, 19 aged for 12 months, and five aged for 3 months. Three studies aged the samples for 2 years, and another three aged the samples for 18 months. Two studies aged the samples for 9 months. Ageing for 3 days, 1 week, 15 days, 15 months and 5 years were used in one study each. Eleven studies have used thermocycling for ageing; five studies used 1000 cycles and

two studies used 5000 cycles. 2500, 3000, 10000, and 25000 cycles were used in one study each.

Regardless of the ageing effect, 12 MMP inhibitors (chitosan, EGCG, GM6001, MA, MA, caffeic acid, propolis, Res, SDF, E-64 and DMA) used showed no favourable trend in either increasing or decreasing the bond strength. On the other hand, 19 MMP inhibitors (CHX, DCC, QUE, HES, RUT, NAR, PRO, MAP, green tea extract, riboflavin, BAI, GD, ACR, EDC, MMP8-I, rosmarinic acid, BAC, PVPA, galardin and $FeSO_4$) showed a slight favourable trend towards increasing the bond strength. This was calculated in relation to the relevant control group for each MMP inhibitor used.

Concerning the pretreatment duration, regardless of both the ageing period and the MMP inhibitor type, a slight favourable trend in bond strength can be observed in the durations between 30s through 60s to 120s. No apparent trend was observed when the inhibitors were applied for less than 30s or more 120s, in correspondence to the relevant control group.

Regardless of both the inhibitor application duration and whether the inhibitor was used or not, the majority of the studies, 44 studies, showed decrease in bond strength with ageing. Five studies lacked baseline (24hrs), thus they were not included in this part. Out of the 44 studies, 17 showed a decrease in bond strength after 6 months, and another 17 showed a decrease after ageing for 12 months. Three studies showed a decrease after ageing for 18 months, and another three showed a decrease after ageing for 2 years. Two studies showed a decrease after ageing for 3 months. One study showed a decrease in bond strength after ageing for 5 years, and another study showed a decrease after 15 days of ageing. Regarding thermocycling, four studies showed a decrease in bond strength following 1000 thermocycles. Moreover, one

study for each of the following showed a decrease in bond strength: 25000, 10000, 2500 and 3000 thermocycles.

6.4.3. Risk of bias evaluation

Table 2.3 shows the risk of bias content used to evaluate all included studies. Overall, almost half of the included studies showed a medium risk of bias (32 out of 62), while 17 out of 62 studies showed a high risk of bias. The remainder of 13 studies were classified as low risk of bias (Table 2.3). Out of all the included studies, only one study mentioned the blinding of the operator testing for the bond strength (Balloni et al., 2016).

Table 2.2 Demographic and data design of included studies. All included studies used microtensile (μTBS) bond strength tests, except five studies that used microshear bond strength tests (Maha A. El Baz, 2018; Sacramento et al., 2012; Da Silva et al., 2015; Firouzmandi et al., 2020b; Mobarak, 2011).

Paper (year)	MMP inhibitor type (origin)	Duration of dentine pretreatment	Substrate condition	Bonding agent	Ageing solution	Period of ageing	Groups	Bond strength means (SD)	Bond strength (%) increase (+)/ decrease (-) at each ageing period	Bond strength (%) increase (+)/ decrease (-) after aging
1. (Baena et al., 2020)	Chitosan (natural)	60s	Caries-free	Optibond FL (OFL) (Kerr) Scotchbond Universal (3M)	Artificial saliva	24hrs	**Chitosan 0.1% + OFL** Control (OFL) **Chitosan 0.1% + SBU** Control (SBU)	**38.0 (7.7)** 41.3 (14.5) **28.1 (14.3)** 25.0 (16.5)	-7.99% +12.4%	
						10000 thermocycles	**Chitosan 0.1% + OFL** Control (OFL) **Chitosan 0.1% + SBU** Control (SBU)	**29.2 (14.1)** 32.2 (12.9) **33.1 (17.0)** 30.4 (11.8)	-9.32% +8.88%	-23.16% -22% +17.80% +21.6%
2. (Balloni et al., 2016)	Chlorhexidine (CHX) (synthetic)	60s	Caries-free	Clearfil SE bond	Distilled water	24hrs	**CHX 2%** Control	**19.24 (11.89)** 11.97 (9.95)	+60.74%	
						6 months	**CHX 2%** Control	**12.67 (7.43)** 10.22 (5.00)	+23.97%	-34.15% -14.62%
3. (Bravo et al., 2017)	Chlorhexidine (CHX) (synthetic)	20s	Caries-free	Adper Scotchbond 1XT Adper prompt Single Bond Universal	Distilled water	3 days	**CHX 2% + ASB** Control (ASB) **CHX 2% + APP** Control APP **CHX 2% + SBU** Control SBU	**26.28 (9.29)** 28.56 (5.83) **24.21 (7.52)** 20.14 (4.87) **28.43 (9.78)** 29.24 (7.90)	-7.98% +20.21% -2.77%	
						3 months	**CHX 2% + ASB** Control (ASB) **CHX 2% + APP** Control APP **CHX 2% + SBU** Control SBU	**32.26 (10.33)** 19.82 (7.65) **28.51 (13.18)** 20.86 (6.13) **44.11 (12.09)** 23.54 (12.09)	+62.76% +36.67% +87.4%	N/A
						6 months	**CHX 2% + ASB** Control (ASB) **CHX 2% + APP** Control (APP) **CHX 2% + (SBU)** Control SBU	**31.73 (5.18)** 23.39 (5.69) **27.37 (4.40)** 20.51 (5.66) **36.88 (6.65)** 23.62 (7.07)	+35.66% +33.45% +56.14%	N/A
4. (Bueno et al., 2015)	Chlorhexidine (CHX) (synthetic)	60s	Not mentioned	Clearfil SE bond	Distilled water	24hrs	**CHX 2%** Control	**28.0 (8.4)** 24.2 (7.2)	+15.7%	

						6 months	**CHX 2%** Control	**33.4 (9.3)** 21.8 (7.3)	+53.21%	+19.30% -10%
5. (Comba et al., 2020)	N,N'-dicyclohexylcarbodiimide (DCC) (synthetic)	60s	Caries-free	Scotch bond universal (SBU) (Self-etch SE and etch-and-rinse ER)	Artificial Saliva	24hrs	**0.5M DCC SBU (ER)** SBU (ER) **0.5M DCC SBU (SE)** SBU (SE)	**46.0 (5.3)** 37.1 (12.5) **39.4 (11.1)** 26.3 (11.4)	+23.99% +49.81%	
						12 months	**0.5M DCC SBU (ER)** SBU (ER) **0.5M DCC SBU (SE)** SBU (SE)	**33.5 (13.9)** 31.0 (11.0) **35.3 (13.9)** 13.4 (9.1)	+8.06% +163.4%	-27.2% -16.44% -10.41% -50%
6. (Czech et al., 2019)	Chlorhexidine (CHX) (synthetic) EGCG (natural)	60s	Caries-affected	Adper Single Bond 2	Distilled water	24hrs	**EGCG (200 µg/mL)** **CHX 2%** Control	**24.08 (7.20)** **14.64 (7.74)** 23.43 (7.73)	+2.77% -37.52%	
						6 months	**EGCG (200 µg/mL)** **CHX 2%** Control	**18.67 (8.51)** **11.20 (4.79)** 23.43 (7.73)	-20.32% -52.2%	-225% -23.5% 0%
						12 months	**EGCG (200 µg/mL)** **CHX 2%** Control	**16.77 (5.50)** **10.17 (3.02)** 16.28 (9.58)	+3.01% -37.53%	-30.4% -30.53% -30.5%
7. (Davila-Sanchez et al., 2020)	Quercetin (QUE) Hesperidin (HES) Rutin (RUT) Naringin (NAR) Proanthocyanidin (PRO) (All natural)	60s	Caries-affected	Scotchbond Universal (3M)	Distilled water	24hrs	**HES 6.5%** **PRO 6.5%** **QUE 6.5%** **NAR 6.5%** **RUT 6.5%** Control	**18.41 (5.30)** **20.66 (3.92)** **24.58 (4.90)** **24.64 (3.70)** **26.00 (5.51)** 14.42 (4.43)	+27.67% +43.27% +70.46% +70.87% +80.31%	
						25000 thermocycles	**HES 6.5%** **PRO 6.5%** **QUE 6.5%** **NAR 6.5%** **RUT 6.5%** Control	**15.73 (6.07)** **17.20 (2.72)** **12.02 (5.21)** **22.12 (2.92)** **21.08 (4.75)** 9.43 (4.29)	+66.81% +82.4% +27.47% +134.5% +123.5%	-14.6% -16.75% -51% -10.23% -19% -34.6%
8. (de Araújo Costaa et al., 2019)	Chlorhexidine (CHX) (synthetic) EGCG (natural)	60s	Eroded (ERO) and Non-eroded (non-ERO)	Clearfil SE bond	Distilled water	24hrs	**CHX 2% (non-ERO)** **CHX 2% (ERO)** **EGCG 0.1% (non-ERO)** **EGCG 0.1% (ERO)** Control (non-ERO) Control (ERO)	**40.87 (10.23)** **49.30 (9.42)** **53.67 (6.10)** **61.61 (3.17)** 52.44 (8.47) 59.25 (5.91)	-22.06% -16.79% +2.35% +3.98%	
						6 months	**CHX 2% (non-ERO)** **CHX 2% (ERO)** **EGCG 0.1% (non-ERO)** **EGCG 0.1% (ERO)** Control (non-ERO) Control (ERO)	**32.77 (10.67)** **36.91 (9.88)** **50.02 (3.42)** **44.63 (13.26)** 47.64 (11.67) 45.16 (11.87)	-31.21% -18.27% +5% -1.17%	-20% -25% -7% -27.6% -9.15% -23.78%

9. (Maha A. El Baz, 2018)	EGCG (natural)	60s	Caries-free	Primer and Bond one-etch and rinse (Dentsply)	Distilled water	24hrs	**EGCG 0.1%** Control	**18.8 (0.2)** 15.4 (0.7)	+22.08%	
						6 months	**EGCG 0.1%** Control	**17.6 (0.3)** 12.2 (0.9)	+44.26%	-6.4% -20.8%
						5000 thermocycles	**EGCG 0.1%** Control	**22.1 (0.7)** 8.8 (0.8)	+151.1%	+17.55% -42.8%
10. (Fang et al., 2017)	Mussel adhesive protein (MAP) (natural) GM6001 (synthetic)	60s	Caries-free	Gluma Comfort Bond	Distilled water	24hrs	**MAP 1mg/ml** **GM6001 10µM** Control	**19.31 (4.48)** **18.86 (4.2)** 19.25 (4.21)	+0.31% -2.03%	
						2500 thermocycles	**MAP 1mg/ml** **GM6001 10µM** Control	**12.22 (4.49)** **10.87 (4.27)** 6.08 (3.12)	+101% +78.78%	-37% -42.34% -68.4%
11. (Fernandes et al., 2020)	Chlorhexidine (CHX) (synthetic) EGCG (natural)	60s	Caries-free	Clearfil SE Bond Primer	Artificial Saliva	24hrs	**CHX 2%** **EGCG 0.01%** Control	**44.16 (6.81)** **42.76 (7.36)** 40.65 (6.51)	+8.63% +5.19%	
						12 months	**CHX 2%** **EGCG 0.01%** Control	**33.58 (10.49)** **34.91 (7.84)** 33.85 (9.27)	-0.8% +3.13%	-24% -18.4% -16.7%
12. (Fialho et al., 2019)	Chlorhexidine (CHX) (synthetic) EGCG (natural)	60s	Caries-affected	Adper Single Bond 2 (3M)	Distilled water	24hrs	**EGCG 0.2%** **EGCG 2%** **EGCG 0.5%** **CHX 2%** Control	**32.65 (9.97)** **29.16 (11.52)** **28.57 (6.30)** **33.33 (11.26)** 35.81 (8.25)	+8.82% -18.57% -20.22% -6.93%	
						12 months	**EGCG 0.2%** **EGCG 2%** **EGCG 0.5%** **CHX 2%** Control	**22.75 (9.38)** **17.15 (10.61)** **23.65 (7.19)** **19.98 (7.01)** 26.17 (12.28)	-13.07% -34.47% -9.63% -23.65%	-30.32% -41.2% -17.2% -40% -27%
13. (Gerhardt et al., 2016)	Chlorhexidine (CHX) (synthetic) EGCG (natural) Green tea (natural)	60s	Caries-free	Clearfil SE Bond	Deionised water/ distilled water	24hrs	**CHX 2%** **EGCG 2%** **Green Tea 2%** Control	**13.31 (3.36)** **6.93 (3.43)** **10.60 (4.69)** 8.64 (5.52)	+54.05% -19.79% +22.69%	
						6 months	**CHX 2%** **EGCG 2%** **Green Tea 2%** Control	**11.09 (4.98)** **15.96 (5.32)** **17.82 (12.20)** 16.69 (7.20)	-33.55% -4.37% +6.77%	-16.7% +130.3% +68% +93%
14. (Campos et al., 2019)	Chlorhexidine (CHX) (synthetic)	Not mentioned	Caries-affected	Clearfil SE Bond	Distilled water	24 hrs	**CHX 2%** Control	**19.84 (8.11)** 24.89 (9.44)	-20.29%	
						12 months	**CHX 2%** Control (no Tx)	**17.59 (8.85)** 28.30 (11.54)	-37.84%	-11.34% +13.7%
15. (Giacomini et al., 2020)	Chlorhexidine (CHX) (synthetic)	30s	Caries-free	Adper Single Bond 2 (SB)/Adper Single Bond	Artificial saliva	24hrs	**CHX 2% (SB)** Control **CHX 2% (SU-ER)** Control	**28.41 (7.64)** 33.35 (9.01) **33.66 (7.79)**	-14.81% +6.45%	

