ANTIMICROBIAL ENHANCEMENT OF DENTURE BASE RESIN USING MICROCAPSULE DRUG DELIVERY TECHNOLOGY: A CHARACTERIZATION STUDY

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By

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LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
ATR	Attenuated Total Reflectance
CFU	Colony forming unit
C. albicans	Candida albicans
DBR	Denture base resin
DCM	Dichloromethane
DS	Denture stomatitis
DDS	Drug delivery system
EO	Essential oil
ESE	Emulsion and solvent evaporation
FESEM	Field emission scanning electron microscope
FTIR	Fourier-transform infrared
GCMS	Gass chromatography mass spectroscopy
IR	Infrared
MHA	Muller-Hinton agar
PBS	Phosphate buffer solution
PMMA	Polymethyl methacrylate
PLA	Poly(lactic) acid
PVA	Poly(vinyl) alcohol
RDP	Removable dental prosthesis
RT	Retention time
SEM	Scanning electron microscope
SDA	Sabouraud dextrose agar
S. aureus	Staphylococcus aureus
S. mutans	Streptococcus mutans
TTO	Tea tree oil
UV-Vis	Ultraviolet visible spectroscopy
o/w	Oil in water

LIST OF SYMBOLS

%	Percentage
°C	Degree Celsius
μL	Microliter
μm	Micrometre
nm	Nanometre
cm^{-1}	Per centimetre
CFU/ml	Colony-forming unit per millilitre
g	Gram
mg	Milligram
mL	Millilitre
mm^2	Millimetre square
R^2	Correlation coefficient
t	Time
h	Hour
s	Seconds
wt%	Weight percentage
w/v%	Weight per volume percentage
w/w %	Weight per weight percentage

PENAMBAHBAIKAN AKTIVITI ANTIMIKROB KE ATAS RESIN GIGI PALSU MENGGUNAKAN TEKNOLOGI PENGHANTARAN UBAT MIKROKAPSUL: KAJIAN PENCIRIAN

ABSTRAK

Stomatitis gigi palsu muncul sebagai reaksi keradangan yang biasa dalam kalangan pesakit yang memakai gigi palsu. Ditekankan bahawa permintaan yang semakin meningkat untuk gigi palsu, terutamanya di kalangan warga emas, disebabkan oleh faktor seperti populasi yang semakin menua dan masalah kesihatan yang menjejaskan kebersihan mulut. Sebagai alternatif, pembangunan baru penghapusan antimikrob yang secara efektif menghalang pertumbuhan bakteria oral boleh menjadi bidang penyelidikan yang aktif. Tujuan kajian ini adalah untuk menyiasat interaksi antimikrob mikrokapsul dalam resin asas gigi palsu terhadap *Staphylococcus aureus, Streptococcus mutans* dan *Candida albicans.* Satu metodologi penghantaran ubat yang baru menggunakan teknik emulsi dan pemeruapan pelarut (ESE) telah dilakukan untuk mensintesis mikroenkapsul poli(asid laktik) (PLA) yang mengandungi agen antimkirob; minyak pokok teh (TTO). Pencirian enkapsulasi PLA/TTO telah dilakukan menggunakan spektrofotometri sinar ultraviolet dan sinar tampak (UV-Vis), spektroskopi inframerah empat transform (FTIR), kromatografi gas serta spektroskopi jisim (GCMS) dan mikroskop bidang pancaran elektron (FESEM). Pangkalan gigi palsu PMMA yang diubah suai disediakan dalam tiga kepekatan mikrokapsul yang berbeza (TTO:PLA (%w/w)), 10%, 50% dan 100%. PMMA yang diubah suai dinilai untuk aktiviti antimikrob melalui larutan larut lesap pada selang masa hari ke-7, ke-14 dan ke-30 menggunakan ujian penyebaran agar dalam lubang Kirby-Bauer dan ujian membunuh masa. Kemudian, imej melalui pemancaran elektron (SEM) dilakukan

