EVALUATION OF ANTIBACTERIAL PROPERTIES OF PORTLAND CEMENT IN COMBINATION WITH CHITOSAN AGAINST *STREPTOCOCCUS MUTANS*

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by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
<	Less than
°C	Degree Celsius
μL	Microlitre
nm	Nanometer
mL	Millilitre
mm	milimitre
et al.	*et alii* – ‘and others’
× *g*	Gravity
g	Gram
mM	Milimolar
NaCl	Sodium chloride
OD	Optical density
PBS	Phosphate Buffered Saline
rpm	Revolutions per minute
WHO	World Health Organization
MTA	Mineral Trioxide Aggregate
$Ca(OH)_2$	Calcium hydroxide
PC	Portland cement
CHT	Chitosan
PCR	Polymerase Chain Reaction
EPS	Extracellular polysaccharide substance

DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
cDNA	Complementary DNA
CaO	Calcium oxide
w/w	weight per weight
APC	Accelerated Portland cement
SEM	Scanning electron microscope
dNTP	Deoxyribonucleotide triphosphate
SPSS	Statistical Package for the Social Sciences
BHI	Brain heart infusion
DEPC	Diethyl Pyrocarbonate
EDTA	Ethylenediaminetetraacetic acid
TE	Tris-EDTA
LB	Lithium borate
BSC	Biosafety cabinet
CFU/ml	Colony-forming units per millilitre
CLSI	Clinical & Laboratory Standards Institute
C_T	Threshold cycle
R^2	Coefficient correlation
Gtf	Glucostransferase
Gbp	Glucan binding protein
RQ	Relative quantitation
SD	Standard deviation
Bp	Base pair
DCT	Direct contact test

PENILAIAN SIFAT ANTIBAKTERIA SIMEN PORTLAND KOMBINASI DENGAN KITOSAN TERHADAP STREPTOCOCCUS MUTANS

ABSTRAK

Karies adalah satu masalah mulut yang kerap berlaku di seluruh dunia. Bakteria utama yang menyebabkan masalah tersebut dikenali sebagai *Streptococcus mutans*. Karies gigi amat menyakitkan kerana ia boleh menyebabkan keradangan pulpa. Terkini, rawatan pulpa gigi yang terdiri daripada pulpa dan pulpotomi adalah rawatan yang banyak digunakan. Biomaterial yang digunakan dalam rawatan di klinik adalah mineral trioksida agregat (MTA) dan kalsium hidroksida ($Ca(OH)_2$). Namun, bahan-bahan ini mempunyai beberapa kekurangannya. Dalam kajian ini, bahan biomaterial baru yang dikenali sebagai simen Portland (APC) disintesis dengan beberapa kepekatan kitosan (CHT) yang berbeza dan sifat antibakteria bahan ini diuji terhadap *S. mutan*. Bahan telah disediakan seperti berikut: APC, APC-0.6% CHT, APC-1.25% CHT dan APC-2.5% CHT. Ujian *direct contact* menunjukkan penurunan ketara dalam pertumbuhan pada semua kumpulan yang diuji berbanding dengan kumpulan kawalan. Keputusan yang sama diperoleh dalam ujian pembentukan biofilm di mana APC-2,5% CHT menunjukkan kesan pengurangan pertumbuhan tertinggi terhadap *S. mutans*. Analisis taburan bakteria menggunakan pengimbas mikroskop elektron pada APC-2,5% CHT menunjukkan jumlah taburan bakteria berkurangan setelah diuji selama 7 hari. Gen ekspresi menggunakan RT-PCR menunjukkan kumpulan APC-2,5% CHT telah mengurangkan kadar ekspresi gen Beta-glucosteransferase (*gtfB*) dan glucan-binding protein B (*gbpB*) berbanding kumpulan APC. Kesimpulannya, APC dengan kepekatan kitosan yang berbeza menunjukkan kesan antibakteria yang bagus di mana APC-2,5% CHT mempunyai kesan antibakteria tertinggi terhadap *S. mutans.*

