ANTIMICROBIAL EFFECT AND MICROBIAL ADHERENCE TO MAXILLOFACIAL PROSTHETIC MATERIALS

FARHANA RAHMAN

UNIVERSITI SAINS MALAYSIA

2021

ANTIMICROBIAL EFFECT AND MICROBIAL ADHERENCE TO MAXILLOFACIAL PROSTHETIC MATERIALS

by

FARHANA RAHMAN

Thesis submitted in fulfilment of the requirements

for the degree of

Master of Science

March 2021

ACKNOWLEDGEMENT

In the name of ALLAH, the Most Gracious and Most Merciful

First and foremost, Alhamdulillah, all praises to Allah, the Almighty for the strengths and His showers of blessings in completing this study. In the accomplishment of this study, the most grateful to my supervisor, Dr. Yanti Johari for her vital support, for her patience, guidance and encouragement throughout my time as her student that served as the major contributor towards the completion of this study. I am extending my heartfelt thanks to my co-supervisors, Assoc. Prof. Dr. Zaihan Ariffin, Dr. Suharni Mohamad and Dr. Nafij Jamayet for providing invaluable guidance throughout the research. Also I would like to thanks Prof. Dr. Mariatti Jaffar from School of Materials and Mineral Resources Engineering, Universiti Sains Malaysia for helping in the preparation of the modified PMMA (m-PMMA), which held the most vital part of this research. Last but not least, I would like to thanks to the staff at Prosthodontic Laboratory and Craniofacial Science Laboratory with special thanks to Mr. Fairuz Bin Hasbullah, Ms. Asiah Abu Bakar and Mr. Mohamad Ezany Bin Yusoff for their time and valuable efforts. I am grateful to my parent Mrs. Monowara Begum, my brother Mr. Mohammad Mahmudur Rahman for their continuous faith, love care unceasing prayers and encouragement of my efforts over the years. My special thanks goes to my postgraduate friends and research colleagues, Hasan, Mr. Mohammad ezany for their willingness to share their bright ideas and thoughts with me as well as for providing me new knowledge through deep discussions. Last but not least, I thank all those who helped me directly or indirectly in the successful completion of my study.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SYMBOLS AND ABBREVIATIONS

%	Percentage
ºC	Degree celsius
µl	Microlitre
AgNPs	Silver nanoparticles
ATCC	American Type Cell Culture
ANOVA	Analysis of variance
BHI	Brain heart infusion
BPO	Benzoyl peroxide
Cm^2	Centimeter square
C. albicans	*Candida albicans*
CFU	Colony forming unit
CLSI	Clinical and Laboratory Standards Institute
c-PMMA	Commercial polymethyl methacrylate
dl	Deciliter
ºC	Degree celsious
H^+	Hydrogen ion
H_2O	Water
hr	Hour
HTV	Heat vulcanized silicone
HA	Hydroxyapatite crystals
MANOVA	Multivariate analysis of variance
mg	Miligram
mm	Millimolar

mL	Mililitre
m-PMMA	Modified polymethyl methacrylate
MRSA	Methicillin resistant *Staphylococcus aureus*
MHBA	Mueller-Hinton blood agar
OD	Optical density
PBS	Phosphate buffer solution
PLA	Polylactic acid
PDMS	Polydimethylsiloxane
PMMA	Plymethyl methacrylate
Sd	Standard deviation
SDA	Sabouraud dextrose agar
SDB	Sabouraud dextrose broth
SEM	Scanning electron microscope
SPSS	Statistical Package of social sciences
S. aureus	*Staphylococcus aureus*
S. mutans	*Streptococcus mutans*

KESAN ANTIMIKROBIAL DAN PELEKATAN MIKROBIAL PADA BAHAN PROSTETIK MAKSILOFASIAL

ABSTRAK

Prostetik maksilofasial digunakan untuk memulihkan kecacatan fungsi dan anatomi kawasan maksilofasial yang disebabkan oleh trauma atau tumor. Ia dapat memberi manfaat kepada pesakit jika bahan tersebut pembuatan untuk menunjukkan kesan antimikrob dan menahan pelekatan mikrob bagi mengurangkan risiko jangkitan. Objektif kajian ini adalah untuk menilai kesan antimikrob, kekasaran permukaan dan pelekatan mikrob bahan hasil keluaran tempatan iaitu polimetil metakrilat (m-PMMA) yang telah diubahsuai berbanding dengan polimetil metakrilat yang dihasilkan secara komersial (c-PMMA), silikon A-2000 dan silikon A-2186 yang merupakan bahan yang biasa digunakan untuk prostesis maksilofasial. Strain mikrob iaitu *Staphylococcus aureus (S. aureus)*, *Streptococcus mutans (S. mutans)* dan *Candida albicans (C. albicans)* digunakan dalam kajian ini. Kesan antimikrob bahan prostetik maksilofasial ditentukan dengan ujian resapan agar, manakala pelekatan mikrob dianalisa menggunakan kaedah penghitungan koloni secara langsung dan kekasaran permukaan bahan yang diuji ditentukan menggunakan profilometer. Imbasan mikroskop elektron (SEM) juga digunakan untuk memeriksa kekasaran permukaan dan pelekatan mikrob. ANOVA sehala digunakan untuk menganalisis kekasaran permukaan dan analisis varians multivariate (MANOVA) digunakan untuk menganalisa pelekatan mikrob. Untuk aktiviti antimikrob, diperhatikan bahawa semua bahan yang diuji tidak merencat pertumbuhan semua strain mikrob yang diuji. Analisis kekasaran permukaan menunjukkan perbezaan yang signifikan ($p < 0.05$) antara elastomer silikon dan PMMA. Terdapat juga