untuk menilai keterikatan mikroorganisma pada permukaan resin gigi palsu yang diubah suai. Keputusan yang diperoleh daripada analisis pencirian menujukkan mikrokapsul telah berjaya disintesis menggunakan kaedah emulsi dan pemeruapan pelarut di mana kajian profil pembebasan ubatan dan saiz purata mikrokapsul yang terbentuk adalah teragih secara homogen untuk pelepasan ubat terkawal. Aktiviti antimikrob PMMA resin asas gigi yang diubah suai dengan mikrokapsul PLA/TTO menunjukkan kesan antimikrob yang baik (Jadual 4.2/4.3/4.4/4.5) dan secara statistik menunjukkan kesan yang signifikan ($p<0.05$) terhadap pertumbuhan mikroorganisma *S. aureus* dan *C. albicans* di mana zon perencatan meningkat apabila kepekatan meningkat kecuali pada *S. mutans*. Ujian membunuh masa digunakan untuk mengkaji aktiviti antimikrob dari masa ke semasa, aktivti resin gigi palsu yang diubah suai menunjukkan kesan antimikrob yang baik terhadap semua mikroorganisma yang diuji. Dalam kajian morfologi menggunakan FESEM, lampiran sel kulat dan bakteria dalam tempoh 24 jam adalah berkurang apabila kepekatan bertambah. Kajian ini mendedahkan bahawa penggabungan mikrokapsul PLA/TTO dalam resin gigi palsu mempunyai kesan terhadap peningkatan aktiviti antimikrob, dan berpotensi untuk pengguna gigi palsu pada masa hadapan, Namun, kajian lanjut termasuk sifat fizikal dan citotoksisiti dieprlukan untuk disisasat sebelum ujian klinikal.

ANTIMICROBIAL ENHANCEMENT OF DENTURE BASE RESIN USING MICROCAPSULE DRUG DELIVERY TECHNOLOGY: A CHARACTERIZATION STUDY

ABSTRACT

Denture stomatitis (DS) presents as a common inflammatory reaction in denture wearing patients. It is highlighted that the rising demand for dentures, particularly among the elderly, due to factors such as aging populations and associated health issues affecting oral hygiene. Alternatively, new development of antimicrobial elimination that could effectively inhibit the growth of oral bacteria could be an active area of research. The aim of this study is to investigate the antimicrobial interaction of PLA/TTO microcapsules in polymerized PMMA denture base resin against *Staphylococcus aureus, Streptococcus mutans* and *Candida albicans.* A new drug delivery methodology employed via emulsion and solvent evaporation (ESE) techniques was performed to synthesize polylactic acid (PLA) microcapsules containing antimicrobial agents; tea tree oil (TTO). The characterization of TTO/PLA microcapsules were characterized using Ultraviolet-visible (UV-Vis), Fourier-transform Infrared (FTIR), Gas chromatography-mass spectrometry (GCMS) and field emission scanning electron microscope (FESEM). The modified PMMA denture base was prepared in three different concentrations of prepared microcapsules (TTO: PLA (% w/w)), 10%, 50% and 100%. For antimicrobial activity, the modified PMMA was evaluated for antimicrobial activity through leaching solution at the intervals of 7th, 14th and 30th days using the well diffusion test and time-killed assay. Then, FESEM images was performed to assess the attachment of microorganism on the surface of modified denture base. The results obtained from characterization analysis showed the microcapsules were successfully synthesized using emulsion and solvent evaporation

method where the drug release study and the average particle size formed is homogenously distributed for controlled drug release. The antimicrobial activity of modified PMMA base resin incorporated with PLA/TTO microcapsules showed a good antimicrobial effect (Table 4.2/4.3/4.4/4.5) and statistically revealed a significant effect ($p<0.05$) against *S. aureus* and *C. albicans* where the inhibition area increased as the concentration increased except for *S. mutans*. The time killed assay was used to study the antimicrobial agent over the time, it showed that the antimicrobial activity of modified denture base exhibited a good antimicrobial effect against all microorganism tested. In morphological test using FESEM, the attachment of fungal and bacteria cell tested is lesser as the concentration increased within 24 hours studied. The study revealed incorporation of PLA/TTO microcapsules in denture base, has significant impacted on enhanced antimicrobial activity, offering potential usefulness for denture users in the future. However, further studies including physical properties and cytotoxicity are needed to be investigated before the clinical trials.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