EVALUATION OF ANTIBACTERIAL PROPERTIES OF PORTLAND CEMENT IN COMBINATION WITH CHITOSAN AGAINST STREPTOCOCCUS MUTANS

ABSTRACT

Caries is one of the most prevalent oral problems among people around the world. The main pathogenic bacteria that cause the problem is known as *Streptococcus mutans*. Tooth caries can be painful as it can cause pulp inflammation. Vital pulp therapies which include pulp capping and pulpotomy are the most commonly used treatment nowadays. Biomaterials that are commonly utilised in the treatment in clinics include mineral trioxide aggregate (MTA) and calcium hydroxide ($Ca(OH)_2$); however these materials have some drawbacks. In this study, a new material known as Portland cement was synthesized with different concentrations of chitosan and antibacterial properties against *S. mutan* was investigated. Materials was prepared as follows: APC, APC-0.6%CHT, APC-1.25%CHT and APC-2.5%CHT. The direct contact test demonstrated a significant reduction in the growth curve in all treated groups compared to the control group. Similar findings were obtained in the biofilm formation test with APC-2.5%CHT showed the highest inhibition against *S. mutans*. Bacterial distribution analysis by scanning electron microscopy on APC-2.5%CHT showed that after 7 days of treatment, the number of distributed bacteria was decreased. Gene expression by RT-PCR demonstrated APC-2.5%CHT has significantly down-regulated *gtfB* and *gbpB* genes compared to APC alone. In conclusion, APC with different concentrations of chitosan exhibited antibacterial effects with APC-2.5%CHT demonstrated the highest inhibition against *S. mutans*.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Dental caries is one of the most common oral diseases around the world (WHO, 2020). Basically, dental caries is caused by the breakdown of tooth enamel which is caused by bacteria that accumulate on the teeth and the usual suspect is *S. mutans* (Metwalli *et al*., 2013). The main habitats of *S. mutans* are mouth, larynx and intestine. The microorganism has a very strong acid property that can lead to an acidic environment in the oral cavity (Forssten *et al*., 2010). Besides that, *S. mutans* also has other virulence factors that can cause dental caries which include adhesion and acid tolerance (Banas, 2004). *S. mutans* has the ability to form biofilm and colonize the oral cavity via the adhesion mechanism on the surface of the tooth (Krzyściak *et al*., 2013). Acid tolerance property of *S. mutans* makes them capable to get through in an acidic conditions and have a particular interaction with other species of microorganisms that exist in the oral cavity (Krzyściak *et al*., 2013). Dental caries can cause inflammation of the pulp that can be painful.

Dental pulp tissues are encased in the hard tissues of dentin, cementum and enamel. This unique distinction protects the vascular tissue of the pulp from the surrounding microorganisms. The pulp tissues have several functions throughout the life of the tooth such as nutrition delivery, dentinogenesis and immune defence. It is therefore of great importance to preserve the vitality of the pulp for long-term tooth survival. When the infection or necrosis of pulp tissue occurs, the tooth must be treated by post and core restoration and/or root canal treatment, which structurally compromised the tooth and make it more susceptible to fracture and failure (Yu & Abbott, 2007; Ingle *et al*., 2008).

Vital pulp therapy aims to preserve the pulp vitality in mature and immature permanent teeth and induce the apexogenesis to complete the growth of the root complex of the immature tooth through the formation of protective dentinal bridge. The vital pulp therapies are recommended when the tooth has reversible pulpitis symptoms (Al-Hiyasat *et al*., 2006; Aguilar & Linsuwanont, 2011). However, some encouraging results have been shown with irreversible pulpitis symptoms *(Asgary et al.*, 2014). The current approaches of vital pulp therapies include pulp capping and pulpotomy.

Nowadays, biomaterials are used as preventive materials or restorative materials in dentistry. Mineral trioxide aggregate (MTA) was first developed by Torabinejad in 1993 as a dental root repair and root filling material (Asgary *et al*., 2013). MTA has many advantages which include radiopacity, antibacterial and biocompatibility properties, high pH and the ability to aid in the release of bioactive dentin matrix proteins (Hilton, 2009). However, this material also has its own disadvantages such as the tendency for discolouration, long set-up time and expensive (Parirokh & Torabinejad, 2010). The other mostly used material in clinics is known as calcium hydroxide ($Ca(OH)_2$) in which it was first developed by Hermann (Jalan *et al*., 2017). It has become the gold standard for pulp capping with several advantages such as high pH value along with great antibacterial effect (Mostafa & Moussa, 2018). However, the major drawback of it is dissolution which can cause micro leakage (Komabayashi *et al*., 2016).

Portland cement (PC) is also one of the materials used in dentistry. It comes from the calcination of the mixture of limestones from Portland, England and siliconargillaceous materials (Viola *et al*., 2011). PC and MTA are basically similar in their structure even though the latter contains bismuth oxide to provide radiopacity. PC also has similar properties like MTA in terms of biocompatibility, antibacterial activity, tissue response, chemical composition and physical properties (Melo Júnior *et al*., 2015). A previous study by Guerreiro-Tanomaru *et al.* (2012) claimed that all PC-based materials exhibited antimicrobial activity against several bacteria strains (*Micrococcus luteus*, *Streptococcus mutans*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Candida albicans*) (Guerreiro-Tanomaru *et al*., 2012).

Chitosan is also one of the natural biomaterials that is used in many studies. Chitosan has many good properties which makes it very attractive to many scientists in bio-dental applications. These include a broad antibacterial spectrum, biocompatibility, hydrophilicity and biodegradability (Husain *et al*., 2017). According to Erpaçal *et al*. (2019), numerous studies have been conducted in dentistry that used chitosan as preventive materials because it has good antibacterial properties (Erpaçal *et al*., 2019). It is stated that the use of chitosan in low molecular weight can block the adsorption of *S. mutans* to hydroxyapatite and also initiate tooth regeneration (Erpaçal *et al*., 2019). In this study, white PC was selected and combined with the various concentrations of chitosan. This combination is generated for dental pulp capping in order to prevent the pathogenic bacteria from adhering to the teeth and also prevent from dental caries.