perbezaan yang signifikan dalam pelekatan bakteria pada bahan yang diuji di mana unit pembentukan koloni (CFU) *S. aureus* dan *S. mutans* secara signifikannya adalah lebih tinggi pada permukaan yang kasar iaitu elastomer silikon berbanding PMMA ($p>0.017$). Tidak terdapat perbezaan yang signifikan dalam pelekatan *C. albicans* antara elastomer silikon dan PMMA. Ini dapat disimpulkan bahawa kehadiran pengisi dalam m-PMMA mungkin tidak mencukupi untuk merangsang kesan agen antimikrob, namun, m-PMMA menunjukkan kurang pelekatan mikrob berbanding bahan lain yang diuji. Hasil kajian juga menunjukkan bahawa kekasaran permukaan bahan memainkan peranan penting dalam pelekatan mikrob.

ANTIMICROBIAL EFFECT AND MICROBIAL ADHERENCE TO MAXILLOFACIAL PROSTHETIC MATERIALS

ABSTRACT

Maxillofacial prostheses are used to restore the functional and anatomical defects of the maxillofacial region caused by trauma or tumour. It is beneficial to the patients if the materials used for the fabrication of the maxillofacial prostheses could demonstrate antimicrobial effects and resist microbial adherence hence reducing the risk of infection. The objective of this study is to evaluate the antimicrobial effect, surface roughness and microbial adherence of the locally produced material namely modified polymethyl methacrylate (m-PMMA) against commercially produced polymethyl methacrylate (c-PMMA), silicone A-2000 and silicone A-2186 which are commonly used materials for maxillofacial prostheses. The microbial strains namely *Staphylococcus aureus* (*S. aureus*), *Streptococcus mutans* (*S. mutans*) and *Candida albicans* (*C. albicans*) were used in this study. Antimicrobial effect of maxillofacial prosthetic materials was determined by agar diffusion test, whilst microbial adherence was analysed using a direct colony-counting method and surface roughness of tested materials was determined using profilometer. Scanning electron microscopy (SEM) images were also used to examine the surface roughness and microbial adherence. One-way ANOVA was used to analyse surface roughness and Multivariate Analysis Of Variance (MANOVA) was used to analyse microbial adherence. For antimicrobial activity, it was observed that all tested materials did not inhibit the growth of all tested microbial strains. Surface roughness analysis showed significant difference ($p<0.05$) between PMMA and silicone elastomers. There was

also significant difference in bacterial adherence on the tested materials in which significantly higher colony-forming unit (CFU) of *S. aureus* and *S. mutans* were observed on roughened surfaces namely silicone elastomers than that of PMMA (p>0.017). No significant difference was observed in the adherence of *C. albicans* between silicone elastomers and PMMA. It can be concluded that the presence of fillers in m-PMMA may not be adequate to promote the release of antimicrobial agents, however, m-PMMA showed less microbial adherence in comparison to other tested materials. The findings also demonstrate that surface roughness of the materials play an important role in microbial adherence.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Maxillofacial prosthesis is used to replace the missing facial parts which have been lost to accident, disease and trauma around the head and neck areas. The provision of maxillofacial prosthesis is the immediate management for such defects for the replacement of the missing structure with acceptable appearances and improved function (Beumer *et al.*, 1996). They are two types of maxillofacial prosthesis namely extraoral prostheses which includes orbital, nose and ear prosthesis whilst intraoral prostheses includes obturator, stent and speech aid prosthesis.

Many materials have been used for the fabrication of the maxillofacial prosthesis. The material should be flexible, dimensionally stable, light in weight preferably with low thermal conductivity and acceptable strength. It is crucial that the materials should also possess good biological and chemical properties since they are exposed to the unpredictable environmental condition. Apart from possessing a good handling characteristic to assist in the prosthesis fabrication and esthetics value for the appearance, the material should also display its biocompatibility value. Furthermore, the material will be in direct contact with the skin, hence making them susceptible to bacterial colonization (Ariani *et al.,* 2012). Hence, it is beneficial if the material can demonstrate a certain degree of antimicrobial activity and be resistant to microbial colonization.

Due to their unique properties, the polymethyl methacrylate (PMMA) and silicone elastomers materials have become the preferred choice for the fabrication of maxillofacial prosthesis. However, those mentioned materials are not without their shortcomings. The surfaces of the facial prosthesis are susceptible to bacterial adherence that may lead to bacterial colonization (Aziz *et al.*, 2003; Mitra *et al.*, 2014; Sakaguchi and Powers, 2012). Among the properties, the water absorption properties were given emphasis since facial prostheses may absorb saliva or sweat from surrounding facial tissue or during the cleaning of the prosthesis with water. Any absorbed water may affect the physical properties and the perception of color matching to the surrounding facial tissue (Chalian and Phillips, 1974). Besides that, the surface chemistry and the surface topography also have an impact on the adherence of the microorganisms and promote the microbial colony formation in the presence of surface irregularities (Verran and Maryan, 1997).