				Universal etch and rinse (SU-ER)/Adper Single Bond Universal self-etch (SU-SE)			**CHX 2% (SU-SE)** Control	31.62 (8.20) **37.47 (10.68)** 45.62 (12.39)	-17.86%	
						6 months	**CHX 2% (SB)** Control **CHX 2% (SU-ER)** Control **CHX 2% (SU-SE)** Control	**31.55 (6.15)** 32.59 (9.44) **33.79 (6.24)** 32.05 (7.04) **34.25 (11.21)** 40.15 (14.77)	-3.19% +5.43% -14.69%	+11% -2.3% +0.4% +1.36% -8.6% -12%
16. (Grandizoli, 2018)	Chlorhexidine (CHX) (synthetic)	60s	Caries-affected	Clearfil SE bond	Distilled water	24 hrs	**CHX 2%** Control	**21.7 (16.3)** 19.3 (11.9)	+12.44%	
						6 months	**CHX 2%** Control	**1.9 (1.8)** 2.5 (1.2)	-24%	-91.2% -87%
17. (Karrabi and Danesh Kazemi, 2016)	Chlorhexidine (CHX) (synthetic)	120s	Caries-free	Adper Single Bond	Artificial Saliva	6 months	**CHX 2%** Control	**52.67 (6.862)** 28.84 (6.231)	+82.63%	N/A
18. (Kasraei S., 2017)	Riboflavin (natural)	120s light activation	Caries-free	Adper Single Bond	Distilled water	5000 thermocycles	**Riboflavin 0.1%** Control	**12.79 (3.64)** 12.64 (2.35)	+1.19%	N/A
19. (Lenzi et al., 2014)	Chlorhexidine (CHX) (synthetic)	60s	Caries-free And Caries-affected (CA)	Adper Single Bond	Distilled water	24 hrs	**CHX 2%** Control **CHX 2% (CA)** Control (CA)	**32.8 (3.8)** 30.7 (2.2) **25.1 (4.0)** 24.3 (3.8)	+6.84% +3.29%	
						6 months	**CHX 2%** Control **CHX 2% (CA)** Control (CA)	**31.3 (2.6)** 24.2 (3.6) **23.2 (5.2)** 14.3 (5.8)	+29.34% +62.24%	-4.6% -21.2% -7.6% -41%
20. (Li et al., 2018)	Baicalein (BAI) (natural) 5% Glutaraldehyde (GD) (synthetic)	120s	Caries-free	Adper Single Bond 2	Artificial Saliva	24 hrs	**GD 5%** **BAI 2.5 µg/mL** Control	**58.86 (4.29)** **58.32 (3.95)** 41.89 (5.18)	+40.51% +39.22%	
						3 months	**GD 5%** **BAI 2.5 µg/mL** Control	**56.10 (5.8 9)** **52.43 (5.43)** 34.46 (6.22)	+62.8% +52.15%	-4.70% -10% -18%
						6 months	**GD 5%** **BAI 2.5 µg/mL** Control	**51.86 (6.42)** **52.43 (5.43)** 26.82 (5.30)	+93.36% +95.49%	-11.90% -10% -36%
21. (Loguercio et al., 2016a)	Chlorhexidine (CHX) (synthetic)	60s	Caries-free	Primer & Bond (PB) NT Adper Single Bond (SB) 2	Distilled water	24 hrs	**CHX 2% (PB)** Control **CHX 2% (SB)** Control	**33.1 (2.8)** 35.1 (3.1) **43.5 (3.5)** 40.2 (3.3)	-5.7% +8.21%	
						5 years	**CHX 2% (PB)** Control **CHX 2% (SB)** Control	**22.1 (2.2)** 11.0 (2.7) **31.3 (2.7)** 16.1 (2.1)	+100.91% +94.41%	-33.23% -68.6% -28% -60%

22. (Loguercio et al., 2009b)	Chlorhexidine (CHX) (synthetic)	15s/60s	Caries-free	Primer & Bond (PB) 2.1/ Adper Single Bond (SB)	Distilled water	24 hrs	**CHX 2% (PB) 15s** **CHX 0.002% (PB) 15s** Control 15s **CHX 2% (SB) 15s** **CHX 0.002% (SB) 15s** Control 15s **CHX 2% (PB) 60s** **CHX 0.002% (PB) 60s** Control 60s **CHX 2% (SB) 60s** **CHX 0.002% (SB) 60s** Control 60s	**33.1 (6.5)** **25.7 (2.4)** 28.3 (4.3) **43.5 (4.1)** **41.4 (4.8)** 39.2 (5.4) **31.3 (5.1)** **29.2 (3.4)** 32.4 (5.4) **41.2 (4.2)** **43.2 (6.1)** 41.5 (6.4)	+16.96% -9.19% +10.97% +5.61% -3.4% -37.65% -0.72% +4.1%	
						6 months	**CHX 2% (PB) 15s** **CHX 0.002% (PB) 15s** Control 15s **CHX 2% (SB) 15s** **CHX 0.002% (SB) 15s** Control 15s **CHX 2% (PB) 60s** **CHX 0.002% (PB) 60s** Control 60s **CHX 2% (SB) 60s** **CHX 0.002% (SB) 60s** Control 60s	**27.3 (4.2)** **23.2 (4.1)** 20.1 (4.2) **40.1 (5.7)** **37.2 (6.1)** 27.9 (6.2) **28.1 (4.4)** **27.0 (3.6)** 21.2 (3.8) **37.6 (3.3)** **40.1 (3.7)** 25.4 (4.1)	+35.82% +15.42% +43.73% +33.33% +32.55% +27.36% +48.03% +57.87%	-17.5% -9.7% -29% -7.8% -10% -29% -10.22% -7.5% -34.5% -8.7% -7.2% -39%
23. (Maravic et al., 2018)	Acrolein (ACR) (synthetic)	60s	Caries-free	Adper Scotchbond 1XT	Artificial Saliva	24hrs	**ACR 0.01%** Control	**46.6 (3.1)** 46.0 (4.9)	+1.3%	
						12 months	**ACR 0.01%** Control	**39.9 (3.3)** 24.8 (2.4)	+60.89%	-14.4% -46%
24. (Mazzoni et al., 2013)	Carbodiimide (EDC) (synthetic)	60s	Caries-free	Optibond (OB) FL/ Scotchbond (SB) 1XT	Artificial Saliva	24hrs	**EDC 0.3M + OB** Control **EDC 0.3M + SB** Control	**44.5 (9.8)** 43.3 (9.4) **38.8 (9.8)** 40.5 (10.3)	+2.77% -4.2%	
						12 months	**EDC 0.3M + OB** Control **EDC 0.3M + SB** Control	**41.2 (10.1)** 33.1 (7.9) **32.5 (9.6)** 24.8 (8.8)	+24.47% +31.05%	-7.4% -23.5% -16.2% -39%
25. (Mazzoni et al., 2018)	Carbodiimide (EDC) (synthetic)	60s	Caries-free	Clearfil SE primer/ XP Bond	Artificial Saliva	24hrs	**EDC 0.3M + (Clearfil)** Control **EDC 0.3M + (XP bond)** Control	**30.1 (6.3)** 32.8 (4.4) **36.5 (7.1)** 37.6 (5.9)	-8.23% -2.93%	
						12 months	**EDC 0.3M + (Clearfil)** Control **EDC 0.3M + (XP bond)** Control	**26 (8.0)** 21.4 (5.7) **28.6 (6.4)** 18.1 (4.9)	+21.5% +58.01%	-13.6% -34.7% -13% -52%
26. (Mohamed et al., 2020)	Chitosan (natural)	60s	Caries-free		Distilled water	24hrs	**Chitosan 0.2%** **Chitosan 2.5%**	**39.16 (38.62)**	+88.09% -24.93%	

				Universal Single Bond adhesive			Control	**15.63 (14.64)** 20.82 (21.43)		
						3 months	**Chitosan 0.2%** **Chitosan 2.5%** Control	**23.95 (25.08)** **16.89 (17.79)** 21.1 (21.03)	+13.51% -19.95%	-39% +8% +1.3%
						6 months	**Chitosan 0.2%** **Chitosan 2.5%** Control	**25.1 (25.73)** **21.36 (20.94)** 28.76 (28.15)	-12.73% -25.73%	-36% +36.6% +38%
27. (Mosallam et al., 2018)	Green tea (GT) Morus alba leaves (MA) Morus nigra leaves (MN) (All natural)	60s	Caries-free	Tetric N-Bond Universal	Distilled water	24hrs	**GT 20mg/ml (water extract)** **GT 5mg/ml (alcohol extract)** **MA 20mg/ml (water extract)** **MA 5mg/ml (alcohol extract)** **MN 5mg/ml (water extract)** **MN 5mg/ml (alcohol extract)** Control	**29.22 (6.29)** **16.70 (5.30)** **4.01 (1.92)** **26.68 (5.81)** **24.90 (6.74)** **26.68 (5.81)** 28.38 (6.68)	+2.96% -41.16% -85.87% -5.99% -12.26% -5.99%	
						1000 thermocycles	**GT 20mg/ml (water extract)** **GT 5mg/ml (alcohol extract)** **MA 20mg/ml (water extract)** **MA 5mg/ml (alcohol extract)** **MN 20mg/ml (water extract)** **MN 5mg/ml (alcohol extract)** Control	**18.97 (6.66)** **12.73 (6.63)** **2.64 (2.27)** **17.93 (4.82)** **17.83 (6.57)** **17.93 (4.82)** 17.39 (1.71)	+9.09% -26.8% -84.82% +3.11% +2.53% +3.11%	-35% -24% -34% -33% -28.3% -33% -39%
28. (Mosallam et al., 2019)	Morus alba leaves (MA) Morus nigra leaves (MN) (Both natural)	60s	Caries-free	Scotch Bond Universal	Distilled water	24hrs	**MA 20mg/ml (water extract)** **MA 5mg/ml (alcohol extract)** **MN 20mg/ml (water extract)** **MN 5mg/ml (alcohol extract)** Control	**29.30 (7.31)** **17.39 (1.63)** **35.03 (5.24)** **19.72 (8.82)** 28.38 (6.68)	+3.24% -38.72% +23.43% -30.51%	
						1000 thermocycles	**MA 20mg/ml (water extract)** **MA 5mg/ml (alcohol extract)** **MN 20mg/ml (water extract)** **MN 5mg/ml (alcohol extract)** Control	**20.55 (8.85)** **10.26 (8.28)** **20.60 (5.97)** **18.05 (7.84)** 17.39 (1.71)	+18.17% -41% +18.46% +3.8%	-30% -41% -41% -8.5% -39%
29. (Ou et al., 2018a)	Chlorhexidine (CHX) MMP8-I inhibitor (Both synthetic)	30s	Caries-free	Adper Single Bond 2	Distilled water	24hrs	**CHX 2%** **MMP8-I** Control	**42.14 (8.83)** **55.29 (9.71)** 47.18 (11.69)	-10.68% +17.19%	
						6 months	**CHX 2%** **MMP8-I** Control	**41.83 (15.52)** **54.70 (13.66)** 39.06 (9.88)	+7.09% +40.04%	-0.7% -1.1% -17.2%
						12 months	**CHX 2%** **MMP8-I** Control	**39.92 (16.08)** **54.29 (15.26)** 35.82 (19.14)	+11.45% +51.56%	-5.3% -2% -24%
30. (Paulose and Fawzy, 2018)	Carbodiimide (EDC) (synthetic)	60s	Caries-free	Adper Scotchbond multipurpose (SB) /Single	Distilled water	24hrs	**EDC 0.3M + SMP** Control **EDC 0.3M -dry + SU** Control	**40.7 (9.3)** 43.2 (8.1) **39.7 (5.3)** 36.9 (8.7)	-5.79% +7.59%	

				bond Universal adhesive (SBU)			**EDC 0.3M -wet + SU** Control	**30.9 (5.7)** 33.6 (6.1)	-8.04%	
						12 months	**EDC 0.3M + SMP** SMP Control **EDC 0.3 -dry + SU** Control **EDC 0.3M -wet + SU** Control	**30.8 (7.4)** 22.3 (7.3) **26.7 (4.9)** 18.8 (5.9) **11.2 (4.6)** 13.7 (4.6)	+38.12% +42.02% -18.25%	-24% -48.4% -33% -49% -64% -59.2%
31. (Pedrosa et al., 2018)	Caffeic acid (natural)	60s	Caries-free	Adper Scotchbond multipurpose / Clearfil SE bond	Distilled water	24hrs	**CAPE 0.05% (ASB)** **CAPE 0.1% (ASB)** Control **CAPE 0.05% (CSE)** **CAPE 0.1% (CSE)** Control	**34.40 (7.75)** **36.58 (6.16)** 40.67 (8.90) **23.47 (6.91)** **25.73 (5.55)** 31.74 (8.05)	-15.42% -10.06% -26.06% -18.94%	
						12 months	**CAPE 0.05% (ASB)** **CAPE 0.1% (ASB)** Control **CAPE 0.05% (CSE)** **CAPE 0.1% (CSE)** Control	**26.97 (9.88)** **22.88 (4.44)** 25.24 (9.72) **24.20 (7.78)** **26.21 (7.33)** 25.99 (6.79)	+6.85% -9.35% -6.89% +0.85%	-21.6% -37.4% -38% -3.1% -1.8% -18%
32. (Perote et al., 2015)	Chlorhexidine (CHX) (synthetic) Ethanolic Propolis Extract (EPE) (natural) Aqueous Propolis Extract (APE) (natural)	60s	Caries-free	Adper Single Bond 2	Artificial saliva/ deionised water	24hrs	**CHX 0.2%** **EPE 10%** **APE 10%** Control	**31.6 (7.0)** **29.1 (6.9)** **33.0 (6.7)** 28.6 (5.3)	+10.49% +1.75% +15.38%	
						6 months	**CHX 0.2%** **EPE 10%** **APE 10%** Control	**26.5 (4.4)** **23.1 (3.9)** **25.1 (4.8)** 24.0 (3.9)	+10.42% -3.75% +4.58%	-16% -21% -24% -16%
						1000 thermocycles	**CHX 0.2%** **EPE 10%** **APE 10%** Control	**27.0 (3.4)** **25.8 (4.4)** **26.2 (7.2)** 25.4 (6.7)	+6.3% +1.57% +3.15%	-14.5% -11.34% -20.6% -11.20%
33. (Porto, 2018)	Chlorhexidine (CHX) (synthetic) Quercetin (Que) (natural) Resveratrol (Res) (natural)	60s	Caries-free	Single Bond Universal	Distilled water	24hrs	**CHX 2%** **Que (lg ml^{1}) 100** **250** **500** **1,000** **Res (lg ml^{1}) 100** **250** **500** **1,000** **Que + Res (lg ml^{1}) 3:1 100** **250** **500** **1,000** **Que + Res 1:1 100** **250**	**27.78 (6.88)** **32.06 (8.90)** **27.51 (8.70)** **31.21 (9.93)** **31.30 (10.33)** **18.81 (6.07)** **23.90 (7.46)** **23.74 (5.98)** **20.11 (5.31)** **27.40 (7.19)** **19.33 (6.02)** **28.44 (7.07)** **31.38 (8.45)** **18.78 (3.63)** **23.93 (7.20)**	-22.45% -10.5% -23.2% -12.87% -12.62% -47.49% -33.28% -33.72% -43.86% -23.51% -46.04% -20.6% -12.4% -47.57% -33.19%	