A conventional complete or partial denture remains the preferred course of therapy because of the medical and financial reasons even though dental implants are increasingly employed in the treatment of edentulous individuals (Carlsson G.E. Et al., 2010). Polymethylmethacrylate (PMMA) denture base resin is the most used denture base material because of its aesthetics values, simplicity of processing and repair, as well as, low cost (Vivek R et al., 2015). However, this material is not suitable to satisfy the mechanical needs of prosthesis, such as the resilient to fracture (Vivek R et al., 2015). Another concern was the development of stain, plaque, and tartar are detected on dentures and teeth (Pasricha et al., 2014). The conditions can be varied among the patients such as the denture surface properties, the duration of use, saliva composition, oral and dental care hygiene. Failure to maintain denture hygiene may result in the development of halitosis, denture stomatitis and other mucosal infections (Cakan U et al., 2015).

However, the denture hygiene is a concern for the elderly, who requires a high level of care due to limited dexterity skills to handle regular oral hygiene regimens. Antibiotics or antifungal medication can be prescribed for the patient who had denture related infections; but the issues of safety and still remain regarding bacteria resistance (Dalwai et al., 2016). Denture cleaning can be done using mechanical or chemical means, either alone or in combination (Shay K., 1999). Brushes, ultrasonic cleansers can also be used for mechanical cleaning, while disinfectants, diluted acids, alkaline hypochlorites and alkaline peroxide can be used to clean dentures (Aydiner, S. F., 2021). Nevertheless, adding the denture cleansers or antimicrobial agents should not change the cytotoxicity or reduce the dentures' mechanical qualities. Therefore, choosing appropriate denture cleaners or antimicrobial medications is essential for their incorporation into the base material (Chen R. et al., 2017). These chemical agents should not induce any physical or chemical changes to denture base material. Since the dentures will need to be cleaned in several times over their period of effective use, it is crucial that the cleaning agents do not harm the dentures (Andrade IM. Et al., 2014).

The optimum denture base material has biocompatibility and; a high bind strength with accessible denture teeth as well as possessing acceptable physical and mechanical properties (Meng & Latta., 2005). Denture base material fabricated from polymerized polymethyl methacrylate (PMMA) resin is commonly used due to its good physical and aesthetic properties. PMMA denture acrylic resin remains the preferred material for removable full and partial prostheses. The demand for PMMA denture resin materials is based on its affordable cost, convenience of usage and basic processing equipment. Given the fact that the denture users are generally of advanced or elder age, it is reasonable to expect that mechanical methods will not be sufficient to clean dentures (Polat Sagsoz N. et al., 2022). For these reasons, it is advisable to store dentures in chemical solutions, which provide a technique of facilitating denture cleaning. Effervescent tablets are the most often used and recommended chemical agents among denture wearers (Andrade IM. Et al., 2014). Recently, users are encouraged to store the dentures in solutions made with agents in tablet or powder from. This procedure can also be claim to complement the chemical cleaning due to the release of oxygen in the solution containing such a tablet or powder (May J. et al., 2000).

Dentures have considerable variances in PMMA, which rely on the amounts of a crosslink resin and heat activated initiators to enhance the physical properties of the treated materials (Meng & Latta., 2005). Adding filler to enhance the denture base properties is regarded as the most cost effective and appropriate option to improve their clinical application and the longevity of the restoration. Research done by Ilie and colleagues (2021) highlighted the use of graphene oxide, hydroxyapatite, zirconia nanoparticles against *Enterobacter Ludwig* and *Escherichia coli*. The results indicated that zirconia nanoparticles are ineffective as denture filler, whereas graphene oxide nanoparticles are the best filler followed by hydroxyapatite nanoparticles, which may significantly reduce the bacterial load. Up to 700 different microbial species makes up biofilms in the oral, and around 100 to 200 of these species may be found in any healthy oral cavity (Kolenbrander et al., 2010). Many alternative and complementary medicine has been extensively investigated for dental use in the form of dentifrice, mouth rinses (Reddy, V. et al., 2021). Different therapeutic strategies have been used to prevent and cure denture stomatitis, ranging from the use of denture disinfectants and cleaners to the delivery of oral and systemic antifungal drugs. The impact of denture cleansers and disinfectant solutions on the adherence of germs to denture base materials has been examined in several research (Al Thobity et al., 2017; Bahador A. et al., 2016). However, increasing one set of the material's properties without compromising the others remains the fundamental difficulty for researchers and physicians in developing a modified PMMA material for denture applications (Alqutaibi et al., 2023).