The oral cavity and facial areas provide conducive environment for harboring microorganisms. These microorganisms get attached to the desired surface of the materials and they produce extracellular polysaccharide leading to the formation of biofilm (Kröncke *et al.*, 1990). This biofilm is a major source for the attachment and the growth of the microorganisms. In dentistry, microbial contamination often grows in the dental tube due to the flow of water and aerosols. Besides that, the most common biofilm-associated recurring diseases that were commonly found around 11-67% of the denture wearers is the denture stomatitis (Luo *et al.*, 2010). Therefore, the biofilm acts as a harbor for pathogenic organisms which acts as a persistent source of infections (Donlan, 2001).

In this study, the commonly used material for maxillofacial prosthesis fabrication namely PMMA and silicone elastomers will be tested against *Staphylococcus aureus* (*S. aureus*), *Streptococcus mutans* (*S. mutans*) and *Candida albicans* (*C. albicans*). *S. aureus* is a facultative anaerobic gram-positive coccus which is present in the nasal cavity for at least 30% of the normal population, which renders it a major risk factor for infections. *S. mutans* is also a gram-positive, facultative anaerobic bacterium that is commonly found in the oral cavity. *C. albicans* is an opportunistic pathogen in the human oral cavity and a major microbial agent causing denture stomatitis and commonly found in denture wearers; on the surfaces of denture bases and the silicone elastomers (Ramage *et al.*, 2004).

Therefore, the aim of the study is to evaluate the antimicrobial effect, surface roughness and microbial adherence on maxillofacial prosthetic materials. It is hoped that the findings of this study can help the clinicians during the selection of maxillofacial prosthetic materials.

1.2 Problem statement

It has been established previously that the colonization of the microorganisms surrounding the prosthesis both externally and internally can certainly cause some problems to the individuals (Arciola *et al.*, 2012; Campoccia *et al.*, 2010). The adherence of the microorganisms on the surfaces of the maxillofacial prosthetic materials can cause infections to the surrounding tissues, which then lead to discomfort and irritation to the patients. Thus, it is beneficial if the material also possesses the antimicrobial effects in the same instance with the least microbial adherence.

Although PMMA is widely used for fabrication of maxillofacial prosthesis in clinical dentistry indeed it is a major challenge of using this substance is its poor antimicrobial effect (Lee *et al.,* 2018), which increases oral infections caused by microbial adhesion. In this study, there have been modifications on the PMMA by incorporating 0.5% benzoyl peroxide (BPO), 2% hydroxyapatite HA and 2% polylactic acid (PLA) fillers particles in order to achieve antimicrobial effect, therefore less microbial adhesion would occur on the surfaces of modified PMMA (m-PMMA) over commercially obtained c-PMMA, silicone A-2000, silicone A-2186. This m-PMMA is locally produced at the School of Material and Mineral Resources Engineering, Universiti Sains Malaysia (USM). Therefore, it is hoped that the development of methods that could modify the surfaces to prevent the adhesion of the microorganisms, would be a significant advancement in the treatment of clinical dentistry.

1.3 Justification of the study

School of Material and Mineral Resources Engineering, Universiti Sains Malaysia has produced a local m-PMMA which includes 2% HA, 0.5% BPO, 2% PLA. Research on its mechanical and physical properties has been performed involving flexural, impact strength, fracture toughness, and hardness tests. However, the property of the m-PMMA with respect to biocompatibility, antimicrobial effect and microbial adherence has not yet been explored. Thus, this study is aimed to investigate some of the biological components of m-PMMA including its antimicrobial effect and the microbial adherence phenomenon. Surface roughness test was also incorporated into this study to assist in the understanding of the effect of surface irregularities towards microbial adherence.

1.4 Objectives of the study

1.4.1 General objective

To evaluate the antimicrobial effect and microbial adherence of maxillofacial prosthetic materials.

1.4.2 Specific objectives

i. To determine the antimicrobial effect of modified PMMA (m-PMMA) in comparison to commercial PMMA (c-PMMA), silicone A-2000 and silicone A-2186 against *S. aureus*, *S. mutans,* and *C. albicans.*

ii. To investigate the surface roughness of m-PMMA in comparison to c-PMMA, silicone A-2000 and silicone A-2186.

iii. To determine the microbial adherence of *S. aureus*, *S. mutans,* and *C. albicans* on the surfaces of m-PMMA in comparison to c-PMMA, silicone A-2000 and silicone A-2186.

iv. To evaluate the microbial adherence of *S. aureus, S. mutans,* and *C. albicans* and surface topography of m-PMMA in comparison to c-PMMA, silicone A-2000 and silicone A-2186 using scanning electron microscope (SEM).