							500 **1,000** **Que + Res 1:3 100** **250** **500** **1,000** Control	**23.29 (5.23)** **19.10 (5.49)** **22.73 (6.37)** **20.83 (6.61)** **25.99 (7.89)** **23.76 (5.76)** 23.62 (6.71)	-34.98% -46.68% -36.54% -41.85% -27.44% -33.67%	
						3 months	**CHX 2%** **Que (lg ml^{3}) 100** **250** **500** **1,000** **Res (lg ml^{3}) 100** **250** **500** **1,000** **Que + Res (lg ml^{3}) 3:1 100** **250** **500** **1,000** **Que + Res 1:1 100** **250** **500** **1,000** **Que + Res 1:3 100** **250** **500** **1,000** Control	**30.68 (8.71)** **25.29 (8.01)** **34.68 (16.17)** **42.37 (13.59)** **37.40 (11.37)** **31.03 (11.25)** **37.90 (10.11)** **29.77 (7.34)** **26.18 (7.77)** **30.48 (10.16)** **35.38 (13.54)** **31.14 (10.31)** **32.32 (8.39)** **37.13 (12.29)** **32.80 (14.05)** **32.36 (11.43)** **28.13 (8.54)** **28.56 (11.45)** **30.82 (8.77)** **26.55 (7.93)** **31.66 (10.92)** 26.47 (8.26)	+15.9% -4.46% +31.02% +60.07% +41.29% +41.29% +43.18% +12.47% -1.1% +15.15% +33.66% +17.64% +22.1% +40.27% +23.91% +22.25% +6.27% +7.9% +16.43% +0.3% +19.61%	+10.4% -21.1% +26% +35.7% +19.50% +65% +58.6% +25.4% +30.2% +11.24% +83% +9.50% +3% +97.7% +37% +395 +47% +25.6% +48% +2% +33.25% +12%
34. (Prasansuttiporn et al., 2020)	Rosmarinic acid (natural)	5s	Caries-affected	Clearfil SE Bond	Artificial Saliva	24hrs	**Rosmarinic acid 100µM** Control	**35.4 (5.5)** 35.1 (5.3)	+0.85%	
						12 months	**Rosmarinic acid 100µM** Control	**34.2 (4.3)** 30.3 (4.2)	+12.87%	-3.4% -12.7%
35. (Prasansuttiporn et al., 2017)	Rosmarinic acid (natural)	5s	Caries-free	Clearfil SE Bond	Artificial saliva	24hrs	**Rosmarinic acid 100µM** Control	**54.8 (3.9)** 55.2 (4.1)	-0.72%	
						12 months	**Rosmarinic acid 100µM** Control	**52.6 (4.7)** 45.8 (4.0)	+14.85	-4% -17%
36. (Ruksaphon K, 2017)	Chlorhexidine (CHX) (synthetic) Rosmarinic acid (natural)	60s	Caries-free	Clearfil SE Bond OptiBond FL (FL) OptiBond Solo (solo)	Artificial saliva	24hrs	**CHX 2% + (solo)** **CHX 2% + (FL)** **Rosmarinic 100µM + (solo)** **Rosmarinic 100µM + (FL)** Control (solo) Control (FL)	**38.42 (8.04)** **38.46 (7.82)** **36.00 (8.04)** **41.27 (6.76)** 39.60 (7.50) 37.27 (8.45)	-2.98% +3.19% -11.4% +3.51%	

						3 months	**CHX 2% + (solo)** **CHX 2% + (FL)** **Rosmarinic 100µM + (solo)** **Rosmarinic 100µM + (FL)** Control (solo) Control (FL)	**40.75 (7.12)** **41.26 (5.51)** **39.43 (10.12)** **41.27 (6.76)** 32.13 (7.32) 29.45 (8.12)	+26.83% +40.1% -1.3% +13.2%	+6% +7.2% +9.5% 0% -19% -21%
						6 months	**CHX 2% + (solo)** **CHX 2% + (FL)** **Rosmarinic 100µM + (solo)** **Rosmarinic 100µM + (FL)** Control (solo) Control (FL)	**32.83 (6.82)** **29.33 (6.66)** **31.37 (10.24)** **32.79 (7.37)** 30.54 (8.05) 26.46 (6.39)	+7.5% +10.85% -14.92% -8.23%	-14.55% -24% -13% -20.55% -23% -29%
						12 months	**CHX 2% + (solo)** **CHX 2% + (FL)** **Rosmarinic 100µM + (solo)** **Rosmarinic 100µM + (FL)** Control (solo)) Control (FL)	**22.85 (11.72)** **27.82 (11.54)** **28.98 (7.68)** **28.04 (9.09)** 3.10 (8.22) 3.91 (9.20)	+637.1% +611.51% -25.75% -29.19%	-40.5% -28% -19.5% -32% -92% -89.5%
37. (Sacramento et al., 2012)	Chlorhexidine (CHX) (synthetic)	60s	Caries-affected	Clearfil protect Bond (PB) Clearfil (SE) Bond	Distilled water	24hrs	**CHX 2% (SE)** **CHX 2% (PB)** Control (SE) Control (PB)	**12.39 (2.37)** **14.60 (3.65)** 12.28 (2.91) 16.24 (2.71)	+0.9% -10.1%	
						6 months	**CHX 2% (SE)** **CHX 2% (PB)** Control (SE) Control (PB)	**2.88 (1.30)** **3.09 (0.92)** 2.95 (0.77) 2.32 (0.60)	-2.37% +33.19%	-77% -79% -90% -86%
						12 months	**CHX 2% (SE)** **CHX 2% (PB)** Control (SE) Control (PB)	**1.76 (0.35)** **2.34 (0.76)** 1.36 (0.22) 1.11 (0.59)	+29.41% +110.81%	-86% -84% -89% -93%
38. (Sadeghi, 2017)	Chlorhexidine (CHX) (synthetic)	60s	Caries-free	Optibond Solo Plus (OSP)/ Single Bond Universal (SBU)	Distilled water	1 week	**CHX 0.2% + OSP** Control **CHX 0.2% +SBU** Control	**29.84 (5.43)** 34.57 (8.22) **35.75 (8.58)** 58.17 (10.25)	-13.68% -38.54%	N/A
						6 months	**CHX 0.2% + OSP** Control **CHX 0.2% +SBU** Control	**20.59 (5.52)** 22.51 (3.55) **23.28 (3.90)** 33.42 (7.04)	-8.53% -30.34%	N/A
39. (Santiago et al., 2013)	Chlorhexidine (CHX) (synthetic) EGCG (natural)	60s	Caries-free	Adper Single Bond 2	Distilled water	24hrs	**EGCG 0.02%** **EGCG 0.1%** **EGCG 0.5%** **CHX 2%** Control	**31.39 (7.82)** **34.74 (9.14)** **27.11 (7.78)** **34.68 (7.30)** 34.17 (7.75)	-8.14% +1.67% -20.66% +1.49%	

						6 months	**EGCG 0.02%** **EGCG 0.1%** **EGCG 0.5%** **CHX 2%** Control	**31.75 (10.58)** **35.99 (10.91)** **31.18 (9.29)** **31.62 (5.78)** 27.67 (6.98)	+14.75% +30.07% +12.69% +14.28%	+1.15% +3.6% +15% -9% -19%
40. (Shen et al., 2020)	Chlorhexidine (CHX) (synthetic)	60s	Caries-free	Single Bond 2/ Single Bond Universal	Distilled water	24hrs	**CHX 2%** Control	**37.43 (5.29)** 33.00 (3.95)	+13.42%	
						6 months	**CHX 2%** Control	**33.31 (3.28)** 28.36 (4.01)	+17.45%	-11% -14%
41. (Venigalla et al., 2016)	Riboflavin (natural) Carbodiimide (EDC) (synthetic) Proanthocyanidin (PRO) (natural)	120s	Caries-free	Adper Single Bond water wet bonding (WWB)/ Ethanol wet bonding (EWB)	Artificial Saliva	24hrs	**Riboflavin 0.1% + WWB** **EDC 1M +WWB** **PRO 6.5% +WWB** Control **Riboflavin 0.1% + EWB** **EDC 1M + EWB** **PRO 6.5% + EWB** Control	**46.94 (2.17)** **45.14 (1.76)** **41.71 (1.63)** 31.76 (1.51) **52.12 (0.46)** **47.50 (0.78)** **44.38 (0.69)** 41.61 (1.13)	+47.8% +42.13% +31.33% +25.26% +14.16% +6.66%	
						6 months	**Riboflavin 0.1% + WWB** **EDC 1M +WWB** **PRO 6.5% +WWB** Control **Riboflavin 0.1% + EWB** **EDC 1M + EWB** **PRO 6.5% + EWB** Control	**45.14 (1.50)** **42.58 (1.24)** **34.30 (1.21)** 23.96 (1.43) **51.80 (0.32)** **45.27 (0.50)** **41.90 (0.79)** 37.37 (0.58)	+88.4% +77.71% +43.16% +38.61% +21.14% +12.12%	-4% -6% -18% -24.5% -1% -4.7% -5.6% -10.20%
42. (Xu et al., 2020)	Benzalkonium chloride (BAC) (synthetic) Polyvinylphosphonic acid (PVPA) (synthetic) *Proanthocyanidin* (PRO) (natural)	30s	Caries-free	Clearfil SE bond	Distilled water	24hrs	**MDP 5% + BAC 1%** **MDP 5% + PVPA 1000µm/mL** **MDP 5% + PRO 15%** Control **MDP 15% + BAC 1%** **MDP 15% + PVPA1000µm/mL** **MDP 15% + PRO 15%** Control	**29.2 (6.6)** **27.9 (4.1)** **26.5 (6.9)** 26.9 (5.8) **31.7 (4.0)** **30.4 (6.7)** **30.3 (3.5)** 29.3 (3.8)	+8.55% +3.7% -1.50% +8.2% +3.75% +3.4%	
						12 months	**MDP 5% + BAC 1%** **MDP 5% + PVPA 1000µm/mL** **MDP 5% + PRO 15%** Control **MDP 15% + BAC 1%** **MDP 15% + PVPA 1000µm/mL** **MDP 15% + PRO 15%** Control	**25.9 (5.2)** **26.8 (6.3)** **25.6 (4.7)** 26.3 (6.2) **35.2 (6.1)** **31.8 (5.3)** **29.7 (3.6)** 31.5 (6.4)	-1.52% +2% -2.6% +11.75% +0.95% -5.71%	-11% -4% -3.4% -2.23% +11% +4.6% -2% -7.5%
		60s				24hrs	**CHX 2%**	**14.58 (5.048)**	-19%	

43. (Kazemi-Yazdi et al., 2020)	Chlorhexidine (CHX) (synthetic)		Caries-free	Clearfil SE Bond	Distilled water		Control	18.00 (5.54)		
						3000 thermocycles	**CHX 2%** Control	**14.36 (7.44)** 16.71 (8.00)	-14.06%	-1.5% -7.2%
44. (Da Silva et al., 2015)	Chlorhexidine (CHX) (synthetic)	60s	Caries-free	Ambar/ Single Bond 2	Distilled water	24hrs	**CHX 2% (SB)** Control **CHX 2% (Ambar)** Control	**21.7 (6.7)** 11.4 (3.6) **11.2 (5.9)** 12.5 (7.6)	+90.35% -10.4%	
						15 days	**CHX 2% (SB)** Control **CHX 2% (Ambar)** Control	**11.1 (3.6)** 6.3 (2.5) **6.8 (4.2)** 7.7 (3.6)	+76.19% -11.69%	-49% -45% -39.3% -38.4%
45. (Zheng et al., 2015)	Chlorhexidine (CHX) (synthetic) Green tea GT (natural) Ferrous sulfate $FeSO_4$ (synthetic) Galardin (synthetic)	60s	Caries-free	Optibond FL/ Clearfil SE Bond	Artificial saliva	9 months	**CHX 2% (FL)** **GT 0.05% (FL)** **$FeSO_4$ 1mM (FL)** **Galardin 0.2mM (FL)** Control **CHX 2% (SE)** **GT 0.05% (SE)** **$FeSO_4$ 1mM (SE)** **Galardin 0.2mM (SE)** Control	**32.9 (11.3)** **33.2 (14.0)** **25.3 (10.5)** **33.6 (10.5)** 25.3 (11.8) **32.9 (11.3)** **26.1 (14.2)** **25.3 (10.5)** **33.6 (14.1)** 20.3 (13.6)	+30.04% +31.23% 0% +32.81% +28.57% +28.57% +24.63% +65.52%	N/A
46. (Sadek et al., 2010)	Chlorhexidine (CHX) (synthetic)	60s	Not mentioned	Scotchbond multi-purpose (MP)/ Single Bond 2 (SB)/ Experimental ethanol wet-bonding (EWB) adhesive	Artificial saliva	24hrs	**CHX 2% + EWB** Control **CHX 2% + MP** Control **CHX 2% + SB** Control	**46.8 (5.1)** 45.8 (7.2) **41.3 (8.1)** 44.2 (3.5) **42.6 (5.2)** 42.3 (7.4)	+2.18% -6.56% +0.71%	
						9 months	**CHX 2% + EWB** Control **CHX 2% + MP** Control **CHX 2% + SB** Control	**44.6 (5.6)** 44.4 (6.9) **37.4 (5.6)** 37.4 (3.5) **38.2 (4.7)** 44.4 (4.9)	+0.45% 0% -13.96%	-4.7% -3% -9.44% -15.4% -10.33% -5%
						18 months	**CHX 2% + EWB** Control **CHX 2% + MP** Control **CHX 2% + SB** Control	**43.6 (5.5)** 44.2 (7.8) **30.5 (8.0)** 32.6 (7.1) **28.8 (8.3)** 31.5 (4.3)	-1.36% -6.44% -8.57%	-7% -3.5% -26% -26.24% -32.4% -25.5%