Oral treatment drugs are used to kill or supress microorganism in the oral cavity, hence removing foul breath and preventing dental caries and periodontal disease (Peres et al., 2019, Herrera D. et al., 2003). Oral drug treatment contributes to the suppression of oral bacteria, because of the adverse effects, continuous oral hygiene management requires the use of natural oral products that can be safely used for an extended period of time without causing side effects. The use of natural therapies is growing since they are seen to be effective pharmacological alternatives for mild or medium entity problems (Ercan N. et al., 2015). Previous study developed natural remedies as the antimicrobial agents to be incorporated into denture materials. Many herbal formulations, such as *Cinnamon*, *Neem* tree extract, and *Melaleuca alternifolia* oil, have been established as potent antifungal agents that can be used safely (Dalwai et al., 2016). Essential oils, which are secondary metabolites, have been studied for their bioactivities and potential therapeutic application including anticancer, antibacterial, and anti-inflammatory properties (Astani A. et al., 2010). Australian tea tree oil (TTO), derived from *Melaleuca alternifolia* is one of the best-known essential oils. It is commonly used to treat skin, bronchial, dental infections, as well as an antiseptic and disinfectant (Mondello F. et al., 2006). This tea tree oil contains few active compounds, such as terpene hydrocarbons, and their associated alcohols (terpinene-4-ol), which responsible for its antibacterial and antifungal properties (Dalwai et al., 2016).

Failure in ensuring good denture hygiene can contributes to denture stomatitis and its recurrent even following the treatment regimen. The specific antifungal or antibacterial agents such as nystatin, miconazole or natural antifungal sources has been used locally or systematically to prevent bacterial and fungal from developing (Zainal et al., 2021). In addition to characterizing the antimicrobial activity of TTO, the therapeutics effectiveness of tea tree oil has been studied. The effectiveness of tea tree oil in dental applications has been determined whereby the efficacy of 0.2% TTO mouthwash could minimize the numbers of S*treptococcus mutans* in comparison to placebo treatment (Groppo F. et al., 2002). Besides having a role in the treatment of gingivitis, there is some preliminary evidence to suggest that tea tree oil may reduce the levels of several compounds associated with halitosis (Takarada K., 2005). However, because of its stability and reactivity with the denture resin chemistry, adding the bioactive compound to dental adhesive may be difficult and challenging. From Dalwai et al (2016) studies, polymerized heat-polymerizing denture base resin with tea tree oil microcapsules compromised the membrane related function. Thus, microencapsulation drug delivery technology would be a beneficial approach for medication delivery.

Understanding how microbes relate to denture wearing has given the opportunity to improve oral and systemic health of the global elderly population (Redfern et al., 2022). Previous studies had investigated the addition of antimicrobial agents into denture soft lining materials with promising results (Dalwai et al., 2016, Bettencourt et al., 2023). However, since their core base materials are easily degradable by the oral environment, they present a short life cycle and are considered temporary (Bettencourt et al., 2023). Moreover, due to their porous structure, they are prone to intense microbial colonization and need cleaning procedures. To overcome these disadvantages, alternative drug delivery systems based on hard reline materials have been proposed (Bacali et al., 2019). Drug delivery systems (DDS) are engineered technologies to allow the medicinal drugs to be delivered in regulated or targeted doses. The controlled release DDS implies therapeutic release kinetic over a long period of time, has quickly emerged as a superior delivery technology to address the limits of conventional administration (Liu et al., 2016). The approaches used in DDS may be a potential solution for medication release performance. The composition of any natural extract produced influences its mechanism of action against bacteria or fungi. Besides the immersion in solutions, drugs can also be added to a coating system or be loaded to an acrylic matrix during manufacture (Bacali et al., 2019). Loading acrylic resins with chlorhexidine was considered effective and feasible in drug release and microbiological tests against *C. albicans,* however, loading a drug into the polymeric matrix can alter its structure (Gad et al., 2018).