1.5 Research questions

i. Are there any differences in the antimicrobial effect of m-PMMA in comparison to c-PMMA, silicone A-2000 and silicone A-2186 against *S. aureus*, *S. mutans,* and *C. albicans*?

ii. Are there any differences in the surface roughness of m-PMMA in comparison to c-PMMA, silicone A-2000 and silicone A-2186?

iii. Are there any differences in the microbial adherence of *S. aureus*, *S. mutans*, and *C. albicans* on the surfaces of m-PMMA in comparison to c-PMMA, silicone A-2000 and silicone A-2186?

iv. Are there any differences in the microbial adherence of *S. aureus, S. mutans,* and *C. albicans* and surface topography of m-PMMA in comparison to c-PMMA, silicone A-2000 and silicone A-2186?

1.6 Null hypothesis

i. There are no differences in antimicrobial effect of m-PMMA in comparison to c-PMMA, silicone A-2000 and silicone A-2186 against *S. aureus*, *S. mutans*, and *C. albicans*.

ii. There are no differences in the surface roughness of m-PMMA in comparison to c-PMMA, silicone A-2000 and silicone A-2186.

iii. There are no differences in microbial adherence of *S. aureus*, *S. mutans,* and *C. albicans* on the surfaces of m-PMMA in comparison to c-PMMA, silicone A-2000 and silicone A-2186.

iv. There are no differences in microbial adherence of *S. aureus*, *S. mutans,* and *C. albicans* and surface topography of m-PMMA in comparison to c-PMMA, silicone A-2000 and silicone A-2186.

CHAPTER 2

LITERATURE REVIEW

2.1 Maxillofacial prosthesis

Facial deformity can be the result of accidental trauma, treatment of neoplasm or congenital malformation. These defects or deformities are usually replaced or restored by prosthesis in order to maintain the aesthetic function and daily social activities. The prosthesis or maxillofacial prosthesis is indicated when surgical reconstruction is not feasible or inadequate. Maxillofacial prosthesis can be defined as any prosthesis which is used to replace the part or all of any stomatognathic and/or craniofacial structure (Barhate *et al.*, 2015). It can be classified into extraoral and intraoral prosthesis. Extraoral prostheses include ocular, orbital and nasal prostheses, while extraoral prostheses can be obturator or tongue prostheses. Extraoral and intraoral maxillofacial prostheses are widely preferred among patients as the defects of the craniofacial region create an unpleasant condition for the individual to lead a comfortable life (Ahmed *et al.*, 2010).

Success of treatment in maxillofacial prosthodontics mainly depends upon proper diagnosis, treatment planning and materials used in fabrication of prostheses. However, prosthodontists were limited by materials available to construct the ideal prosthesis when dealing with movable tissue, large and heavy facial prosthesis, retention of prostheses as well as patient's expectation of the final outcome (Mahajan and Gupta, 2012).

The material selection presumes great importance in the field of maxillofacial prosthodontics as it provides life like appearance and fine details to many patients who suffer from orofacial deformities (Koran and Craig, 1975). Therefore, the material should satisfy the functionality, biocompatibility, aesthetics, as well as durability to serve as a maxillofacial prosthetic material. Hence, there are still ongoing studies in the field of dental materials in search for the ideal material for maxillofacial prosthesis. The history of making maxillofacial prosthesis to restore defects dated back for centuries when the Egyptians and Chinese used wax and resins to reconstruct the missing portion of the head and neck region. In the beginning of the sixteenth century, a renowned French surgeon named Ambrose Pare introduced the idea of the fabrication of the nasal prosthesis which was considered very important for facial beautification (Beumer *et al.*, 1996; Chalian and Phillips, 1974; Moore *et al.*, 1977). In the year 1862, William Morton used porcelain in the fabrication of nasal prosthesis that matched the exact color of the patient's face which was indeed a revolution in the field of prosthesis (Barhate *et al.*, 2015; Maller *et al.*, 2010). The nasal prosthesis was made of gold, silver, paper and liner cloth that was attached to the face by strings or glued together (Beumer *et al.*, 1996). Another innovation was the combination of nasal and palatal prosthesis in which the obturator portion was a prime part of nasal prosthesis (Barhate *et al.*, 2015; Maller *et al.*, 2010).

2.2 Maxillofacial prosthetic materials

There are a variety of materials, such as wood, wax and metals which have been used for the fabrication of facial prosthesis from ancient times (Beumer *et al.*,1996; Moore *et al.*,1977). However, there has been a gradual improvement in the material selection for facial prosthesis in which polymers have been highlighted as the material of choice in recent years. In order to achieve clinical success and patient's acceptance, the operator must have a thorough understanding of the characteristics of the materials being used for specific defects. Maxillofacial prosthetic materials are a combination of extraoral (vinyl chloride polymers, polymethylmethacrylate, silicones, and polyurethane) and intraoral (silicones, polymethylmethacrylate, tantanum, ticonium, vitallium) prosthetic materials. Biocompatibility is a major concern for maxillofacial prostheses which needs to be considered before fabricating the prostheses (Beumer *et al.*, 1996). Materials should not contain any toxic components or carcinogenic agents that can harm the underlying tissues (Roberts, 1971). However, studies on these materials are still ongoing to overcome their toxicity and to come out with a new exclusive material that can be labelled as the "ideal maxillofacial prosthetic material".

The completed facial prostheses should be unnoticeable in public; faithfully reproducing lost structures in the finest detail. The color, texture, form, and translucency must duplicate that of missing structures and adjacent tissues.