47. (Breschi et al., 2010a)	Galardin (synthetic)	30s	Caries-free	Adper Scotchbond 1XT (SB1XT)	Artificial saliva	24hrs	**Galardin 0.2mM** Control	**44.1 (7.3)** 41.4 (5.9)	+6.52%	
						12 months	**Galardin 0.2mM** Control	**32.4 (6.6)** 22.6 (5.4)	+43.36%	-26.5% -45.4%
48. (Stanislawczuk et al., 2009)	Chlorhexidine (CHX) (synthetic)	60s	Caries-free	Prime & Bond NT/ Single Bond (SB) 2	Distilled water	24hrs	**CHX 2% + Prime & Bond** Control **CHX 2% + (SB)** Control	**21.9 (4.7)** 22.0 (9.7) **23.4 (2.1)** 14.6 (3.1)	-0.45% +60.27%	
						6 months	**CHX 2% + Prime & Bond** Control **CHX 2% + (SB)** Control	**31.1 (3.1)** 27.2 (6.1) **31.1 (2.6)** 20.4 (2.1)	+14.34% +52.45%	+42% +23.6% +33% +39.7%
49. (Firouzmandi et al., 2020b)	Silver diamine fluoride (SDF) (synthetic)	180s	Caries-free and Caries-affected (CA)	Adper single Bond 2	Distilled water	24hrs	**SDF 30%** Control **SDF 30% (CA)** Control	**17.08 (4.88)** 18.37 (4.71) **17.63 (4.19)** 12.20 (2.34)	-7.02% +44.51%	
						6 months	**SDF 30%** Control **SDF 30% (CA)** Control	**15.72 (2.34)** 14.72 (3.51) **10.30 (3.78)** 11.53 (2.66)	+6.79% -10.67%	-8% -20% -41.58% -5.5%
50. (Giacomini et al., 2017)	Chlorhexidine (CHX) E-64 (Both synthetic)	60s	Caries-free Eroded (ERO) and Caries-affected (CA)	Adper Single Bond Universal	Artificial Saliva	24hrs	**CHX 2%** **CHX 2% (ERO)** **CHX 2% (CA)** **E-64 5µM** **E-64 5µM (ERO)** **E-64 5µM (CA)** Control Control (ERO/water) Control (CA/water)	**28.36 (5.88)** **22.53 (4.76)** **18.31 (3.50)** **28.33 (5.42)** **30.23 (6.51)** **24.51 (4.41)** 35.32 (5.30) 29.85 (4.77) 23.42 (4.95)	-19.71% -24.52% -21.82% -19.79% +1.27% +4.65%	
						6 months	**CHX 2%** **CHX 2% (ERO)** **CHX 2% (CA)** **E-64 5µM** **E-64 5µM (ERO)** **E-64 5µM (CA)** Control Control (ERO/water) Control (CA/water)	**16.50 (3.89)** **20.13 (4.62)** **16.50 (3.90)** **20.80 (3.71)** **27.70 (5.32)** **20.80 (3.71)** 27.45 (5.33) 26.07 (4.96) 20.28 (3.55)	-39.89% -22.78% -18.64% -24.23% +6.25% +2.56%	-42% -11% -10% -26.5% -8.37% -15.14% -22.3% -12.6% -13.4%
51. (Sabatini et al., 2014)	Chlorhexidine (CHX)	60s	Caries-free	Adper Single Bond Plus	Artificial Saliva	24hrs	**CHX 2%** **BAC 0.5%** **BAC 1.0%** Control	**38.3 (10.3)** **36.4 (8.4)** **51.4 (7.9)** 34.3 (7.8)	+11.66% +6.12% +49.85%	

	Benzalkonium chloride (BAC) (Both synthetic)					6 months	**CHX 2%** **BAC 0.5%** **BAC 1.0%** Control	**34.3 (5.2)** **36.6 (6.2)** **53.9 (6.9)** 27.4 (6.2)	+25.18% +33.58% +96.72%	-10.44% +0.55% +4.8% -20%
52. (Carvalho et al., 2016)	Chlorhexidine (CHX) (synthetic) EGCG (natural)	60s	Caries-affected	Adper Single Bond 2	Distilled water	24hrs	**EGCG 2%** **CHX 2%** Control	**23.0 (6.3)** **23.3 (6.0)** 24.3 (8.6)	-5.35% -4.12%	
						6 months	**EGCG 2%** **CHX 2%** Control	**35.7 (8.4)** **23.0 (7.2)** 21.6 (6.4)	+65.28% +6.48%	+55.2% -1.3% -21.2%
53. (Loguercio et al., 2016b)	Chlorhexidine (CHX) (synthetic)	15s	Caries-free	Prime & Bond NT/ Adper Single Bond 2	Distilled water	24hrs	**CHX 2% (PB)** Control **CHX 2% (SB)** Control	**44.2 (4.3)** 42.3 (3.4) **50.3 (5.6)** 46.2 (4.7)	-21.43% -8.44% +8.87%	
						2 years	**CHX 2% (PB)** Control **CHX 2% (SB)** Control	**36.3 (5.1)** 23.6 (5.3) **43.3 (3.5)** 32.3 (4.5)	+12.38% -26.93% +34.06%	-18% -44.2% -14% -30%
54. (Cova et al., 2011)	Riboflavin (natural)	60s	Caries-free	XP Bond adhesive (Dentsply)	Artificial saliva	24hrs	**Riboflavin 0.1%** Control	**44.4 (10.4)** 37.3 (10.3)	+19.03%	
						6 months	**Riboflavin 0.1%** Control	**35.6 (11.2)** 22.0 (7.0)	+61.82%	-20% -41%
						12 months	**Riboflavin 0.1%** Control	**30.9 (12.2)** 17.7 (9)	+74.58%	-30.4% -52.55%
55. (Mobarak, 2011)	Chlorhexidine (CHX) (synthetic)	60s	Caries-free and Caries-affected (CA)	Self-etch primer adhesive (Clearfil SE Bond)	Artificial saliva	24hrs	**CHX 2%** **CHX 5%** Control **CHX 2% (CA)** **CHX 5% (CA)** Control	**23.79 (5.9)** **25.94 (6.4)** 24.33 (5.1) **20.84 (6.2)** **20.59 (5.1)** 21.73 (6.0)	+9.48% +19.37% +11.97% -4.1% -5.25%	
						2 years	**CHX 2%** **CHX 5%** Control **CHX 2% (CA)** **CHX 5% (CA)** Control	**8.74 (3.2)** **10.98 (3.3)** 9.46 (3.4) **9.99 (3.4)** **14.67 (4.5)** 9.97 (3.5)	-12.34% +10.13% -5.12% +0.2% +47.14%	-63.3% -58% -61% -52% -29% -54%
56. (Manso et al., 2014)	Chlorhexidine (CHX) (synthetic)	30s	Caries-free	All Bond 3 (Bisco)/ Excite (Ivoclar Vivadent)	Distilled water	24hrs	**CHX 2%/water (Bisco)** Control **CHX 2%/ ethanol (Bisco)** Control **CHX 2%/ water (Excite)** Control	**46.96 (3.6)** 51.07 (3.6) **54.67 (3.6)** 59.41 (3.6) **40.05 (5.4)** 49.51 (5.4)	-8.05% -7.98% -19.11%	

							CHX 2%/ ethanol (Excite) Control	**53.37 (5.4)** 49.67 (5.4)	+7.45%	
						6 months	**CHX 2%/ water (Bisco)** Control **CHX 2%/ ethanol (Bisco)** Control **CHX 2%/ water (Excite)** Control **CHX 2%/ ethanol (Excite)** Control	**50.69 (3.6)** 57.13 (3.6) **52.17 (3.6)** 56.41 (3.6) **36.78 (5.4)** 42.10 (5.4) **57.47 (5.4)** 44.56 (5.4)	-11.27% -7.52% -12.64% +28.97%	+8% +12% -4.6% -5% -8.16% -15% +7.7% -10.30%
						15 months	**CHX 2%/ water (Bisco)** Control **CHX 2%/ ethanol (Bisco)** Control **CHX 2%/ water (Excite)** Control **CHX 2%/ ethanol (Excite)** Control	**46.07 (4.4)** 47.29 (4.4) **39.58 (4.4)** 44.41 (4.4) **40.87 (6.6)** 45.51 (6.6) **49.55 (6.6)** 42.48 (5.4)	-2.58% -10.88% -10.2% +16.64%	-2% -17.22% -27.6% -25.25% +2% -8% -7% -14.5%
57. (Breschi et al., 2010b)	Chlorhexidine (CHX) (synthetic)	30s	Caries-free	Adper Scotchbond 1XT	Artificial saliva	24hrs	**CHX 2%** **CHX 0.2%** Control	**41.2 (9.6)** **39.2 (9.3)** 40.8 (8.7)	+0.98% -3.92%	
						2 years	**CHX 2%** **CHX 0.2%** Control	**28.5 (7.2)** **32.6 (8.3)** 13.4 (4.9)	+112.69% +143.28%	-31% -17% -67%
58. (Montagner et al., 2015)	Chlorhexidine (CHX) (synthetic)	60s	Caries-free	Adper Single Bond 2	Distilled water	24hrs	**CHX 2%** Control	**25.3 (6.2)** 26.7 (10.0)	-5.24%	
						18 months	**CHX 2%** Control	**20.1 (10.3)** 14.8 (9.4)	+35.81%	-20.55% -44.6%
59. (Li et al., 2020)	Dopamine methacrylamide (DMA) (synthetic)	60s	Caries-free	Adper Single Bond 2	Deionised water	24hrs	**DMA 0.1 mM** **DMA1.0 mM** **DMA10 mM** Control	**28.73 (5.19)** **30.76 (7.57)** **27.06 (7.53)** 29.96 (6.43)	-4.11% +2.67% -9.68%	
						1000 thermocycles	**DMA 0.1 mM** **DMA1.0 mM** **DMA10 mM** Control	**23.84 (7.06)** **29.19 (6.58)** **23.34 (7.36)** 16.24 (6.90)	+46.8% +79.74% +43.72%	-17% -5.1% -14% -46%
60. (Hass et al., 2016)	Proanthocyanidin (PRO) (natural) Riboflavin (natural)	60s	Caries-free	Adper Single Bond 2 (SB)/ Tetric N-Bond (TN)	Distilled water	24hrs	**PRO 6.5% (SB)** **Riboflavin 0.1% (SB)** **GD 5% (SB)** Control **PRO 6.5% (TN)** **Riboflavin 0.1% (TN)**	**36.2 (5.5)** **37.1 (9.7)** **38.5 (2.4)** 39.5 (7.9) **29.2 (1.2)** **31.5 (6.9)**	-8.35% -6.08% -2.53% -20.65% -14.4%	

	Glutaraldehyde (GD) (synthetic)						**GD 5% (TN)** Control	**35.7 (1.9)** 36.8 (4.7)	-2.99%	
						18 months	**PRO 6.5% (SB)** **Riboflavin 0.1% (SB)** **GD 5% (SB)** Control **PRO 6.5% (TN)** **Riboflavin 0.1% (TN)** **GD 5% (TN)** Control	**31.9 (4.3)** **31.6 (3.5)** **29.7 (2.6)** 13.9 (1.8) **27.6 (6.3)** **25.1 (1.3)** **24.2 (1.4)** 13.9 (1.8)	+129.5% +127.3% +133.6% +98.56% +80.58% +74.1%	-12% -15% -23% -65% -5.5% -20.3% -32.2% -62.2%
61. (Kalagi et al., 2020)	Chlorhexidine (CHX) (synthetic)	5s	Caries-free	Adper Scotchbond Multipurpose (SBMP)	Distilled water	24hrs	**CHX 2%** Control	**66.4 (8.8**) 49.1 (12.6)	+35%	
						6 months	**CHX 2%** Control	**71.9 (14.7)** 41.6 (10.6)	+72%	-8.9% -15.3%
62. (Tekce et al., 2016)	Chlorhexidine (CHX) (synthetic)	60s	Caries-free	Single Bond Universal (SBU) All Bond Universal (ABU)	Distilled water	24hrs	**CHX 2% (SBU)** Control **CHX 2% (ABU)** Control	**45.22 (6.32)** 43.33 (3.41) **38.92 (4.01)** 43.81 (3.61)	+4.36% -11.16%	
						12 months	**CHX 2% (SBU)** Control **CHX 2% (ABU)** Control	**41.19 (3.98)** 37.67 (3.40) **31.37 (5.97)** 38.54 (6.19)	+9.34% -18.6%	-9% -13% -19.4% -12%

Table 1.3 Quality assessment and risk of bias.

This table demonstrated the quality assessment and risk of bias as reported in the materials and methods section.

Paper (year)	Randomi -sation	Substrate condition	Dentine pretreatment duration	Manufacturer instruction	Storage medium	Interface surface area	Restorative procedure performed by same operator	Sample size calculation	Blinding of the bond strength test operator	Risk of bias
(Baena et al., 2020)	N	Y	Y	Y	Y	Y	N	N	N	High
(Balloni et al., 2016)	Y	Y	Y	Y	Y	Y	N	N	Y	Medium
(Bravo et al., 2017)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Bueno et al., 2015)	Y	N	Y	Y	Y	Y	N	N	N	High
(Comba et al., 2020)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Czech et al., 2019)	Y	Y	Y	Y	Y	Y	Y	N	N	Medium
(Davila-Sanchez et al., 2020)	Y	Y	Y	Y	Y	Y	Y	Y	N	Low
(de Araújo Costaa et al., 2019)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Maha A. El Baz, 2018)	N	Y	Y	Y	Y	Y	N	Y	N	Medium
(Fang et al., 2017)	N	Y	Y	Y	Y	Y	N	N	N	High
(Fernandes et al., 2020)	Y	Y	Y	Y	Y	Y	N	Y	N	Medium
(Fialho et al., 2019)	Y	Y	Y	Y	Y	Y	Y	Y	N	Low

(Gerhardt et al., 2016)	Y	Y	Y	Y	Y	N	N	N	N	High
(Campos et al., 2019)	Y	Y	N	Y	Y	Y	Y	N	N	Medium
(Giacomini et al., 2020)	Y	Y	Y	Y	Y	Y	N	Y	N	Medium
(Grandizoli, 2018)	Y	Y	Y	Y	Y	Y	N	Y	N	Medium
(Karrabi and Danesh Kazemi, 2016)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Kasraei S., 2017)	Y	Y	Y	N	Y	Y	N	N	N	High
(Lenzi et al., 2014)	Y	Y	Y	N	Y	Y	N	N	N	High
(Li et al., 2018)	Y	Y	Y	N	Y	Y	N	N	N	High
(Loguercio et al., 2016a)	Y	Y	Y	N	Y	Y	N	N	N	High
(Loguercio et al., 2009b)	Y	Y	Y	Y	Y	Y	Y	N	N	Medium
(Maravic et al., 2018)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Mazzoni et al., 2013)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Mazzoni et al., 2018)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Mohamed et al., 2020)	N	Y	Y	Y	Y	Y	N	N	N	High
(Mosallam et al., 2018)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Mosallam et al., 2019)	Y	Y	Y	Y	Y	N	N	N	N	High
(Ou et al., 2018a)	Y	Y	Y	Y	Y	N	N	N	N	High