1.2 Problem statement

Nowadays, biomaterials have important roles in dental treatment. It is crucial for dentists to choose suitable materials in order to have a good long-term effect on patients' teeth. Although $Ca(OH)_2$ and MTA are commonly used in dentistry, these materials however still have several disadvantages. A study by Arandi (2017), $Ca(OH)_2$ has its own drawbacks in which it has low elastic modulus and low compressive strength that limits its usage in thin layer. Hence, it is not favourable in restoration support. Also, due to its high solubility and water sorption, $Ca(OH)_2$ can lead to liner softening and loss of material if the tooth restoration is not sealed properly. Other disadvantage of $Ca(OH)_2$ is that this material cannot seal exposed pulp from external environment in which it may cause a failure of the treatment (Jalan *et al*., 2017). Meanwhile, the disadvantages of MTA including presence of toxic elements in the material composition, difficult handling characteristics, discolouration potential, long setting time and also high material cost (Monisha *et al*., 2013). Thus a new novel material with a good physicochemical and biological properties must be investigated.

1.3 Justification of the study

There are some studies that use PC as a root filling material. To date, there is no study describes the effects of a combination of these two biomaterials, PC and chitosan on antibacterial properties against *S. mutans*. Since chitosan have been widely studied previously for various purposes, this study is interested to evaluate the effect of combination of PC and chitosan which might exhibit a good synergistic effect against bacteria. This combination could be the potential agent for antibacterial material that may enhance the variation of vital pulp treatment modalities.

1.4 Objectives of the study

1.4.1 General objective

To evaluate antibacterial properties of PC in combination with various concentrations of chitosan on *S. mutans*.

1.4.2 Specific objectives:

1. To determine the effect of PC in combination with various concentrations of chitosan on *S. mutans* growth curve.
2. To assess the effect of PC in combination with various concentrations of chitosan on biofilm formation of *S. mutans.*
3. To determine the adhesion and distribution of *S. mutans* on biomaterial surface by using SEM.
4. To evaluate the *S. mutans* adhesion genes expression by using real time PCR.

1.5 Hypothesis

PC in combination with chitosan exhibits antibacterial properties against *S. mutans.*

1.6 Research question

1. Does PC in combination with chitosan inhibit bacteria growth?
2. Does PC in combination with chitosan reduce biofilm formation of *S. mutans*?
3. Does PC in combination with chitosan inhibit the adhesion and distribution of *S. mutans* on biomaterial surface?
4. Does PC in combination with chitosan reduce the expression of *S. mutans* adhesion genes?

CHAPTER 2

LITERATURE REVIEW

2.1 Oral health

Oral health is a key indicator of overall health and quality of life. The general well-being of a person depends on their oral health in which it becomes a reason to keep their mouth healthy every single day. According to Glick *et al*. (2016), the definition of oral health is multi-faceted as it plays an important role in a person's daily activities such as talking, smelling, tasting, chewing and so on. Healthy oral can affect a person's emotion which can be observed through facial expression. A person can always practice a good oral hygiene since young by brushing teeth using a fluoride toothpaste, gargling with mouthwash and flossing the teeth. Also, it is important to replace the toothbrush every three to four months and have regular dental check-ups (American Dental Association, n.d.).

2.2 Dental caries

Dental caries, which is the most leading type of oral problem worldwide is also known as tooth decay. It has publicly become a major health problem and is 4 to 5 times more frequent than asthma (Heng, 2016). According to WHO (2020) reports, dental caries globally affects over 530 million children in their primary teeth and about 2.3 billion people have the same problem in their permanent teeth. In Malaysia, it is stated about 90% prevalence of tooth decay among adults in which it affects more than 10 teeth (Esa *et al*., 2014). Other study by Kaur *et al.* (2015) revealed that dental caries among children has given Malaysia a prevalence of about 70-90%. Those who live in the rural area are more likely to suffer dental caries compared to those who live in urban area (Esa *et al*., 2014). Usually, people who have lower socioeconomic status suffer such problem more than those who have higher socioeconomic. This is might be due to the lack of education, awareness as well as poor hygiene practices in their daily life (Kaur *et al*., 2015).

2.2.1 Types of dental caries

According to Yadav & Prakash (2016), there are different types of dental caries. First is primary caries or also known as incipient caries. This caries occur at the site of teeth that has no experience of decay. The other type of caries is known as secondary caries or recurrent caries. It happens at the site of tooth that has previously experienced decay. Tooth that has been restored with fillings and other restorative materials is commonly the site for secondary caries. The last type of caries is known as arrested caries. This caries is no longer active and has stopped progressing. In addition, dental caries is categorised into several class based on their progression rate, affected hard tissue and sites. The classification by the site is called G.V Black's classification. There are seven classes altogether in G.V Black's classification while progression rate and affected hard tissue have two and three classes, respectively as listed in Table 2.1 (Yadav & Prakash, 2016).

Table 2.1 Classification of dental caries (Yadav & Prakash, 2016).