2.3 Materials of preference worldwide

For the last 50 years, silicone elastomers are the primary selection for the fabrication of maxillofacial prosthesis (Han *et al.*, 2008). According to the American Academy of Maxillofacial Prosthetics (AAMP), the country ranked the highest for the rate of responses in choosing the silicone elastomers in the field of maxillofacial prosthetics is the United States (86%), followed by Australia (5%), Canada, Asia and then Europe (about 1%). In a survey, it showed that the majority of clinician was using room temperature-vulcanized (RTV) silicone products. The reason for selecting RTV silicone material includes the use of stone molds, ease of manipulation, and ease of coloring (Montgomery and Kiat-Amnuay, 2010).

Next to silicone elastomers, the acrylic (polymethylmethacrylate) is preferable in the tissue bedding area where the least movement takes place during functioning. Therefore, both acrylic and silicones were given preferences in the field of dentistry.

2.4 Acrylic resins (PMMA)

The evolution of acrylic resins started in 1940 and since then, it has been one of the most acceptable materials besides silicone for maxillofacial prosthesis. Like other prosthetic materials, acrylic resins also have both advantages and disadvantages. The advantages include offering good retention, possibility of repair and relining, color stability and reasonable good shelf life of about two years (Khindria *et al.*, 2009; Maller *et al.*, 2010). However, discoloration of acrylic resin was seen as a disadvantage. With the presence of the new generation of the acrylic monomer, oligomers and macromere are hoped to reduce the shortcomings of traditional acrylic copolymers. Acrylic resins are divided into heat cure, cold cure and light activated (Maller *et al.*, 2010).

2.4.1 Modified polymethylmethacrylate

There are some shortcomings of PMMA, such as polymerisation shrinkage, low flexural strength, low impact strength and low fatigue resistance. Hence, Mallikarjuna *et al.* (2015) proposed few methods to improve the properties of PMMA resin such as:

(i) Usage of polycarbonates and polyamides as substitutes for PMMA,

(ii) Chemical alteration of PMMA which was carried out by the addition of copolymers, cross-linking agents and rubber substances within the form of butadiene styrene and

(iii) The incorporation of fillers such as fibers, metal or ceramic into the denture bases as fillers.

According to Rajul and Romesh (2015), adding fillers to PMMA in order to reinforce properties is considered to be the most effective and reasonable method. Those fillers include resin, metal and ceramic forms to increase the mechanical, thermal and chemical properties of PMMA. A number of studies had been carried out to evaluate the mechanical properties of PMMA reinforced with different fillers (Alhareb *et al.*, 2015; Hamizah *et al.*, 2012; Vivek and Soni, 2015). Besides the incorporation of fillers to improve the mechanical properties, some studies are looking at incorporation of antimicrobial agents into PMMA (Luo *et al.*, 2010; Lyutakov *et al.*, 2015; Prokopovich *et al.*, 2015). One of the studies showed the antimicrobial activity and biocompatibility of polyurethane iodine complexes which exhibited potent antimicrobial activity against Gram-negative and Gram-positive bacteria (including methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* and bacterial spores), fungi, and viruses, as well as inhibited surface bacterial colonization and biofilm-formation (Luo *et al.*, 2010).

Another study demonstrated that PMMA films doped with either silver ions, silver nanoparticles (AgNPs) or silver-imidazole polymeric complexes displayed varying degrees of antibacterial activity against both *Staphylococcus epidermidis* and *Escherichia coli* (Lyutakov *et al.*, 2015). In early 2014, Prokopovich *et al.* (2015) also demonstrated that when oleic acid capped silver nanoparticles were encapsulated into PMMA-based bone cement samples, they exhibited antimicrobial activity against MRSA, *S. epidermidis* and *Acinetobacter baumannii* at nanoparticles concentrations as low as 0.05% (w/w). Shi *et al.* (2000) further noted that four materials, including PMMA, polyurethane, polystyrene, and silicone which are all used in the fabrication of maxillofacial prostheses, exhibited bacterial adherence in great numbers on their surfaces. Bacterial adherence to maxillofacial prostheses contributes to skin infections around the region of the prostheses, gradually leading to patient's refusal to use the prostheses.

In this research, m-PMMA was locally produced at School of Material and Mineral Resources Engineering Universiti Sains Malaysia which includes 2% HA, 0.5% BPO and 2% PLA. This material was subjected to antimicrobial testing and microbial adherence assay. The fillers that were added helped increase the mechanical properties of PMMA and were believed to contain antimicrobial properties as described below.

2.4.1(a) Hydroxyapatite

Hydroxyapatite (HA) is an amorphous calcium phosphate which has calcium phosphorus (Ca:P) ratio of 10:6 with chemical formula $Ca_{10}(PO_4)_6(OH)_2$. It was introduced in 1975 as a filling material for intrabody defects. Besides that, HA is considered as bioactive filler because of its similarity toward the biological HA in impure calcium phosphate form which can be discovered in human bone and teeth. It is an attractive biomedical material owing to its excellent biocompatibility, osteoconductivity, osteophilic and non-toxic chemical components (Kantharia *et al.*, 2014). HA has almost similar composition to the mineral component of human bone and teeth. This is the reason why most of the dental and medical profession tends to use HA as biomaterial for medical and dental applications (Dorozhkin and Epple, 2002).