(Paulose and Fawzy, 2018)	Y	Y	Y	Y	Y	Y	N	N	N	High
(Pedrosa et al., 2018)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Perote et al., 2015)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Porto, 2018)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Prasansuttiporn et al., 2020)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Prasansuttiporn et al., 2017)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Ruksaphon K, 2017)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Sacramento et al., 2012)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Sadeghi, 2017)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Santiago et al., 2013)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Shen et al., 2020)	Y	Y	Y	N	Y	Y	N	N	N	High
(Venigalla et al., 2016)	Y	Y	Y	Y	Y	N	N	N	N	High
(Xu et al., 2020)	Y	Y	Y	N	Y	Y	N	N	N	High
(Kazemi-Yazdi et al., 2020)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Da Silva et al., 2015)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Zheng et al., 2015)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Sadek et al., 2010)	Y	N	Y	Y	Y	Y	N	N	N	High
(Breschi et al., 2010a)	Y	Y	Y	Y	Y	Y	N	N	N	Medium

(Stanislawczuk et al., 2009)	Y	Y	Y	N	Y	Y	Y	N	N	Medium
(Firouzmandi et al., 2020b)	N	Y	Y	Y	Y	N	N	N	N	High
(Giacomini et al., 2017)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Sabatini et al., 2014)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Carvalho et al., 2016)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Loguercio et al., 2016b)	Y	Y	Y	Y	Y	Y	Y	N	N	Medium
(Cova et al., 2011)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Mobarak, 2011)	N	Y	Y	Y	Y	N	N	N	N	High
(Manso et al., 2014)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Breschi et al., 2010b)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Montagner et al., 2015)	Y	Y	Y	Y	Y	Y	Y	N	N	Medium
(Li et al., 2020)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Hass et al., 2016)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Kalagi et al., 2020)	Y	Y	Y	Y	Y	Y	N	N	N	Medium
(Tekce et al., 2016)	Y	Y	Y	Y	Y	Y	Y	N	N	Medium

6.5. Discussion

This systematic review aimed to collect and analyse available evidence on the influence of different MMP inhibitors, as dentine surface pretreatment, on the immediate and long-term bond strength of direct coronal composite restorations. Based on the results, it was found that the type of the MMP inhibitor affects the bond strength. For instance, some inhibitors demonstrated no noticeable effect on the bond strength, while others presented a slight favourable trend towards an increase in bond strength. This may be due to the different MMP inhibitor's modes of action. As mentioned in the results, 31 different types of MMP inhibitors (synthetic or naturally derived) were used in this review. Some of these inhibitors inhibit MMP's activity via Zn^{2+} or Ca^{2+} cation-chelating properties, such as CHX (Ou et al., 2018b). Others such as EGCG bind to collagen via hydrogen bonding and hydrophobic interaction, crosslinking collagen and blocking collagenase's action at the treated site (Jung et al., 2011).

Additionally, the duration at which these MMP inhibitors were applied varied, which may affect the outcome. It was shown in this review that as the duration of application increases (between 30s to 120s), the bond strength increase. This could be due to the inhibitory effect on the MMP's enzyme, which preserves the integrity of the collagen fibrils, consequently preserving or not negatively affecting the bond strength. Hypothetically, application for less than 30s would not be enough for the inhibitory effect to occur. On the other hand, application for more than 120s may lead to either evaporation or dissolution of the inhibitor. Nonetheless, this again could be influenced

by the type of MMP inhibitor used. For instance, some studies suggest that CHX can cause MMP activity inhibition and increase in bond strength when applied for 15s only (Loguercio et al., 2009a). While other studies, that used SDF as an MMP inhibitor, demonstrated a decrease bond strength when applied for 180s (Firouzmandi et al., 2020a).

As expected and as the results suggested in the majority of the studies, the ageing factor affects the bond stability by decreasing its strength. This may be attributed to the hydrolysis effect of the storage medium on the resin part of the hybrid layer, as suggested by previous studies (Mazzoni et al., 2015; Tjäderhane et al., 2013).

The ageing solution is another essential factor to be considered. The majority of the included studies used water as a gold standard. Other included studies used artificial saliva as they closely resemble the oral physiological environment. However, it is worth noting that water use has been shown to underestimate the degradation activity of MMPs. Artificial saliva on the other hand, contains Ca and Zn ions which may overestimate the MMP's degrading activity (Tezvergil-Mutluay et al., 2010; De Munck et al., 2005b).

There are various limitations to the presented systematic review. No statistical analysis for the obtained data was conducted. Additionally, the study that incorporated MMP inhibitors in the adhesive systems were not included Nevertheless, another systematic review currently under process by the same authors is currently looking at this.. Another limitation to be considered is that this review only included in-vitro studies. In-vivo studies and clinical trials would provide stronger evidence, as it can evaluate the safety, toxicity and efficacy of a given intervention in a complex model (Rezaee and Abdollahi, 2017). However, very few in-vivo studies were found in the literature and

accordingly, they were excluded. Moreover, the effect of various bonding agents used was not included in the final data interpretation in relation to the bond strength.

Regarding the risk of bias, out of all the included studies, only one study mentioned the blinding of the operator testing for the bond strength. Although the results may exhibit the standard level of bond strength reporting, reporting whether the person performing the bond strength tests was blinded or not should at least be reported. This will increase the credibility of the study and make it clear in assessing the risk of bias. Moreover, out of all the 62 studies included, only six studies calculated the sample size and demonstrated a power analysis out of all the studies. The main purpose of using power analysis is to aid the researcher to ascertain the smallest sample size suitable to identify the effect of a given test at the desired level of significance, hence including it would be essential (Cohen, 1992).

The data provided in this systematic review may aid clinicians in predicting the outcome for their restoration's bond strength and give them support based on evidence.

6.6. Conclusion

Different MMP inhibitors demonstrated different effects on bond strength of direct coronal composite restorations. The duration of the MMP inhibitor's application positively correlates to the bond strength as long as it is between 30s and 120s. The bond strength was negatively affected by ageing.

7. CHAPTER SEVEN: Conclusion, study limitations and future remarks

7.1. General conclusion and future prospects

In chapter 3, concerning adhesion and sealing, the optimized formulation was shown to form resin-tags to caries-affected dentine of primary teeth. The infiltrated resins tags were longer covering a large area of the carious substrate than commercial filling materials. In chapter 6, the optimized formulation was shown to inhibit the proteolytic (degrading) dentine enzyme matrix-metalloproteinase (MMP). The reduction was higher when compared to commercial fillings.

As mentioned previously, caries affects roughly 2.5 billion people globally, which is 37% of the world population, according to the world health organization (Cooper, 2018). With these numbers, a wide range of consequences arises affecting paediatric patient's quality of life and healthcare system expenditure. The optimized formulation presented could be added to the dentist's toolbox and utilized in treating children's teeth effectively. Since the restoration can be applied as a single step, it significantly reduces the procedure time. In return, this will make it easy for a general dental practitioner to apply it in an anxious child, thus, reducing the number of referrals to speciality clinics. Moreover, since children are treated early and in a short time, the chance of caries progression will be low, which lowers the chances of children requiring hospitalization for dental extractions. This, in return, would decrease the expenditure on the management of caries in children, which, as stated before, costs the NHS billions of pounds.

7.1.1. Study limitations

In chapter 3, the ability of the optimised formulation to form long resin tags was demonstrated qualitatively. Nonetheless, this experiment would benefit from quantitative results to accurately demonstrate the variations in resin tag lengths across

a larger surface area along the substrate. This is essential, as assessing it from 2D surfaces will not be accurately represented. In addition, the sample size of the experiments was small, and thus a larger sample size would be ideal.

In chapter 5, the ability of the optimised formulation to inhibit MMP activity was demonstrated both qualitatively and quantitatively. Although it was argued that both PLS and MCPM work reciprocally to aid in the MMP inhibition, it is vital to determine which one directly cause the effect. Therefore, another group of the optimized formulation with and without PLS will be more beneficial in determining this.

Finally, the optimized formulation will require more testing regarding durability and longevity. Nonetheless, experiments using thermocycling are being performed in the lab to evaluate this. Moreover, the material will undergo more prolonged clinical trials in children's teeth at the next Clinical Trial phase, and accordingly, this will provide more understanding of the durability of the novel composite.

7.1.2. Current updates for the optimized formulation and future work

7.1.2.1. Scaling-up and manufacture

Two companies, Schottlander™ and DMG™, which both have an ISO 13485, are scaling up and manufacturing the optimized formulation. The company assess the stability and the reproducibility of the restoration. Concerning sealability, the company is aiming to increase this reduction scale from 10g batches to 2kgs. Furthermore, Schottlander™ registered a trademark for the optimized formulation calling it '*Renewal MI*™'.

7.1.2.2. Cytotoxicity and carcinogenicity test

In order to market products to be used in humans, rigorous tests on cytotoxicity and carcinogenicity are required. These tests were performed in our lab previously; nonetheless, they are required to be reproduced and obtained by other labs with Good Laboratory Practice (GLP). ENVIGO^{TM} lab is the lab responsible for repeating the cytotoxicity and carcinogenicity tests for the optimized composite formulation.

7.1.3. Application for a clinical trial

For products that do not have a CE mark, application for ethical and regulatory approvals. The applications are made through the integrated research approval system (IRAS) and the Medicines and Healthcare products Regulatory Agency (MHRA). Various processes will then need to be provided to the MHRA. These include a summary of benchmark tastings, biological assessments, investigator's brochure, instructions for use, essential evidence for the medical device directive (MDD) conformity, clinical investigation plan and consent forms.

Appendix : Biobank forms and certificates

University College London Hospitals NHS
NHS Foundation Trust
UCL/UCLH Biobank for Studying Health and Disease

Risk assessment form for Projects using Human Tissues

This document should be completed in line with UCL EDI HTA SOP Assessing risk for Human Tissue Projects

Form:	**Risk Assessment form for Projects using Human Tissues**		
Version Number:	**01**	**Effective Date:**	**10.12.2017**
Superseded Version Number & Date:	**N/A**	**Review Date:**	
Prepared by:	**Prof Yuan Ling Ng (DI)**		
Authorised by:	**Prof Yuan Ling Ng (DI)**		
HTA licence Number:	**12277**		

1. Project Details

Project Title	Remineralising antibacterial composites & adhesives for more durable, conservative & painless tooth restoration	Principal Investigator	YOUNG
Ethical approval number	/	Human tissue(s) used in the study	Teeth
Project start date	Sep. 2018	Name of the Assessor Date of assessment	[redacted] 21/10/19

University College London Hospitals NHS
NHS Foundation Trust
UCL/UCLH Biobank for Studying Health and Disease

5 = likely

2. Identification of Risks

Procedure	Potential Risks Identified	Control Measures	Likelihood (1-5)	Severity (1-5)	Score (L*S) (1-25)
Acquisition	Consent not or inappropriately obtained for tissue samples	Training given + flowchart	1	2	3
	No ethical/governance approvals for project		1	2	3
	Work continues after donor withdraws consent	in-built in	1	2	3
	Lack of material transfer agreement for tissues obtained externally	Audited by dept lead	1	2	3
Transportation *(If human tissue is not being transported as part of this project please ignore this section)*	Delay or loss in Transit	Protocol in place	1	1	2
	Damage of tissue in Transit		1	1	2
	Packaging Failure	Very short distances	1	2	3
	Loss of sample traceability to site of donation		1	2	3
Use	Sample used without explicit written consent from donor	See measures for acquisition	1	2	3
	Samples used for other purposes not indicated on consent form		1	2	3
	Tissues handled by individual not part of the research team		1	3	4
	Procedure damages tissue sample		1	1	2
	Equipment malfunction		1	1	2
Storage	Incorrect storage conditions used	Audited by dept lead	1	1	2
	Malfunction of storage equipment		1	1	2
Disposal	Sample disposed in error	Training given	1	1	2
	Disposal of sample not tracked or incorrectly recorded		1	2	3

Principal Investigator	**Signed** [redacted]	**Date** 21/01/19
DI/PD	**Signed**	**Date**

Human tissue samples stored on UCL premises

1. Do you have any human tissue samples stored on UCL premises? **Yes**.

Name: Hasan Jamal Department: Paediatric Department at The Eastman Dental Hospital
Email: hasan.jamal.18@ucl.ac.uk

If the answer to this question is no, please email the form to y.ng@ucl.ac.uk now, there is no need to complete the remaining sections. If you do have stored human tissue samples, please answer the questions below before returning the form to y.ng@ucl.ac.uk.

2. What is the purpose for which the tissue is stored?
Research.

If applicable, please state the title of the research project,

A novel atraumatic self-bonding and self-healing dental composite to restore carious primary dentition

**Note: If you have tissue collections stored for a range of different purposes/projects then it may be easiest to complete a separate copy of this form for each one.*

3. Who is responsible for the stored tissue (please list if more than one person is involved)?

Name: same as above Email: same as above Phone: same as above

4. What type of tissue is stored (e.g. teeth, saliva, etc.)? **Teeth**.

5. How many samples are currently held, and in what form (e.g. tissue sections, frozen fluids, fixed explants etc.)? 15 teeth (stored in deionised water after being disinfected via 1% Chloramine T.)

6. Where exactly are the tissue samples stored (building, room number, fridge/freezer etc.)?
Eastman Dental Institute, Alexander Wing building, 2nd Floor, Laser Laboratory room no. 220, HTA fridge, Enclosed in a transparent plastic box, with the biobank approval document.

7. Is the tissue stored as part of an NHS REC approved project? **No**
8. Is the tissue stored as part of an EDI Biobank approved project **Yes**
If yes, please supply title, reference number and start/end dates.
Ref. no. **1304**.
(1304-19-001, 1304-19-002, 1304-19-003, 1304-19-004, 1304-19-005, 1304-19-006, 1304-19-007, 1304-19-008, 1304-19-009, 1304-19-010, 1304-19-011, 1304-19-012, 1304-19-013, 1304-19-014, 1304-19-015).
Start/ End date **Sep. 2018 – Sep. 2021.**
9. If the answers to (7) and (8) were 'no', please answer the following questions:
(a) was consent obtained for the storage of the tissue? **Yes**
(b) are the tissue samples identifiable? **Yes**
10. Any other comments you have about your storage of human tissue? Disinfected samples.

Prof Yuan Ling (Paula) Ng
Designated Individual for Human Tissue Act
UCL Eastman Dental Institute
256 Gray's Inn Road London WC1X 8LD

References

Addy M, Prayitno S, Taylor L, et al. (1979) An in vitro study of the role of dietary factors in the aetiolgy of tooth staining associated with the use of chlorhexidine. *Journal of Periodontal Research* 14(5): 403-410.

Alshahni RZ, Shimada Y, Zhou Y, et al. (2019) Cavity adaptation of composite restorations prepared at crown and root: Optical assessment using SS-OCT. *Dental Materials Journal*. 2018-2265.

Apolonio FM, Mazzoni A, Angeloni V, et al. (2017) Effect of a one-step self-etch adhesive on endogenous dentin matrix metalloproteinases. *European Journal of Oral Sciences* 125(2): 168-172.