On the basis of	Classification	Description
Rate of progression	Acute	Signifies a quickly developing condition
	Chronic	Signifies an extended time to developing condition
Affected hard tissue	Enamel	Early in its development and may affect only enamel.
	Dentinal	The extent of decay reaches the deeper layer of dentin
	Cementum	The decay on roots of teeth
Site (G.V Black)	Class I	Pit and fissure caries (anterior or posterior teeth)
	Class II	Approximal surfaces of posterior teeth
	Class III	Approximal surfaces of anterior teeth without incisal edge involvement
	Class IV	Approximal surfaces of anterior teeth with incisal edge involvement
	Class V	Gingival/cervical surfaces on the lingual or facial aspect (anterior or posterior)
	Class VI	Incisal edge of anterior teeth or cusp heights of posterior teeth

2.2.2 Causes of dental caries

Caries is nothing new to humankind as it has already existed since long time ago. It is reported that caries has been discovered during an ancient time like Paleolithic and Mesolithic era. The skulls of human ancestors have already shown signs of decay in their teeth and it is suggested that caries is very likely linked to the high intake of dietary foods that rich in carbohydrates (Ruby *et al*., 2010; Oxilia *et al*., 2015). Basically, the requirement factors for the formation of caries are host which include saliva and teeth, cariogenic bacteria, dietary food and time (Gupta *et al*., 2013). These factors can be simply elucidated in a Venn's diagram where all circles intersect with each other to cause the caries formation (Rathee & Sapra, 2019). Sugar in dietary food is one of major components in the development of caries. Cariogenic bacteria that live in the mouth utilize free sugars to help them bind to the tooth surface (Yadav & Prakash, 2017). This will cause the production of acid that will dissolve the tooth structure which eventually leads to tooth demineralisation. Tooth decay is progressed in several stages (Figure 2.1) where it starts with the appearance of white spots and ends with inflammation of pulp and abscess formation.

Saliva plays a very significant role in protecting the teeth from caries development. It is stated that saliva contains 99% of water and 1% of various proteins and electrolytes that contributed to the functionality of the saliva (Dodds *et al*., 2015). Interestingly, there are many functions that saliva can serve in human body. Tiwari (2011) mentioned a few main functions of saliva that include lubricating and binding, digestion of food and maintaining oral hygiene. Also, saliva helps to regulate the pH in the oral cavity. When there is production of acid by the bacteria, saliva will serve as a buffer so that demineralisation of tooth is reduced (Pedersen & Belstrøm, 2019).

Proteins in saliva such as proline-rich proteins and mucins help to give protection to the tooth surface and can aid in the remineralisation of tooth (Hegde *et al*., 2019).

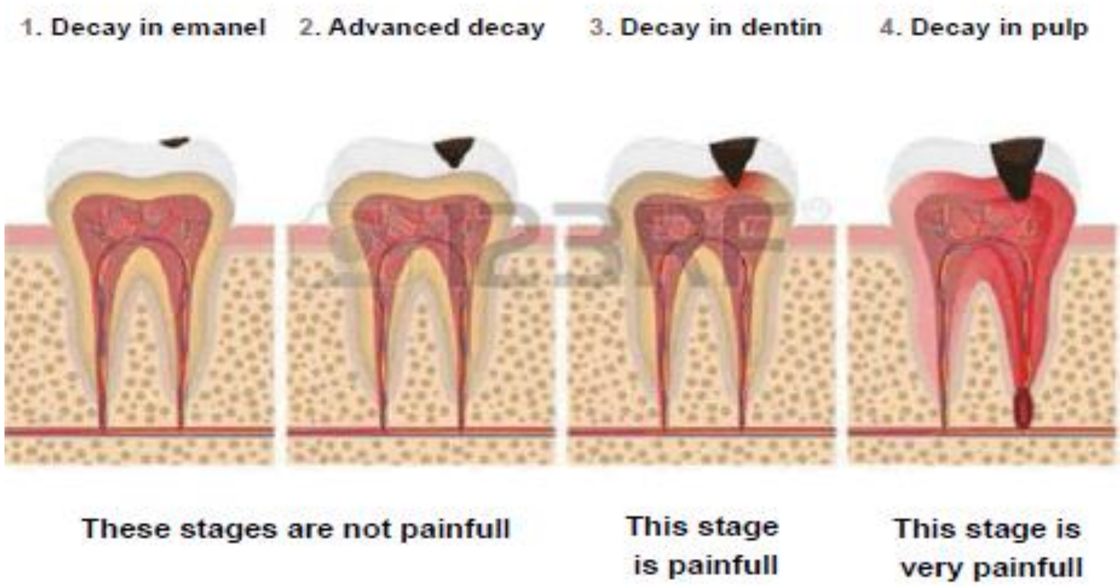

Figure 2.1 Stages of tooth decay (Adapted from Yadav & Prakash, 2017).

2.3 Biofilm formation

Literally, biofilm is an accumulation of numerous microorganisms that permanently attached on a surface that are embedded with a matrix of extracellular polymeric substances (EPS) produced by them (Jamal *et al*., 2018). EPS is a special matrix that gives defence to these microorganisms from any damage which can be due to fluid flow (Tuson & Weibel, 2013). Bacterial biofilm can be harmful to human as it can cause nosocomial infection (Muhsin *et al*., 2015). A study by Chandki *et al.* (2011) pointed out that biofilms are classified into two basis which are location and pathogenicity. On the basis of location, there are two classes of biofilms which include supragingival (biofilm that appears on coronal to gingival margin) and subgingival (biofilm that appears on apical to gingival margin). Meanwhile, the classification of biofilm on the basis of pathogenicity is divided into two which are cariogenic and periopathogenic. Cariogenic is referred to acidogenic and gram positive bacteria while periopathogenic is referred to basophilic and gram negative bacteria. There are mainly two kinds of growth mode for bacteria cells which are planktonic cells and sessile cells. Planktonic cells refer to free-swimming cells while sessile bacteria is the term used for bacteria within the biofilm (Berlanga & Guerrero, 2016; Muhsin *et al*., 2015).