Chemically, HA contains $Ca\ (OH)_2$ that has been established as a medicament for over 40 years. It was reported that $Ca(OH)_2$ has a wide range of benefits as an antimicrobial and antifungal medicaments and is also considered the best medicament in reducing residual microbial flora (Blanscet *et al.*, 2008; Morrier *et al.*, 2003).

2.4.1(b) Polylactic acid microsphere

Polylactic acid (PLA) is aliphatic polyester which has an outstanding advantage compared to other polymers. In the early 1970's, PLA products were approved by the U.S Food and Drug Administration (FDA) for direct contact with biological fluids. Hence, PLA is safe to be used for oral application (Li *et al.*, 2019). PLA and its degradation products, H_2O and CO_2 are non-toxic and non-carcinogenic to the human body. With this property, PLA has been used in many biomedical applications including clips, sutures, and drug delivery systems (DDS). PLA is often used as an antimicrobial carrier for antimicrobial packaging and coating. PLA ([CH (CH3) COO] n) was derived from lactic acid monomer. Lactic acid is released by lactic acid bacteria as an important antibacterial agent to fight against pathogens and spoilage microorganisms. However, the antibacterial effect of pure PLA is not remarkable (Li *et al.*, 2019).

2.4.1(c) Benzoyl peroxide

Benzoyl peroxide (BPO) is a medication and industrial chemical (Gollnick *et al.*, 2015). Usually, 5% BPO is used for acne treatment sufficient to control acne grade I-II (Worret and Fluhr, 2006). In this research, BPO is used as an initiator in modified PMMA.

2.4.1(d) Alternative materials for PMMA

(i) Polyvinylchloride and copolymer

The polymers for maxillofacial applications showed some properties like flexibility, adaptability to both intrinsic and extrinsic staining. To produce an elastomeric effect, plasticizers are added at room temperature. Other ingredients include cross-linking agents which are added to increase the strength and ultraviolet stabilizers for color stability.

(ii) Polyurethane elastomer

Epithane-3 and Calthane are the only polyurethane materials which are available for facial prosthesis. Due to the flexible properties of the material, the margin can be made thin without compromising the strength and help in obtaining optimal aesthetics. However, they exhibit disadvantages such as poor color stability, poor compatibility and moisture sensitivity leading to formation of gas bubbles (Mitra *et al.*, 2014).

(iii) Chlorinated polyethylene

This material resembles to polyvinylchloride in its chemical composition and physical properties. Chlorinated polyethylene elastomer possesses some properties like less irritation to the mucosa, less toxic and non carcinogenic which makes this material an acceptable substitute for silicones. Chlorinated polyethylene elastomer is also a suitable substitute in the fabrication of extraoral maxillofacial prosthesis where cost of silicone is prohibitive (Mitra *et al.*, 2014).

2.4.2 Commercial polymethylmethacrylate

Polymethyl methacrylate is a hydrophobic polymer with properties like tough, leathery, and flexible. Its properties depends on the polymerisation time, temperatures, pressure applied during curing and the rate of cooling after processing (Doğan *et al.*, 1995). However, if the polymerisation time is cut short and the temperatures increased before complete monomer consumption, the unpolymerised monomer will vaporise and cause porosity in the polymer matrix that eventually deteriorates its mechanical strength and the excess residual monomer (Davy and Braden, 1991) gives a toxic effect in service. The advantages of PMMA include excellent cosmetic results, color stability, and compatibility with most adhesive systems. Due to its low cost, mechanical strength and minimal inflammatory responses, PMMA has been chosen as the frequently used material for maxillofacial prosthesis (Lyutakov *et al.*, 2015).

2.5 Silicone

Due to some good physical properties like tear and tensile strength, easy manipulation, higher range of chemical inertness, low degree of toxicity, high degree of thermal and oxidative stability, silicone has been the most acceptable material as maxillofacial prosthesis since 1960 and has a wide range of acceptability over other materials (Mitra *et al.*, 2014). Silicone is generally a combination of organic and inorganic compounds where chemically, the molecule of siloxane bonds Si-O-Si acts as the main chain and Si-C bond is the side group bonded to either side of silicone which makes it extremely flexible (Mitra *et al.*, 2014). The interactions between polydimethylsiloxane (PDMS) chain and silica fillers affect the physical properties of the silicone-based maxillofacial prosthesis. Silicone can be produced in the form of rubber, fluids or resins. According to the vulcanizing reaction, silicone is classified into two groups namely room temperature vulcanized silicone (RTV-silicone) and heat vulcanized silicone (HTV-silicone) (Beumer *et al.*, 1996; Chalian and Phillips, 1974; Moore *et al.*, 1977).

2.5.1 RTV-silicone

RTV-silicone is further divided into two groups:

(i) Cross-linking by condensation reaction

The condensation reaction of cross-linking requires cross-linking agents, for example, tetraethyl silicate and a catalyst, such as dibutyltin dilaurate. For example: Medical adhesive type A (Dow corning).

(ii) Cross-linking of polysiloxanes by addition reaction

These silicones are not truly room vulcanized silicones due to the curing temperature which requires heating the material at 150°C for an hour. The advantages are color stability and biological inertness. For examples: Silastic 382, 399, 891, MDX4-4210, Cosmesil, A-2186, and A-2186F (Mitra *et al.*, 2014; Mohammad *et al.*, 2010).