Araujo MW, Lipman RD and Platt JA (2019) Amalgam: Impact on oral health and the environment must be supported by science. *The Journal of the American Dental Association* 150(10): 813-815.

Babich H, Wurzburger B, Rubin Y, et al. (1995) Anin vitro study on the cytotoxicity of chlorhexidine digluconate to human gingival cells. *Cell biology and toxicology* 11(2): 79-88.

Baena E, Cunha SR, Maravić T, et al. (2020) Effect of chitosan as a cross-linker on matrix metalloproteinase activity and bond stability with different adhesive systems. *Marine Drugs* 18(5): 263.

Bakhsh TA, Sadr A, Shimada Y, et al. (2011) Non-invasive quantification of resin–dentin interfacial gaps using optical coherence tomography: validation against confocal microscopy. *Dental Materials* 27(9): 915-925.

Bakhsh TA, Sadr A, Shimada Y, et al. (2020) Does lining class-II cavities with flowable composite improve the interfacial adaptation? *Journal of Adhesion Science and Technology* 34(4): 400-416.

Balloni ECP, Amaral FLBd, França FMG, et al. (2016) Influence of chlorhexidine in cavities prepared with ultrasonic or diamond tips on microtensile bond strength. *Journal of Adhesion Science and Technology* 31(10): 1133-1141.

Banerjee A (2015) The contemporary practice of minimally invasive dentistry. *Faculty Dental Journal* 6(2): 78-85.

Barbosa C, Kato M and Buzalaf M (2011) Effect of supplementation of soft drinks with green tea extract on their erosive potential against dentine. *Australian Dental Journal* 56(3): 317-321.

Beech DR (1973) Improvement in the adhesion of polyacrylate cements to human dentine. *British Dental Journal* 135(10): 442-445.

Benetti AR, Michou S, Larsen L, et al. (2019) Adhesion and marginal adaptation of a claimed bioactive, restorative material. *Biomater Investig Dent* 6(1): 90-98.

Berkovitz BK, Holland GR and Moxham BJ (2017) *Oral Anatomy, Histology and Embryology E-Book.* Elsevier Health Sciences.

Biswas N, Dey A, Kundu S, et al. (2013) Mechanical properties of enamel nanocomposite. *ISRN Biomaterials* 2013.

Bjorklund G, Hilt B, Dadar M, et al. (2019) Neurotoxic effects of mercury exposure in dental personnel. *Basic & Clinical Pharmacology & Toxicology* 124(5): 568-574.

Black GV (1884) *The Formation of Poisons by Micro-organisms: A Biological Study of the Germ Theory of Disease.* P. Blakiston, son & Company.

Black GV (1895) An Investigation of the Physical Characters of the Human Teeth in Relation to their Diseases, and to Practical Dental Operations, together with the Physical Characters of Filling-materials. *Dent. Cosmos* 37: 469-484.

Boaro LCC, Campos LM, Varca GHC, et al. (2019) Antibacterial resin-based composite containing chlorhexidine for dental applications. *Dental Materials* 35(6): 909-918.

Bohns FR, Degrazia FW, de Souza Balbinot G, et al. (2019) Boron nitride nanotubes as filler for resin-based dental sealants. *Scientific reports* 9(1): 1-8.

Bonsor SJ and Pearson G (2012) *A clinical guide to applied dental materials.* Elsevier Health Sciences.

Bowen RL, Paffenbarger GC and Millineaux AL (1968) A laboratory and clinical comparison of silicate cements and a direct-filling resin: a progress report. *Journal of Prosthetic Dentistry* 20(5): 426-437.

Braga RR, Ballester RY and Ferracane JL (2005) Factors involved in the development of polymerization shrinkage stress in resin-composites: a systematic review. *Dental Materials* 21(10): 962-970.

Bravo C, Sampaio CS, Hirata R, et al. (2017) In-vitro Comparative Study of the use of 2 % Chlorhexidine on Microtensile Bond Strength of Different Dentin Adhesives: A 6 Months Evaluation. *International Journal of Morphology* 35(3): 893-900.

Breschi L, Maravic T, Cunha SR, et al. (2018) Dentin bonding systems: From dentin collagen structure to bond preservation and clinical applications. *Dental Materials* 34(1): 78-96.

Breschi L, Martin P, Mazzoni A, et al. (2010a) Use of a specific MMP-inhibitor (galardin) for preservation of hybrid layer. *Dental Materials* 26(6): 571-578.

Breschi L, Mazzoni A, Nato F, et al. (2010b) Chlorhexidine stabilizes the adhesive interface: a 2-year in vitro study. *Dental Materials* 26(4): 320-325.

Bueno MdFT, Basting RT, Turssi CP, et al. (2015) Effect of 2% chlorhexidine digluconate application and water storage on the bond strength to superficial and deep dentin. *Journal of Adhesion Science and Technology* 29(12): 1258-1267.

Bullard RH, Leinfelder KF and Russell CM (1988) Effect of coefficient of thermal expansion on microleakage. *Journal of the American Dental Association* 116(7): 871-874.

Buonocore MG (1955) A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. *Journal of Dental Research* 34(6): 849-853.

Campos R, Oliveira C, Macedo J, et al. (2019) Effect of zinc chloride added to self-etching primer on bond strength to caries-affected dentin and chemical-physical-mechanical properties of adhesives. *International Journal of Adhesion and Adhesives* 95: 102412.

Carvalho C, Fernandes FP, Freitas Vda P, et al. (2016) Effect of green tea extract on bonding durability of an etch-and-rinse adhesive system to caries-affected dentin. *J Appl Oral Sci* 24(3): 211-217.

Chaudhari PR, Shashikiran N, Hadkar S, et al. (2020) Comparative Evaluation of Antibacterial Efficacy and Microhardness after Adding Different Combinations of Triple Antibiotic Powder in Conventional Glass Ionomer Cement Restorative Cement. *Journal of Clinical & Diagnostic Research* 14(3).

Chaussain-Miller C, Fioretti F, Goldberg M, et al. (2006) The role of matrix metalloproteinases (MMPs) in human caries. *Journal of Dental Research* 85(1): 22-32.

Cheng L, Zhang K, Weir MD, et al. (2015) Nanotechnology strategies for antibacterial and remineralizing composites and adhesives to tackle dental caries. *Nanomedicine (Lond)* 10(4): 627-641.

Chesterman J, Jowett A, Gallacher A, et al. (2017) Bulk-fill resin-based composite restorative materials: a review. *British Dental Journal* 222(5): 337-344.

Chheda A and Vernekar M (2015) A natural preservative ε-poly-L-lysine: fermentative production and applications in food industry. *International Food Research Journal* 22(1).

Chiego DJ (2018) *Essentials of Oral Histology and Embryology E-Book: A Clinical Approach.* Elsevier Health Sciences.

Chowdhary N and Reddy VS (2010) Dentin comparison in primary and permanent molars under transmitted and polarised light microscopy: An in vitro study. *Journal of Indian Society of Pedodontics and Preventive Dentistry* 28(3): 167.

Cleghorn BM, Boorberg NB and Christie WH (2010) Primary human teeth and their root canal systems. *Endodontic Topics* 23(1): 6-33.

Cohen J (1992) Statistical power analysis. *Current directions in psychological science* 1(3): 98-101.

Comba A, Maravic T, Villalta V, et al. (2020) Effect of an ethanol cross-linker on universal adhesive. *Dental Materials* 36(12): 1645-1654.

Cooper C (2018) Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet* 392(10159): 1789-1858.

Cova A, Breschi L, Nato F, et al. (2011) Effect of UVA-activated riboflavin on dentin bonding. *Journal of Dental Research* 90(12): 1439-1445.

Czech R, Oliveira C, França F, et al. (2019) 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* 33(22): 2430-2442.

Da Silva EM, Glir DH, Gill AW, et al. (2015) Effect of Chlorhexidine on Dentin Bond Strength of Two Adhesive Systems after Storage in Different Media. *Brazilian Dental Journal* 26(6): 642-647.

Daculsi G, Menanteau J, Kerebel LM, et al. (1984) Length and shape of enamel crystals. *Calcified Tissue International* 36(5): 550-555.

Davidson CL and Feilzer AJ (1997) Polymerization shrinkage and polymerization shrinkage stress in polymer-based restoratives. *Journal of Dentistry* 25(6): 435-440.

Davila-Sanchez A, Gutierrez MF, Bermudez JP, et al. (2020) Influence of flavonoids on long-term bonding stability on caries-affected dentin. *Dental Materials* 36(9): 1151-1160.

Dawes C (2003) What is the critical pH and why does a tooth dissolve in acid? *Journal-Canadian Dental Association* 69(11): 722-725.

de Araújo Costaa CAG, Passosb VF, Neric JR, et al. (2019) Effect of Metalloproteinase Inhibitors on Bond Strength of a Self-etching Adhesive on Erosively Demineralized Dentin. *Journal of Adhesive Dentistry* 21(4).

de Assuncao IV, da Costa Gde F and Borges BC (2014) Systematic review of noninvasive treatments to arrest dentin non-cavitated caries lesions. *World J Clin Cases* 2(5): 137-141.

de Carvalho RV, Vieira EO, Gaviolli E, et al. (2020) Doxycycline as a dentin pretreatment agent for MMP-2 inhibition and maintaining hybrid layer stability over time. *International Journal of Adhesion and Adhesives* 98: 102510.

De Munck J, Van Landuyt K, Peumans M, et al. (2005a) A critical review of the durability of adhesion to tooth tissue: methods and results. *Journal of Dental Research* 84(2): 118-132.

De Munck Jd, Van Landuyt K, Peumans M, et al. (2005b) A critical review of the durability of adhesion to tooth tissue: methods and results. *Journal of dental research* 84(2): 118-132.

Degrazia FW, Leitune VCB, Samuel SMW, et al. (2017) Boron nitride nanotubes as novel fillers for improving the properties of dental adhesives. *Journal of Dentistry* 62: 85-90.

DeVito-Moraes AG, Francci C, Vidal CM, et al. (2016) Phosphoric acid concentration affects dentinal MMPs activity. *Journal of Dentistry* 53: 30-37.

Dickens SH, Flaim GM and Takagi S (2003) Mechanical properties and biochemical activity of remineralizing resin-based Ca-PO4 cements. *Dental Materials* 19(6): 558-566.

Ekstrand KR, Gimenez T, Ferreira FR, et al. (2018) The International Caries Detection and Assessment System–ICDAS: A Systematic Review. *Caries Research* 52(5): 406-419.

Elamin F, Abdelazeem N, Salah I, et al. (2019) A randomized clinical trial comparing Hall vs conventional technique in placing preformed metal crowns from Sudan. *PloS One* 14(6): e0217740.

Fang H, Li QL, Han M, et al. (2017) Anti-proteolytic property and bonding durability of mussel adhesive protein-modified dentin adhesive interface. *Dental Materials* 33(10): 1075-1083.

Farkhondeh T, Afshari R, Mehrpour O, et al. (2020) Mercury and Atherosclerosis: Cell Biology, Pathophysiology, and Epidemiological Studies. *Biological Trace Element Research* 196(1): 27-36.

Fatemeh K, Mohammad Javad M and Samaneh K (2017) The effect of silver nanoparticles on composite shear bond strength to dentin with different adhesion protocols. *J Appl Oral Sci* 25(4): 367-373.

Fathi M and Mortazavi V (2004) A review on dental amalgam corrosion and its consequences.

Featherstone JD (2000) The science and practice of caries prevention. *Journal of the American Dental Association* 131(7): 887-899.

Fejerskov O and Kidd E (2009) *Dental caries: the disease and its clinical management.* John Wiley & Sons.

Fernandes FP, Adorno CC, da Silva TM, et al. (2020) Addition of EGCG to self-etching primer: effect on adhesive properties and bond stability to dentin. *Journal of Adhesion Science and Technology*. 1-14.

Fialho MPN, Hass V, Nogueira RP, et al. (2019) 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* 91: 398-405.

Firouzmandi M, Mohaghegh M and Jafarpisheh M (2020a) Effect of silver diamine fluoride on the bond durability of normal and carious dentin. *Journal of Clinical and Experimental Dentistry* 12(5): e468.

Firouzmandi M, Mohaghegh M and Jafarpisheh M (2020b) Effect of silver diamine fluoride on the bond durability of normal and carious dentin. *J Clin Exp Dent* 12(5): e468-e473.

Frassetto A, Breschi L, Turco G, et al. (2016) Mechanisms of degradation of the hybrid layer in adhesive dentistry and therapeutic agents to improve bond durability—A literature review. *Dental Materials* 32(2): e41-e53.

Fredin B (1994) Mercury release from dental amalgam fillings. *International Journal of Risk & Safety in Medicine* 4(3): 197-208.

Frencken JE (2009) Evolution of the the ART approach: highlights and achievements. *J Appl Oral Sci* 17 Suppl(SPE): 78-83.

Frencken JE, Pilot T, Songpaisan Y, et al. (1996) Atraumatic restorative treatment (ART): rationale, technique, and development. *Journal of Public Health Dentistry* 56(3 Spec No): 135-140; discussion 161-133.

Garg C, Sharma AK, Gupta A, et al. (2019) Anisamido-Polyethylenimines as Efficient Nonviral Vectors for the Transport of Plasmid DNA to Sigma Receptor–Bearing Cells In Vitro. *Journal of pharmaceutical sciences* 108(4): 1552-1558.

Gerhardt K, Oliveira C, França F, et al. (2016) 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* 71: 23-27.

Giacomini MC, Scaffa P, Chaves LP, et al. (2017) Role of Proteolytic Enzyme Inhibitors on Carious and Eroded Dentin Associated With a Universal Bonding System. *Operative Dentistry* 42(6): E188-E196.

Giacomini MC, Scaffa PMC, Goncalves RS, et al. (2020) Profile of a 10-MDP-based universal adhesive system associated with chlorhexidine: Dentin bond

strength and in situ zymography performance. *Journal of the Mechanical Behavior of Biomedical Materials* 110: 103925.

Grandizoli SLP (2018) Influence of Protease Inhibitors on Bond Degradation of Self-Etch Adhesive Systems to Caries-Affected Dentin: An in Vitro Study. *Advances in Biological Chemistry* 8.

Griffin SO, Oong E, Kohn W, et al. (2008) The effectiveness of sealants in managing caries lesions. *Journal of Dental Research* 87(2): 169-174.

Guo X, Cheng Q, Yu G, et al. (2020) The functions of hydrophobic elastic polyurethane combined with an antibacterial triclosan derivative in the dentin restoration interface. *Journal of the Mechanical Behavior of Biomedical Materials* 102: 103471.