The process of biofilm formation is very complex and has a few main stages (Figure 2.2). The initial stage is the formation of acquired pellicle followed by the primary attachment of bacteria onto the tooth surface and the growth of micro-colonies. The next stage is the maturation of biofilm and then ended with dispersal or detachment of bacteria from the surface which may further create new formation on other region (Crouzet *et al*., 2014). In the first stage, a thin layer of pellicle is formed after the tooth surface has been cleaned. The pellicle layer is generally a protein film that comes from saliva glycoprotein like proline-rich proteins, α-amylase, statherin, mucins, and agglutinin, in which it can act as a ligand for bacterial receptor (Kriebel *et al*., 2018; Valen & Scheie, 2018). There are many interaction forces between those glycoproteins and tooth surface. Huang *et al.* (2011) clarified that there are three types of interaction forces among those components that include long-range forces, medium-range forces and short-range forces. For long-range forces, it is about 50 to 100 nm between the interacting components while medium-range forces and short-range forces are about 10 to 50 nm and less than 5 nm, respectively. Van der Waals forces, dipole-dipole hydrophobic interactions and Coulumb interaction are grouped in long-range forces while hydrophobic interactions are included in the medium-range forces. As for short-range forces, there are several interactions: hydrogen bonds, covalent bonds, Lewis acid-base interactions, ionic interactions and electrostatistic interactions. Adhesion of bacteria to the tooth surface will occur after the alteration of charge surface by the pellicle layer (Kriebel *et al*., 2018).

In the next stage of biofilm formation, bacteria in planktonic form attach to the binding proteins of the acquired pellicle layers via their structures such as pili and flagella (Jamal *et al*., 2018). Other study alluded that these bacteria attach on the surface via fimbriae and fibrils (Huang *et al*., 2011). These appendages are very important as they are able to give support and strength to the bacteria during attachment. According to Berger *et al*. (2018), there are many ways for the bacteria to attach on the tooth surface. Besides using their appendages, they can also attach through proteins and polysaccharide adhesins. Attachment of bacteria can also be determined by environmental factors for instance, pH and temperature (Achinas *et al*., 2019). During this stage, the attachment can still be reversible where some bacteria can detach from the surface since they do not produce EPS matrix yet. After the attachment of the bacteria on the surface, they begin to form a group of colonies called micro-colonies. The bacteria produce EPS matrix, making them become irreversible to detach from the surface (Krzyściak *et al*., 2016). Micro-colonies contain many diverse kind of microorganisms.

Maturation stage occurs when the colonies continue to increase and co-aggregate with one another within the EPS matrix where it retains water, nutrients and enzymes in the biofilm so that continuous metabolism of the colonies will always take place (Jamal *et al*., 2018). Biofilm formation stages end with the dispersion of the cells via different modes that involve seeding, erosion and sloughing. The detachment of biofilm cells can be either single or in a group of cells (Huang *et al*., 2011). Based on a study by Kaplan (2010), this stage can be categorised into three different phases. First, the bacteria cells detach from the biofilm formation. Second, the cells translocate to other site and lastly, the cells bind to a new substrate in the new site. Kaplan also added that there are two mechanisms for the bacteria to disperse and these include active and passive. Active mechanism is when the bacteria disperse by themselves while passive mechanism is when the bacteria disperse by other external forces particularly abrasion among the particles in biofilm and fluid shear.

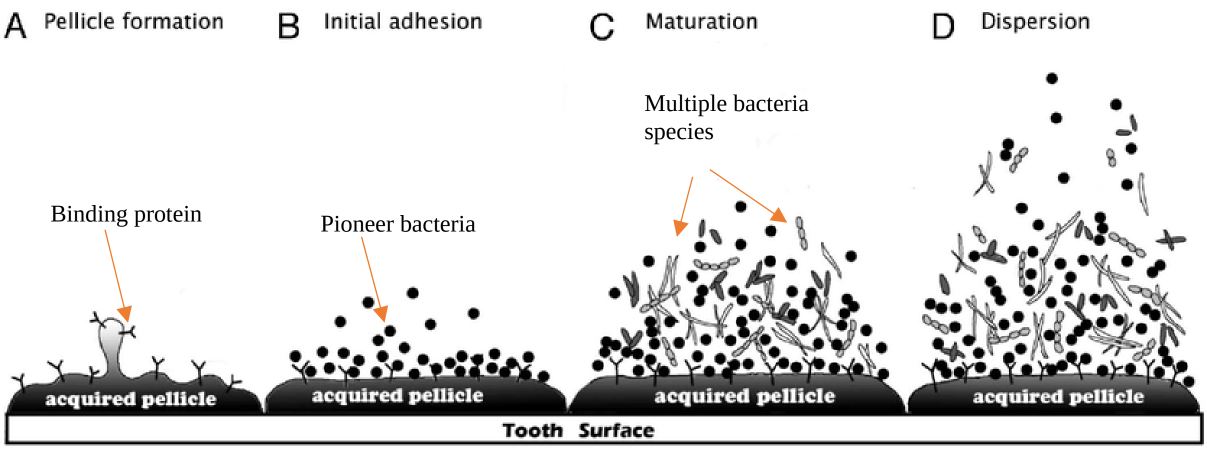

Figure 2.2 Stages of biofilm formation. A) Acquired pellicle formation. B) Primary adhesion of bacteria on the surface. C) Bacteria maturation. D) Dispersion of biofilm cells. (Adapted from Huang *et al*., 2011).