(a) Silicone A-2000

Silicone A-2000 was introduced in the year 2000, which was specially formulated for maxillofacial prosthetics. The viscosity of this material can be controlled by the clinicians while working with the material (Mitra *et al.*, 2014).

(b) Silicone A-2186

Silicone A-2186 was the first commercial platinum-catalysed silicone elastomers, introduced by factor II and incorporated in year 1986. It is a pourable silicone which is supplied in two parts; Part A (base): Part B (catalyst) in a ratio of 10:1, in clear to translucent form. The curing process usually occurs at room temperature. However, curing time can be reduced with elevated temperature. The advantages of silicone A-2186 include a higher amount of platinum content which shows a higher rate of polymerization (Mitra *et al.*, 2014).

2.5.2 HTV-silicone

HTV-silicone is occasionally used for maxillofacial prosthesis for white opacity and a putty-like consistency. The processing temperature of HTV-silicone is 180° to 220°C for about 30 minutes under pressure in a metallic mold. Despite poor aesthetic properties, less elasticity and high technique sensitivity, it exhibits great advantages such as high tensile and tear strength, chemical stability with excellent thermal and colour properties (Mitra *et al.*, 2014). Examples of HTV-silicone are silastic S-6508, 370, 372, 373, 382, 379, Q7-4650, Q7-4635, Q7-4650, Q7-4735 and SE-4524U (Mitra *et al.*, 2014).

2.5.3 Alternative materials for silicone

(i) **Foaming silicones:** Silastic 386 is a type of RTV materials. One of the main advantages of foaming silicones is the reduction in the weight of the prosthesis, however it has reduced strength and hence, easily teared (Mitra *et al.*, 2014).

(ii) **Silicone block polymer:** Silicone block polymers are used to overcome the hydrophobicity caused by silicone (Lewis and Castleberry, 1980).

(iii) **Polyphosphazene:** Polyphosphazenes with little or no fillers and decreasing the ratio of acrylic to rubber yields a softer rubber which is similar to human skin (Mitra *et al.*, 2014).

(iv) **Siphenylene:** Siphenylene is preferred for achieving a better edge strength, low modulus of elasticity and color stability (Lewis and Castleberry, 1980).

2.6 Characteristics of an ideal material

An ideal prosthetic material should have physical and mechanical properties similar to the human tissue that is being replaced. It should be compatible with human tissue, non-toxic, non-allergic, easy to clean and allows intrinsic and extrinsic means of coloring or staining (Lewis and Castleberry, 1980). Adherence to the human tissue, by adhesive or other mechanical means is also one of the ideal characteristics of maxillofacial prosthetic materials. It should also possess antimicrobial property in order to prevent infection. Besides that, it should maintain a relatively simple polymerization process, not sensitive to minor processing variables, and require materials and molding procedures commonly used in dentistry (Andres *et al.*, 1992).

2.7 Properties of maxillofacial prosthetic materials

The success of a maxillofacial prosthesis depends on multiple factors. This includes ability to restore the lost part inconspicuously. Apart from that, it should also be practical so that it is socially acceptable for the patient to be worn on a daily basis. Besides that, a good maxillofacial prosthesis has the adaptability in changing shape and color, so that as the complexion changes with time, it can be changed and minor changes in the tissue can be compensated without having to re-create the entire prosthesis (Khindria *et al.*, 2009).

2.7.1 Aesthetic properties

The color, texture, form, and translucency play a vital role in the successful acceptance of the prosthesis by the patient. Therefore, it should be constructed to resemble the missing structures and the adjacent skin as close as possible (Khindria *et al.*, 2009).

2.7.2 Physical properties

The material used to construct maxillofacial prosthesis should be dimensionally stable and demonstrate high strength, low surface roughness, low surface tension, good retention, able to maintain a minimum thickness as well as has a long shelf life (Khindria *et al.*, 2009).

2.7 .3 Mechanical properties

The mechanical properties of maxillofacial materials are characterised by flexural strength, tensile strength, hardness and elongation. Flexural strength is one of the most important mechanical properties required for denture base resin materials. In addition, it has been reported that acrylic resin with incomplete polymerisation will have lower mechanical properties compared to those with complete polymerisation (Harrison and Huggett, 1992). The tensile strength of PMMA resins can be increased by reinforcing it with HA filler. Calabrese *et al.* (2016) demonstrated that PMMA/HA composite showed the maximum flexural properties at 20 wt% of HA.

Fracture toughness is an indication of the amount of stress required to propagate a pre-existing flaw. However, one of the major shortcomings of PMMA resins is low fracture toughness which can be improved by adding fillers to PMMA composite (Puri *et al.*, 2008). Hardness is a measure of how resistant solid matter is to various kinds of permanent shape change when a compressive force is applied. It is also important in denture base because the hardness of the surface helps facilitate easy finishing or polishing (Moussa *et al.*, 2016). Impact strength is the capability of the material to withstand a suddenly applied load and is expressed in terms of energy. It was reported that incorporation of PMMA resin and filler results in impact strength improvement (Mathew *et al.*, 2014).