Haak R, Hahnel M, Schneider H, et al. (2019) Clinical and OCT outcomes of a universal adhesive in a randomized clinical trial after 12 months. *Journal of Dentistry* 90: 103200.

Habib E, Wang R, Wang Y, et al. (2016) Inorganic Fillers for Dental Resin Composites: Present and Future. *ACS Biomater Sci Eng* 2(1): 1-11.

Hadzovic A (2018) *Solutions Manual to Accompany Inorganic Chemistry 7th Edition.* Oxford University Press.

Handelman SL, Washburn F and Wopperer P (1976) Two-year report of sealant effect on bacteria in dental caries. *Journal of the American Dental Association* 93(5): 967-970.

Hass V, Luque-Martinez IV, Gutierrez MF, et al. (2016) Collagen cross-linkers on dentin bonding: Stability of the adhesive interfaces, degree of conversion of the adhesive, cytotoxicity and in situ MMP inhibition. *Dental Materials* 32(6): 732-741.

Henn S, de Carvalho RV, Ogliari FA, et al. (2012) Addition of zinc methacrylate in dental polymers: MMP-2 inhibition and ultimate tensile strength evaluation. *Clinical Oral Investigations* 16(2): 531-536.

Hiraki J, Ichikawa T, Ninomiya S-i, et al. (2003) Use of ADME studies to confirm the safety of ε-polylysine as a preservative in food. *Regulatory Toxicology and Pharmacology* 37(2): 328-340.

Hussein I, Al Halabi M, Kowash M, et al. (2020) Use of the Hall technique by specialist paediatric dentists: a global perspective. *British Dental Journal* 228(1): 33-38.

Hyldgaard M, Mygind T, Vad BS, et al. (2014) The antimicrobial mechanism of action of epsilon-poly-l-lysine. *Applied and Environmental Microbiology* 80(24): 7758-7770.

Inada E, Saitoh I, Hayasaki H, et al. (2012) Comparison of normal permanent and primary dentition sagittal tooth-crown inclinations of Japanese females. *CRANIO*® 30(1): 41-51.

Innes NP, Clarkson JE, Douglas GVA, et al. (2020) Child Caries Management: A Randomized Controlled Trial in Dental Practice. *Journal of Dental Research* 99(1): 36-43.

Innes NP, Evans DJ and Stirrups DR (2011) Sealing caries in primary molars: randomized control trial, 5-year results. *Journal of Dental Research* 90(12): 1405-1410.

Ishan, Shivlingesh KK, Agarwal V, et al. (2017) Anxiety Levels among Five-Year-Old Children Undergoing ART Restoration- A Cross-Sectional Study. *J Clin Diagn Res* 11(4): ZC45-ZC48.

Ishioka S and Caputo A (1989) Interaction between the dentinal smear layer and composite bond strength. *The Journal of prosthetic dentistry* 61(2): 180-185.

Jamal H (2016a) Tooth organ bioengineering: cell sources and innovative approaches. *Dentistry journal* 4(2): 18.

Jamal HA (2016b) Tooth Organ Bioengineering: Cell Sources and Innovative Approaches. *Dent J (Basel)* 4(2): 18.

Jun SK, Yang SA, Kim YJ, et al. (2018) Multi-functional nano-adhesive releasing therapeutic ions for MMP-deactivation and remineralization. *Scientific Reports* 8(1): 5663.

Jung I-H, Yun J-H, Cho A-R, et al. (2011) Effect of (-)-epigallocatechin-3-gallate on maintaining the periodontal ligament cell viability of avulsed teeth: a preliminary study. *Journal of periodontal & implant science* 41(1): 10-16.

Kalagi S, Feitosa SA, Münchow EA, et al. (2020) Chlorhexidine-modified nanotubes and their effects on the polymerization and bonding performance of a dental adhesive. *Dental Materials* 36(5): 687-697.

Kanchanavasita W, Anstice HM and Pearson GJ (1997) Water sorption characteristics of resin-modified glass-ionomer cements. *Biomaterials* 18(4): 343-349.

Karrabi M and Danesh Kazemi A (2016) Comparison Of Chlorhexidine 2 And Sodium Hypochlorite 5 As Rewetting Agents On Resin-Dentin Micro Tensile Bond Strength. *Journal of Dental Materials and Techniques* 5(4): 189-195.

Kasraei S. MM, Zahra Khamverdi, and Maryam Mojtahedi (2017) The Efficacy of Riboflavin for Collagen Cross-Linking and Optimizing the Bond Strength of an Etch and Rinse Adhesive System to Dentin. *Avicenna J Dent Res*.

Kazemi-Yazdi H, Saeed-Nezhad M and Rezaei S (2020) Effect of Chlorhexidine on durability of two self-etch adhesive systems. *J Clin Exp Dent* 12(7): e663-e669.

Kessler R (2013) The Minamata Convention on Mercury: a first step toward protecting future generations. National Institute of Environmental Health Sciences.

Kidd E and Fejerskov O (2004) What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms. *Journal of Dental Research* 83(1_suppl): 35-38.

Kim J, Uchiyama T, Carrilho M, et al. (2010) Chlorhexidine binding to mineralized versus demineralized dentin powder. *Dental Materials* 26(8): 771-778.

Kirsch J, Tchorz J, Hellwig E, et al. (2016) Decision criteria for replacement of fillings: a retrospective study. *Clin Exp Dent Res* 2(2): 121-128.

Kivanc M, Barutca B, Koparal AT, et al. (2018) Effects of hexagonal boron nitride nanoparticles on antimicrobial and antibiofilm activities, cell viability. *Materials Science & Engineering. C: Materials for Biological Applications* 91: 115-124.

Langhorst SE, O'Donnell JN and Skrtic D (2009) In vitro remineralization of enamel by polymeric amorphous calcium phosphate composite: quantitative microradiographic study. *Dental Materials* 25(7): 884-891.

Larsen T and Fiehn NE (2017) Dental biofilm infections–an update. *APMIS* 125(4): 376-384.

Launey ME, Buehler MJ and Ritchie RO (2010) On the mechanistic origins of toughness in bone. *Annual review of materials research* 40: 25-53.

Lenzi TL, Tedesco TK, Soares FZM, et al. (2014) Chlorhexidine application for bond strength preservation in artificially-created caries-affected primary dentin. *International Journal of Adhesion and Adhesives* 54: 51-56.

Lenzi TL, Tedesco TK, Soares FZM, et al. (2012) Chlorhexidine does not increase immediate bond strength of etch-and-rinse adhesive to caries-affected dentin of primary and permanent teeth. *Brazilian Dental Journal* 23(4): 438-442.

Letzel H, van 't Hof MA, Vrijhoef MM, et al. (1989) A controlled clinical study of amalgam restorations: survival, failures, and causes of failure. *Dental Materials* 5(2): 115-121.

Li J, Chen B, Hong N, et al. (2018) Effect of Baicalein on Matrix Metalloproteinases and Durability of Resin-Dentin Bonding. *Operative Dentistry* 43(4): 426-436.

Li K, Sun Y, Tsoi JKH, et al. (2020) The application of mussel-inspired molecule in dentin bonding. *Journal of Dentistry* 99: 103404.

Liao K-H, Lin Y-S, Macosko CW, et al. (2011) Cytotoxicity of graphene oxide and graphene in human erythrocytes and skin fibroblasts. *ACS applied materials & interfaces* 3(7): 2607-2615.

Liu JX, Werner J, Kirsch T, et al. (2018) Cytotoxicity evaluation of chlorhexidine gluconate on human fibroblasts, myoblasts, and osteoblasts. *Journal of bone and joint infection* 3(4): 165-172.

Lloyd HM (2020) *The use of child oral-health-related quality of life measures in a randomised control trial of the Hall crown technique in a primary care setting*. University of Otago.

Loesche WJ (1976) Chemotherapy of dental plaque infections. *Oral Sciences Reviews* 9: 65-107.

Loguercio AD, Hass V, Gutierrez MF, et al. (2016a) Five-year Effects of Chlorhexidine on the In Vitro Durability of Resin/Dentin Interfaces. *Journal of Adhesive Dentistry* 18(1): 35-42.

Loguercio AD, Stanislawczuk R, Malaquias P, et al. (2016b) Effect of Minocycline on the Durability of Dentin Bonding Produced with Etch-and-Rinse Adhesives. *Operative Dentistry* 41(5): 511-519.

Loguercio AD, Stanislawczuk R, Polli LG, et al. (2009a) Influence of chlorhexidine digluconate concentration and application time on resin-dentin bond strength durability. *European Journal of Oral Sciences* 117(5): 587-596.

Loguercio AD, Stanislawczuk R, Polli LG, et al. (2009b) Influence of chlorhexidine digluconate concentration and application time on resin-dentin bond strength durability. *European Journal of Oral Sciences* 117(5): 587-596.

Lutz H, Engel T, Lischewsky B, et al. (2017) A new great ape with startling resemblances to African members of the hominin tribe, excavated from the Mid-Vallesian Dinotheriensande of Eppelsheim. First report (Hominoidea, Miocene, MN 9, Proto-Rhine River, Germany).

Lygidakis NN, Allan E, Xia W, et al. (2020) Early Polylysine Release from Dental Composites and Its Effects on Planktonic Streptococcus mutans Growth. *J Funct Biomater* 11(3).

Maha A. El Baz aKA (2018) The effect of green tea extract as a matrix metalloproteinase inhibitor on the bond strength of resin composite. *Egyptian Dental Journal* 64.

Makvandi P, Gu JT, Zare EN, et al. (2020) Polymeric and inorganic nanoscopical antimicrobial fillers in dentistry. *Acta Biomaterialia* 101: 69-101.

Malik S, Ruddock FM, Dowling AH, et al. (2018) Graphene composites with dental and biomedical applicability. *Beilstein J Nanotechnol* 9(1): 801-808.

Manso AP, Grande RHM, Bedran-Russo AK, et al. (2014) Can 1% chlorhexidine diacetate and ethanol stabilize resin-dentin bonds? *Dental Materials* 30(7): 735-741.

Maravic T, Breschi L, Comba A, et al. (2018) Experimental use of an acrolein-based primer as collagen cross-linker for dentine bonding. *Journal of Dentistry* 68: 85-90.

Marovic D, Sariri K, Demoli N, et al. (2016) Remineralizing amorphous calcium phosphate based composite resins: the influence of inert fillers on monomer conversion, polymerization shrinkage, and microhardness. *Croatian Medical Journal* 57(5): 465-473.

Marovic D, Tarle Z, Hiller KA, et al. (2014) Reinforcement of experimental composite materials based on amorphous calcium phosphate with inert fillers. *Dental Materials* 30(9): 1052-1060.

Marsh PD (1994) Microbial ecology of dental plaque and its significance in health and disease. *Advances in Dental Research* 8(2): 263-271.

Mazzoni A, Angeloni V, Apolonio FM, et al. (2013) Effect of carbodiimide (EDC) on the bond stability of etch-and-rinse adhesive systems. *Dental Materials* 29(10): 1040-1047.

Mazzoni A, Angeloni V, Comba A, et al. (2018) Cross-linking effect on dentin bond strength and MMPs activity. *Dental Materials* 34(2): 288-295.

Mazzoni A, Mannello F, Tay FR, et al. (2007) Zymographic analysis and characterization of MMP-2 and -9 forms in human sound dentin. *Journal of Dental Research* 86(5): 436-440.

Mazzoni A, Tjäderhane L, Checchi V, et al. (2015) Role of dentin MMPs in caries progression and bond stability. *Journal of dental research* 94(2): 241-251.

Miller W (1890) Microorganisms of the Human Mouth Philadelphia: SS White Dental Mfg. Co.

Mjör I (1987) The safe and effective use of dental amalgam. *International Dental Journal* 37(3): 147-151.

Mjor IA, Moorhead JE and Dahl JE (2000) Reasons for replacement of restorations in permanent teeth in general dental practice. *International Dental Journal* 50(6): 361-366.

Mobarak EH (2011) Effect of chlorhexidine pretreatment on bond strength durability of caries-affected dentin over 2-year aging in artificial saliva and under simulated intrapulpal pressure. *Operative Dentistry* 36(6): 649-660.

Mohamed AM, Nabih SM and Wakwak MA (2020) Effect of chitosan nanoparticles on microtensile bond strength of resin composite to dentin: An in vitro study. *Brazilian Dental Science* 23(2): 10 p.-10 p.

Moher D, Shamseer L, Clarke M, et al. (2015) Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev* 4(1): 1.

Montagner AF, Pereira-Cenci T and Cenci MS (2015) Influence of cariogenic challenge on bond strength stability of dentin. *Brazilian Dental Journal* 26(2): 128-134.

Montagner AF, Sarkis-Onofre R, Pereira-Cenci T, et al. (2014a) MMP inhibitors on dentin stability: A systematic review and meta-analysis. *Journal of dental research* 93(8): 733-743.

Montagner AF, Sarkis-Onofre R, Pereira-Cenci T, et al. (2014b) MMP Inhibitors on Dentin Stability: A Systematic Review and Meta-analysis. *Journal of Dental Research* 93(8): 733-743.

Mosallam R, Younis N, Farouk H, et al. (2018) Effect of green tea and two mulberry leaf extracts on micro-tensile bond strength to dentin. *Future Dental Journal* 4(2): 150-155.

Mosallam R, Younis N, Farouk H, et al. (2019) Effect of Matrix Metalloproteinase Inhibitor from Mulberry fruit extract on the Microtensile Bond Strength Stability: An in vitro Study. *Egyptian Dental Journal* 65(1-January (Fixed Prosthodontics, Dental Materials, Conservative Dentistry & Endodontics)): 541-550.

Nagase H, Visse R and Murphy G (2006) Structure and function of matrix metalloproteinases and TIMPs. *Cardiovascular Research* 69(3): 562-573.

Najeeb S, Khurshid Z, Zafar MS, et al. (2016) Modifications in glass ionomer cements: Nano-sized fillers and bioactive nanoceramics. *International Journal of Molecular Sciences* 17(7): 1134.

Nakabayashi N, Nakamura M and Yasuda N (1991) Hybrid layer as a dentin-bonding mechanism. *Journal of Esthetic and Restorative Dentistry* 3(4): 133-138.

Nakatomi M, Wang X-P, Key D, et al. (2010) Genetic interactions between Pax9 and Msx1 regulate lip development and several stages of tooth morphogenesis. *Developmental Biology* 340(2): 438-449.

Nanci A (2018) Ten Cate’s Oral Histology. St. Louis. Elsevier.

Nizami MZI, Nishina Y, Yamamoto T, et al. (2020) Functionalized graphene oxide shields tooth dentin from decalcification. *Journal of dental research* 99(2): 182-188.