2.4 *Streptococcus mutans*

There are abundant of microorganisms living inside human oral cavity however, not all of them are considered pathogenic. Some of them are commensal organisms but sometimes can also become pathogenic due to some conditions (Avila *et al*., 2009). Tooth decay is one of the oral problems that result from the activity of pathogenic bacteria. Generally, there are several types of bacteria that cause dental caries which include gram positive cocci such as *S. mutans*, gram positive rods like *Lactobacillus fermentum*, gram negative cocci such as *Nesseria* spp and gram negative rods like *Escherishia coli*. Among these bacteria species, *S. mutans* is picked as the most cariogenic pathogens and are responsible in causing dental caries since they are highly acidogenic (Yadav & Prakash, 2017). Due to their acidogenic property, EPS matrix in the biofilm can be formed with the presence of sugars (Forssten *et al*., 2010).

2.4.1 Genes associated with *S. mutans* adhesion

Choi *et al*. (2010) suggested that there are many kinds of proteins that this bacteria species has which can catalyse the sugars to cause the production of acid. According to Hoshino *et al.* (2012), this bacteria also uses the proteins to enable adherance to the tooth surface. Adhesion can be done by utilizing adhesive glucans where these glucans can be produced from sugars with the help of glucosyltransferase (Gtf). Three Gtfs can be made by *S. mutans* strain, GtfB, GtfC and GtfD. All of them serve different roles in the formation of dental plaque (Lemos *et al*., 2019). These proteins are encoded by their respective genes which are *gtfb*, *gtfc* and *gtfd* genes. In addition, *S. mutans* can also produces other protein known as glucan-binding protein (Gbp). This protein is believed to facilitate the bacteria in adhesion (Matsumoto-Nakano, 2017). Lynch *et al.* (2013) hypothesized that three types of Gbp proteins, GbpA, GbpB and GbpC play major role in the cariogenicity of *S. mutans*. However, in other study by Matsumoto-Nakano (2017), he stated that there are at least four types of Gbps produced by *S. mutans* which include GbpA, GbpB, GbpC and GbpD. All of the proteins contribute to different roles in promoting adhesion. Both the Gtf and Gbp proteins have significant association in promoting the adhesion of the bacteria on the tooth surface (Matsumoto-Nakano, 2017).

2.5 Pulp Therapies

Untreated caries lesion can lead to the exposure of pulp whereby it can later cause inflammation and eventually result in necrosis, abscess and worse, tooth loss (Bjørndal *et al*., 2019). This condition can become severely painful and left people feeling uncomfortable all the time. Pulp exposure can also result from trauma and mechanical sources. Komabayashi *et al.* (2016) alluded that pulp exposure from trauma is caused by the injury that happens at the coronal part of the tooth while pulp exposure that results from mechanical sources happens due to the accident during tooth preparation. Hence, pulp treatment known as vital pulp therapy (VCT) is introduced which currently consists of three different techniques; pulpotomy, pulpectomy and pulp capping (Smaïl-Faugeron *et al*., 2018). These therapies are used depending on the seriousness of the pulp exposure.

Ghoddusi *et al.* (2013) suggested that there are a few factors that can determine the successfulness of a VCT. One of them is that there must be a sufficient amount of blood supply in the pulp so that it can sustain the vitality of the pulp. Besides that, VCT can be successful with the existence of healthy periodontium in which moderate to severe teeth wound cannot be proceed with treatment. The other factor for a success

VCT is the control of haemorrhage (Ghoddusi *et al*., 2013). Generally, pulpotomy is a technique used to remove the coronal part of the teeth and maintain the remaining radicular pulp (Solomon *et al*., 2015). According to Tang *et al.* (2017), the clinical success for pulpotomy in deciduous teeth or young permanent teeth is between 83%-100% and it is used for the teeth that have no necrosis. Meanwhile, pulpectomy is a technique used to remove the pulp tissue from the crown and root entirely (McTingue, 2019). It is performed when the carious lesions or traumatic injury have led the pulp tissue to irreversibly infected and necrotic.

2.5.1 Pulp capping

There are two types of pulp capping; direct and indirect. Direct pulp capping is a technique of putting the material directly on the exposed pulp for the maintenance of vital pulp and to promote the development of regenerative dentin (Ghoddusi *et al*., 2013). Meanwhile, indirect pulp capping is a technique of putting the material onto a thin layer of dentin that are near to the pulp (Ghoddusi *et al*., 2013). Indirect pulp capping is done when there is no visible exposure of vital pulp while direct pulp capping is done for the totally exposed vital pulp (Parisay *et al*., 2015). According to Dhaimy *et al.* (2019), before proceeding with direct pulp capping, dentist should do a few recommended tests that are quick, pain-free and consistent to diagnose the degree of pulpal wound. These ideal tests include thermal tests, doppler flow measurement and pulsatile oximetry. They also added that a local anaesthetics ought to be done first before performing indirect pulp capping.