2.7.4 Biological properties

The material should remain stable when exposed to environmental assaults, adhesives, and their solvents. Ideal biological properties of the maxillofacial materials include non-allergenic, cleansable with disinfectants, color stability, inert to solvents and skin adhesives, and resistant to growth of microorganisms. It should exhibit good life of at least 6 months without significant compromise of aesthetic and physical properties (Gonzalez *et al.*, 1978).

2.7.5 Antimicrobial properties

Antimicrobial activity of different types of maxillofacial prosthetic materials has been a subject of great interest among researchers. A study conducted by

Lyutakov *et al*. (2015) demonstrated that PMMA films doped with either silver ions nanoparticles AgNPs or silver-imidazole polymeric complexes displayed varying degrees of antimicrobial activity against both *S. epidermidis* and *E. coli*. Prokopovich *et al*. (2014) also demonstrated that oleic acid capped silver nanoparticles AgNPs encapsulated into PMMA-based bone cement samples exhibited antimicrobial activity against MRSA, *S. epidermidis* and *A. baumannii* at AgNPs concentrations as low as 0.05% (w/w). Luo *et al*. (2010) reported that polyurethane-iodine complexes material exhibited potent antimicrobial activity against gram-negative and gram-positive bacteria (including MRSA, vancomycin-resistant *E. faecium*, and bacterial spores), fungi, and viruses, as well as inhibited surface bacterial colonisation and biofilm-formation.

2.8 Microbial adherence

The intraoral and extraoral region is a unique and suitable environment which comprises a variation of hard, soft, artificial and natural ecological niche. To stay alive in the intraoral and extraoral region, microorganisms tend to adhere to either soft or hard tissues (Shemesh *et al.*, 2010). The process of microbial adherence can be described as "a complex phenomenon like physiochemical interaction between the microorganisms and the surfaces manipulating the balance of attraction and repulsive forces of both sides". Initial stages of bacterial colonisation involve bacterial adherence to the surfaces and production of extracellular polysaccharide, which then leads to the biofilm formation. Once the microorganisms are matured, it will detach and gradually disperse singly and again will adhere to the desired surface in favourable conditions (Veerachamy *et al.*, 2014).

From the microbial adherence, the micro colonies that are formed on the surfaces of the material are enclosed by an extracellular polysaccharide which is responsible for the binding and cell adherence. During the phase of accumulation, the microbes multiply, forming several layers of cell clusters on the surfaces of the foreign body (Veerachamy *et al.*, 2014). However, adherence mechanisms can be mediated by specific and non-specific adherence mechanisms. Specific mechanisms include adherence receptor interactions, which allow binding of the bacteria to particular surfaces, whereas, non-specific adherence mechanisms include metabolic processes such as synthesis or excretion of polymeric substances, finally resulting in physically irreversible attachment (Hasty *et al.*, 1992; Rutter *et al.*, 1984).

2.8.1 Factor influencing microbial adherence

Many factors contribute to the complexity of microbial adherence process. These factors can be related to the environment, microbial features and substances related characteristics such as the serum proteins or antibiotic, ionic strength of the surrounding liquid medium, surface free energy and the cellular process of the microorganisms.

(i) Surface energy

There are contrary reports on effect of surface energy of substrates on bacterial adhesion. Surface energy has a great impact on microbial adherence. The surface charge of bacteria varies according to bacterial species and is influenced by growth medium, bacteria age, and bacterial surface structure. In some studies, it is proved that microbial adherence is activated with the increase in the free surface energy, which resulted in an increase in the number of cell adherence (Hogt et al., 1985; Kurtulmus *et al.*, 2010; Waters *et al.*,1997; Yuan *et al.*, 2017).

(ii) Hydrophobicity

Hydrophobic surfaces attract microorganisms more than the hydrophilic surfaces. Silicone elastomers are hydrophobic in character and acrylic resin is hydrophilic in character (Waters *et al.*, 1999). The cell wall of the bacteria is encapsulated coagulase-negative which shows surface hydrophobicity and according to some studies hydrophobic surfaces of the biomaterials have the affinity to attract the hydrophobic surfaces of the microorganisms (Kröncke *et al.,* 1990 and Satou *et al.,* 1988).

(iii) Surface roughness

Surface roughness has a great influence on microbial adherence. Some studies demonstrated that a surface with irregularities or roughness and porosities tends to increase microbial adherence (McAllister *et al.*, 1993; Waters *et al.*, 1999). Silicone elastomers promote more bacterial adherence due to surface roughness or surface irregularities than acrylic resin (Sousa *et al.*, 2009). Thus, the surface roughness can induce immobilisation and irreversible attachment of the bacteria on the surfaces of the material (Dantas *et al.*, 2016; Sousa *et al.*, 2009; Taylor *et al.*, 1998).

2.9 Microorganisms

There are abundant microorganisms that are frequently present in the oral cavity and skin, such as *Viridans streptococci, S. mutans, C. albicans, Prevotella intermedia, Porphyromonas gingivalis* and many more. The anaerobic bacterium is often responsible for oral infections, periodontal diseases and dental caries (Gendron *et al.*, 2000). The skin, as well as the oral cavity harbor microorganisms. *S.*