Oliveira SS, Pugach MK, Hilton JF, et al. (2003) The influence of the dentin smear layer on adhesion: a self-etching primer vs. a total-etch system. *Dental Materials* 19(8): 758-767.

Oong EM, Griffin SO, Kohn WG, et al. (2008) The effect of dental sealants on bacteria levels in caries lesions: a review of the evidence. *Journal of the American Dental Association* 139(3): 271-278; quiz 357-278.

Orsini G, Ruggeri Jr A, Mazzoni A, et al. (2009) A review of the nature, role, and function of dentin non-collagenous proteins. Part 1: proteoglycans and glycoproteins. *Endodontic Topics* 21(1): 1-18.

Osorio R, Erhardt MC, Pimenta LA, et al. (2005) EDTA treatment improves resin-dentin bonds' resistance to degradation. *Journal of Dental Research* 84(8): 736-740.

Ou Q, Hu Y, Yao S, et al. (2018a) Effect of matrix metalloproteinase 8 inhibitor on resin-dentin bonds. *Dental Materials* 34(5): 756-763.

Ou Q, Hu Y, Yao S, et al. (2018b) Effect of matrix metalloproteinase 8 inhibitor on resin–dentin bonds. *Dental Materials* 34(5): 756-763.

Page MJ, McKenzie JE, Bossuyt PM, et al. (2021) The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 372.

Panpisut P, Liaqat S, Zacharaki E, et al. (2016) Dental composites with calcium/strontium phosphates and polylysine. *PloS One* 11(10): e0164653.

Park E-J, Yi J, Kim Y, et al. (2010) Silver nanoparticles induce cytotoxicity by a Trojan-horse type mechanism. *Toxicology in vitro* 24(3): 872-878.

Pashley DH (1992) Smear layer: overview of structure and function. *Proceedings of the Finnish Dental Society* 88 Suppl 1: 215-224.

Pashley DH, Tay FR, Yiu C, et al. (2004) Collagen degradation by host-derived enzymes during aging. *Journal of Dental Research* 83(3): 216-221.

Paulose NE and Fawzy AS (2018) Effect of carbodiimide on the bond strength and durability of resin-dentin interface. *Journal of adhesion science and Technology* 32(9): 931-946.

Pedrosa VO, Franca FMG, Turssi CP, et al. (2018) Effects of caffeic acid phenethyl ester application on dentin MMP-2, stability of bond strength and failure mode of total-etch and self-etch adhesive systems. *Archives of Oral Biology* 94: 16-26.

Perote LC, Kamozaki MB, Gutierrez NC, et al. (2015) Effect of Matrix Metalloproteinase-inhibiting Solutions and Aging Methods on Dentin Bond Strength. *Journal of Adhesive Dentistry* 17(4): 347-352.

PHE PHE (2013) Oral health survey of three-year-old children. Dental Public Health Epidemiology Programme. A report on the prevalence and severity of dental decay.

PHE PHE (2017a) Guidance Health matters: child dental health.

PHE PHE (2017b) Oral health survey of five-year-old children 2017. National Dental Epidemiology Programme for England: oral health survey of five-year-old children. A report on the inequalities found in prevalence and severity of dental decay. .

Pietrokovski Y, Nisimov I, Kesler-Shvero D, et al. (2016) Antibacterial effect of composite resin foundation material incorporating quaternary ammonium polyethyleneimine nanoparticles. *Journal of Prosthetic Dentistry* 116(4): 603-609.

Porto NT, Oliveira JMS, Freitas PH, Haimeur A, Franca R (2018) Use of polyphenols as a strategy to prevent bond degradation in the dentin–resin interface. *European Journal of Oral Sciences* 126.

Prasansuttiporn T, Thanatvarakorn O, Mamanee T, et al. (2020) Effect of antioxidant/reducing agents on the initial and long-term bonding performance of a self-etch adhesive to caries-affected dentin with and without smear layer-deproteinizing. *International Journal of Adhesion and Adhesives* 102: 102648.

Prasansuttiporn T, Thanatvarakorn O, Tagami J, et al. (2017) Bonding Durability of a Self-etch Adhesive to Normal Versus Smear-layer Deproteinized Dentin: Effect of a Reducing Agent and Plant-extract Antioxidant. *Journal of Adhesive Dentistry* 19(3): 253-258.

Proffitt E (2020) What will be the new normal for the dental industry? *British Dental Journal* 228(9): 678-680.

Pulpdent ACTIVA KIDS BioACTIVE RESTORATIVE https://www.pulpdent.com/products/activa-kids-bioactive-restorative/.

Pulpdent PulpDent ACTIVA BioACTIVE RESTORATIVE. https://www.pulpdent.com/products/activa-bioactive-restorative/.

Pulpdent (2018) *Instructions for use*. Available at: https://www.pulpdent.com/wp-content/uploads/2019/09/XP-VK-IN-03w.pdf (accessed 8th of November).

Radhika M, Sajjan GS, Kumaraswamy B, et al. (2010) Effect of different placement techniques on marginal microleakage of deep class-II cavities restored with two composite resin formulations. *Journal of conservative dentistry: JCD* 13(1): 9.

Rezaee R and Abdollahi M (2017) The importance of translatability in drug discovery. *Expert opinion on drug discovery* 12(3): 237-239.

Rios D, Honório HM, Araújo PAd, et al. (2002) Wear and superficial roughness of glass ionomer cements used as sealants, after simulated toothbrushing. *Pesquisa Odontológica Brasileira* 16(4): 343-348.

Ruksaphon K SP (2017) Efficacy of chlorhexidine and rosmarinic acid to prevent resin/dentine interface degradation. *Dent Oral Craniofac Res* 4(2).

Sabatini C, Kim JH and Ortiz Alias P (2014) In vitro evaluation of benzalkonium chloride in the preservation of adhesive interfaces. *Operative Dentistry* 39(3): 283-290.

Sabatini C and Pashley DH (2015) Aging of adhesive interfaces treated with benzalkonium chloride and benzalkonium methacrylate. *European Journal of Oral Sciences* 123(2): 102-107.

Sabatini C and Patel SK (2013) Matrix metalloproteinase inhibitory properties of benzalkonium chloride stabilizes adhesive interfaces. *European Journal of Oral Sciences* 121(6): 610-616.

Sacramento PA, de Castilho AR, Banzi EC, et al. (2012) Influence of cavity disinfectant and adhesive systems on the bonding procedure in demineralized dentin - a one-year in vitro evaluation. *Journal of Adhesive Dentistry* 14(6): 575-583.

Sadeghi (2017) Effect of Chlorhexidine Application on Dentin Bond Strength Durability of Two

Etch-and-Rinse Adhesive versus a Universal Bond System. *Journal of Dentistry and Oral Care Medicine* 3(2).

Sadek FT, Braga RR, Muench A, et al. (2010) Ethanol wet-bonding challenges current anti-degradation strategy. *Journal of Dental Research* 89(12): 1499-1504.

Sanderson S (2019) Next steps in theMercury Regulations.

Santiago SL, Osorio R, Neri JR, et al. (2013) Effect of the flavonoid epigallocatechin-3-gallate on resin-dentin bond strength. *Journal of Adhesive Dentistry* 15(6): 535-540.

Schwendicke F (2017) Contemporary concepts in carious tissue removal: A review. *J Esthet Restor Dent* 29(6): 403-408.

Schwendicke F, Walsh T, Lamont T, et al. (2021) Interventions for treating cavitated or dentine carious lesions. *Cochrane Database of Systematic Reviews*. DOI: 10.1002/14651858.CD013039.pub2.(7).

Shen J, Xie H, Wang Q, et al. (2020) Evaluation of the interaction of chlorhexidine and MDP and its effects on the durability of dentin bonding. *Dental Materials* 36(12): 1624-1634.

Shirling ET and Gottlieb D (1966) Methods for characterization of Streptomyces species. *International Journal of Systematic Bacteriology* 16(3): 313-340.

Siddiqi KS, Husen A and Rao RA (2018) A review on biosynthesis of silver nanoparticles and their biocidal properties. *Journal of nanobiotechnology* 16(1): 14.

Skrtic D, Antonucci JM, Eanes ED, et al. (2000) Physicochemical evaluation of bioactive polymeric composites based on hybrid amorphous calcium phosphates. *Journal of Biomedical Materials Research* 53(4): 381-391.

Song M, Yu B, Kim S, et al. (2017) Clinical and Molecular Perspectives of Reparative Dentin Formation: Lessons Learned from Pulp-Capping Materials and the Emerging Roles of Calcium. *Dental Clinics of North America* 61(1): 93-110.

Spencer P, Wang Y, Walker M, et al. (2001) Molecular structure of acid-etched dentin smear layers-in situ study. *Journal of Dental Research* 80(9): 1802-1807.

Stanislawczuk R, Amaral RC, Zander-Grande C, et al. (2009) Chlorhexidine-containing acid conditioner preserves the longevity of resin-dentin bonds. *Operative Dentistry* 34(4): 481-490.

Sulkala M, Tervahartiala T, Sorsa T, et al. (2007) Matrix metalloproteinase-8 (MMP-8) is the major collagenase in human dentin. *Archives of Oral Biology* 52(2): 121-127.

Sulkala M, Wahlgren J, Larmas M, et al. (2001) The effects of MMP inhibitors on human salivary MMP activity and caries progression in rats. *Journal of Dental Research* 80(6): 1545-1549.

Tekce N, Tuncer S, Demirci M, et al. (2016) Do matrix metalloproteinase inhibitors improve the bond durability of universal dental adhesives? *Scanning* 38(6): 535-544.

Tezvergil-Mutluay A, Agee KA, Hoshika T, et al. (2010) The requirement of zinc and calcium ions for functional MMP activity in demineralized dentin matrices. *Dental Materials* 26(11): 1059-1067.

Tjaderhane L, Larjava H, Sorsa T, et al. (1998) The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions. *Journal of Dental Research* 77(8): 1622-1629.

Tjäderhane L, Nascimento FD, Breschi L, et al. (2013) Optimizing dentin bond durability: control of collagen degradation by matrix metalloproteinases and cysteine cathepsins. *Dental Materials* 29(1): 116-135.

Tjaderhane L, Palosaari H, Wahlgren J, et al. (2001) Human odontoblast culture method: the expression of collagen and matrix metalloproteinases (MMPs). *Advances in Dental Research* 15(1): 55-58.

Tucker A and Sharpe P (2004) The cutting-edge of mammalian development; how the embryo makes teeth. *Nature Reviews Genetics* 5(7): 499.

Turkistani A, Almutairi M, Banakhar N, et al. (2018) Optical Evaluation of Enamel Microleakage with One-Step Self-Etch Adhesives. *Photomedicine and Laser Surgery* 36(11): 589-594.

US Food and Drug Administration (2004) Polylysine Food Additive Safety.

van Dijken JWV, Pallesen U and Benetti A (2019) A randomized controlled evaluation of posterior resin restorations of an altered resin modified glass-ionomer cement with claimed bioactivity. *Dental Materials* 35(2): 335-343.

Van Meerbeek B, De Munck J, Yoshida Y, et al. (2003) Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. *Operative Dentistry* 28(3): 215-235.

Van Meerbeek B, Yoshihara K, Van Landuyt K, et al. (2020) From Buonocore's Pioneering Acid-Etch Technique to Self-Adhering Restoratives. A Status Perspective of Rapidly Advancing Dental Adhesive Technology. *Journal of Adhesive Dentistry* 22(1): 7-34.

Venigalla BS, Jyothi P, Kamishetty S, et al. (2016) Resin bond strength to water versus ethanol-saturated human dentin pretreated with three different cross-linking agents. *J Conserv Dent* 19(6): 555-559.

Visse R and Nagase H (2003) Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circulation Research* 92(8): 827-839.

Warwick D, Young M, Palmer J, et al. (2019) Mercury vapor volatilization from particulate generated from dental amalgam removal with a high-speed dental drill–a significant source of exposure. *Journal of Occupational Medicine and Toxicology* 14(1): 22.

Wegst UG, Bai H, Saiz E, et al. (2015) Bioinspired structural materials. *Nat Mater* 14(1): 23-36.

Weir M, Chow L and Xu HH (2012) Remineralization of demineralized enamel via calcium phosphate nanocomposite. *Journal of Dental Research* 91(10): 979-984.

White S (2017) Health Matters: Child dental health - Public health matters.

Wu Q, Shan T, Zhao M, et al. (2019) The inhibitory effect of carboxyl-terminated polyamidoamine dendrimers on dentine host-derived matrix metalloproteinases in vitro in an etch-and-rinse adhesive system. *R Soc Open Sci* 6(10): 182104.

Xu H, Weir M, Sun L, et al. (2010) Strong nanocomposites with Ca, PO4, and F release for caries inhibition. *Journal of Dental Research* 89(1): 19-28.

Xu HH, Weir MD and Sun L (2007) Nanocomposites with Ca and PO4 release: effects of reinforcement, dicalcium phosphate particle size and silanization. *Dental Materials* 23(12): 1482-1491.

Xu J, Li M, Wang W, et al. (2020) A novel prime-&-rinse mode using MDP and MMPs inhibitors improves the dentin bond durability of self-etch adhesive. *Journal of the Mechanical Behavior of Biomedical Materials* 104: 103698.

Xu W-P, Zhang L-C, Li J-P, et al. (2011) Facile synthesis of silver@ graphene oxide nanocomposites and their enhanced antibacterial properties. *Journal of Materials Chemistry* 21(12): 4593-4597.

Xu X and Burgess JO (2003) Compressive strength, fluoride release and recharge of fluoride-releasing materials. *Biomaterials* 24(14): 2451-2461.

Yaghmoor RB, Xia W, Ashley P, et al. (2020) Effect of Novel Antibacterial Composites on Bacterial Biofilms. *J Funct Biomater* 11(3): 55.

Yoshiyama M, Tay F, Doi J, et al. (2002) Bonding of self-etch and total-etch adhesives to carious dentin. *Journal of Dental Research* 81(8): 556-560.

Yudovin-Farber I, Beyth N, Weiss EI, et al. (2010) Antibacterial effect of composite resins containing quaternary ammonium polyethyleneimine nanoparticles. *Journal of Nanoparticle Research* 12(2): 591-603.

Zhang L, Weir MD, Chow LC, et al. (2016) Novel rechargeable calcium phosphate dental nanocomposite. *Dental Materials* 32(2): 285-293.

Zheng P, Zaruba M, Attin T, et al. (2015) Effect of different matrix metalloproteinase inhibitors on microtensile bond strength of an etch-and-rinse and a self-etching adhesive to dentin. *Operative Dentistry* 40(1): 80-86.