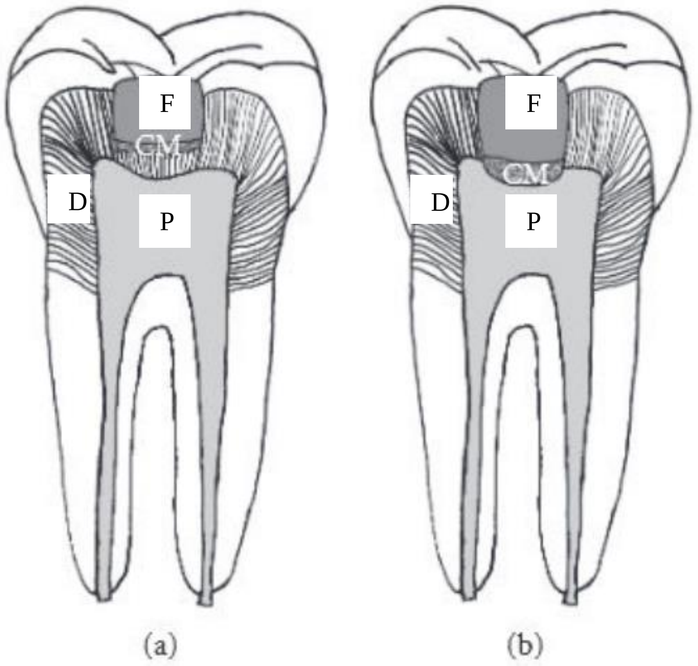

Figure 2.3 Pulp capping treatment. (a) Indirect pulp capping. (b) Direct pulp capping. D: Dentin; P: Pulp; CM: Capping Material; F: Filler material. (Adapted from Dhaimy *et al*., 2019).

2.6 Dental root repair materials

Basically, biomaterial is defined as a natural or synthetic based material that is used in clinical applications to interact with biological systems of human body (Pandey *et al*., 2018). It serve many great functions for human who have experienced injury and disease (NIH, 2017). Many kinds of elements on earth can used as a biomaterial including metals, plastics, ceramics and glasses. Interestingly, living cells and tissues can also be used as a biomaterial (NIH, 2017). Biomaterial is not only used in dental application but also in other medical applications such as joint replacements, bone plates, skin repair, heart valves and others (Tathe *et al*., 2010). Since the main purpose for pulp therapies is to maintain the vitality of the tooth, selection of a good biomaterial is essential as so that the healing process is successful and last long (Nowicka *et al*., 2016). There have been a lot of studies in many years ago regarding the ideal materials for pulp capping. Hanna *et al.* (2020), have listed the best possible characteristics that a biomaterial must possess. First, the biomaterial must obviously has the ability to sustain the vitality of pulp, must be able to promote the development of reparative dentin and must be bactericidal or bacteriostatic as well as provide bacteria tight seal. Based on another study by Ghoddusi *et al*. (2013), they claimed that a perfect dressing material is a must for pulp capping with some special properties such as biocompatible, non-cytotoxic, and antibacterial. However, in reality there are still no material that can wholly satisfy the need of a dentist to treat the pulp (Hilton, 2009).

Based on a report by Komabayashi *et al*. (2016), the history of first ever pulp capping therapy was recorded in 1756 by a dentist named Pfaff where he used a gold foil as the material for the treatment and later in 1930, a material known as $Ca(OH)_2$ was found to be a good material for pulp capping by Hermann. Generally, $Ca(OH)_2$ is an odourless material that has a molecular weight of 74.08 and also is a strong base material with pH value of 12.5 (Baranwal *et al*., 2016). According to another study by El-Mal *et al.* (2019), before the discovery of $Ca(OH)_2$, there were a few choices of material which provide satisfactory result in pulp capping such as eugenol and zinc oxide. However, $Ca(OH)_2$ was used due to its biocompatibility and antibacterial activities. The material has once become the gold standard treatment for pulp capping and is being used for many decades (El-Mal *et al*., 2019). It is stated that $Ca(OH)_2$ is beneficial as a remineralizing agent in direct pulp capping since it can dissociate both calcium and hydroxide ion (Gandolfi *et al*., 2015). Mohammadi & Dummer (2011) suggested that $Ca(OH)_2$ can give lethal effect to the bacteria on tooth through the release of its hydroxyl ion by several mechanisms which include the rupture of bacteria cytoplasmic membrane, the denaturation of bacteria protein as well as the damage of bacteria DNA..

Besides that, there is another valuable material known as MTA which has also been used in many pulp therapies due to its special properties. It was first introduced in 1990 by Torabinejad and White (El-Mal *et al*., 2019). MTA consists of mainly a combination of calcium silicates that contained calcium oxide (CaO) (50–75% w/w) and silicon dioxide (SiO_2) (15–25% w/w) (Ha et al., 2017). This material is available in two types of colors including white and gray (Macwan & Deshpande, 2014). A study conducted by Akabri *et al.* (2012) reported that MTA has initially only one colour which is gray, however this gray color can cause tooth discoloration. Therefore, white MTA is introduced as alternative option. They also stated that gray MTA and white MTA are differentiated by their chemical components in which both types of materials have different concentrations of Al_2O_3, MgO, and FeO. It is believed that this material provides satisfactory result in pulp therapies because of its small size particle and it is claimed to have the ability to promote pulpal cell proliferation (Bogen *et al*., 2008). Since MTA contains calcium, it has ability to inhibit the growth of microorganisms. When hydrolysis of MTA occurs, it can produce $Ca(OH)_2$ by-product in which later the ions can dissociate to enhance the cell proliferation as well as inhibit the growth of microorganisms due to its elevated pH value (Khan *et al*., 2014).

2.6.1 White Portland cement (PC)

In general, white PC is a hydraulic bonding material in white colour. It is the product of grinded white clinker acquired from white clay and limestone as well as calcium sulphate (Temiz *et al*., 2013). It consists of four main compounds which are dicalcium silicate, tetracalcium aluminoferrite, tricalcium aluminate, and tricalcium silicate with the silicates play the major role in giving the strength of hydrated cement paste (Negm *et al*., 2016). It is stated that this material is a part of MTA where both of them seem almost the same when view in microscopic and using X-ray analysis, however they are not really the same (Bidar *et al*., 2014). In addition, there is only one notable difference between both materials which is PC has lower radiocapacity (Vilimek *et al*., 2018). Ravi *et al.* (2011) suggested that PC has five different types with three of the first types having subtypes. The first type is a normal PC. Type I PC is the one that usually used in dental application (Ravi *et al*., 2011).Type II PC is used for water and soil that consist of sulphate. Type III PC is used for high strengths since it is a highly strength cement. Type IV PC is used to maintain a minimum amount and rate of heat generation (low heat PC). Type V is a sulphate resistant PC which is used for high alkaline water and soil (Levy, 2012).

PC exhibits many great properties just like MTA. It has the ability to give tissue response in pulp capping as well as being able to act as antimicrobial with low genotoxicity (Borges *et al*., 2014). According to Gonçalves *et al*. (2010), there are several properties which make PC a good biomaterial and these include the ability to seal and the solubility of the PC, antimicrobial activity marginal adaptation, dentine barrier formation, dimensional stability and moisture tolerance. Sealing ability of a biomaterial can be achieved when it has antimicrobial activity (Ravi *et al*., 2011). Since PC has no radio opacity additives, so discoloration can be reduced (Vilimek *et al*., 2018). MTA has been reported to have shown some disadvantages, so many researchers have been aiming to focus more on evaluating PC as the substitute for MTA in dental application. Also, PC is said to be less expensive than MTA which makes it more favourable to use (Salama *et al*., 2015). A previous study by Guerreiro-Tanomaru et al. (2012) claimed that all PC-based materials exhibited antimicrobial activity against several bacteria strains (*Micrococcus luteus*, *Streptococcus mutans, Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Candida albicans*) (Guerreiro-Tanomaru *et al*., 2012). Another study by Zarrabi *et al.* (2009) exposed that PC has shown more antimicrobial effect against *S. mutans* than against *E. coli* and *Candida*.

2.6.2 Chitosan

Basically, chitosan is produced by deacytylation of a chitin (Elieh-Ali-Komi & Hamblin, 2016). Chitin is initially comes from crustacean's exoskeleton such as lobsters, crabs and shrimps (Husain *et al*., 2017). Chitosan is a linear and semi-crystalline polysaccharide that is made up of (1 → 4)-2-acetamido-2-deoxy-β-D-glucan and (1 → 4)-2-amino-2-deoxy-β-D-glucan units (Figure 2.4) (Croisier & Jérôme, 2013). Based on a study by Tanikonda *et al.* (2014), there are two different ways of chitosan preparation from chitin. The first one is the heterogeneous deacetylation of solid chitin and the other one is the homogeneous deacetylation of pre-swollen chitin under vacuum. Between those two ways, the first one is the preferable way in industrial medication.

Chitosan has been used in many healthcare applications such as in tissue engineering, wound healing, dentistry, ophthalmology and cancer diagnosis (Elieh-Ali-Komi & Hamblin, 2016). This is because chitosan exhibits many magnificent properties as a biomaterial. For chemical properties, this material has been reported to have reactive amino groups (-NH2) and act as a chelating agent in many metal ions (Pokhrel *et al*., 2016). It is believed that plaque formation can be prevented with chitosan due to its positively charged amino groups (Tanikonda *et al*., 2014). There is a variety of biological properties of chitosan mentioned by Pokhrel *et al*. (2016). These include biocompatibility, antitumor and anticancer activity, able to antagonistically adhere mammalian cells and microbial cells, haemostatic, able to regenerate connective gum tissue and many more. In dentistry, chitosan is very common in preventing dental caries since it has the antimicrobial activity and promotes regeneration. It is stated that chitosan has low molecular weight which can inhibit *S. mutans* from adsorption to hydroxyapatite (Erpaçal *et al*., 2019). Furthermore, chitosan is not only popular in healthcare application but also in other application such as food industry, textile and paper industries, agriculture, packaging industry as well as cosmetology (Morin-Crini *et al*., 2019). Combination of chitosan with other materials have been studied previously. For example, a study Mousavi *et al.* (2020) revealed that combination of chitosan, zinc oxide and silver inhibited the growth of fungi as well as bacteria in tissue conditioners (Mousavi *et al.* 2020). Another study by Gristch *et al.* (2019) claimed that combination of chitosan and hydroxyapatite along with copper and strontium has a great potential as scaffolds in bone tissue engineering (Gristch *et al*., 2019).