Encapsulation technologies are an option for preserving the bioactive components and controlling their activity in the medium where they are inserted (Silva et al., 2021). Microencapsulation is a process that involves embedding one substance (the core) into another (the shell) to create a microcapsule complex. Polymer microcapsules are one method for preserving the long-term bioactivity of natural extracts while also protecting them from potential environmental deterioration (Yourdkhani et al., 2017). Microencapsulation is a flexible technology used in a variety of applications, including medicine administration and to isolate, preserve and regulate the distribution of active core materials. Microcapsules have also been employed to provide beneficial antibacterial compounds over the time. The versatility of microcapsules is demonstrated by the ability to release the core material in response to a range of environmental triggers (Dubey et al., 2009).

Recently, a study analysing the dental materials microencapsulation of bioactive substances has opened up the strategy for enhancing the performance of dental materials and denture treatments (Yourdkhani et al., 2017). The preparation and the characterization of TTO in β-cyclodextrin microcapsules was done by P. Kong et al. (2023) gives a high yield encapsulation efficiency. Many studies have been working on incorporating the antimicrobial agents into the dental materials, which focusing on coating of the acrylic surface, aiming to improve the antimicrobial activity of the denture base resin (DBR) (Dalwai et al., 2016; Zhu. Z. et al., 2022) However, the effects of incorporating the antimicrobial agents into the denture base have not yet received much attention.

This study investigates the interaction of biodegradable microcapsules containing organic antimicrobial agents; tea tree oil into denture base materials as a new drug delivering method. For this study, three specific microorganisms were selected consists of two bacteria *(Staphylococcus aureus*, *Streptococcus mutans*) and one fungus (*Candida albicans*). These two bacteria and *Candida albicans* are the most common microorganisms in the oral cavity and they can easily colonize in the surfaces of the denture (Tong et al., 2012). Results from the study on microcapsules containing organic antimicrobial agents which is tea tree oil; into denture base materials as a new drug delivering method and antimicrobial effects will give better benefit to the dental clinician and patient.

1.2 Problem statement

Denture base may serve as reservoir for bacterial growth, which potentially worsening the fungal infection and increasing the dental caries and stomatitis. The formation of denture biofilm can be hindered by factors such as the changes of pH levels and presence of the oral microorganisms which can disrupt the natural flow of saliva. Hence, the prescription of the uncontrolled antibiotics used in the oral environment can promote the establishment of resistant microbial strains. Therefore, more research is needed to create novel and innovative methods. In this this study, incorporating the organic antimicrobial substances; tea tree oil through microencapsulation may be able to carry and release the ideal dose for antimicrobial effect intro denture base that would improve the long-term efficiency. The aim of the research study is to focus on the interaction of the new modified denture base for the microbial interaction and cellular morphology that play an important role in forming a barrier against future colonising of the microorganism. Then, direct delivery application of the TTO through microencapsulation to the site of infection may reduce the risk of systemic side effects or drug-drug interactions. It may also reduce the uncontrolled antibiotic use that contribute to the growth of resistant microbial strains.

1.3 Justification of study

The dental industry is constantly researching new materials and techniques to improve the properties and performance of denture bases. Denture stomatitis is caused by the inflammatory reaction of the underlying soft tissues in the removable dental prosthesis wearers. The poor oral hygiene and long-term denture users have been strongly linked to this. Denture stomatitis refers to the inflammatory and erythematous formations in the oral mucosa in contact with the denture base. This condition, dominated by *Candida albicans* fungi, was reported to feature colonisations mostly on the inner surface of the base plate (Zarb G.A. et al., 2013). Natural products have been proven as an alternative to synthetic chemical substances and drug delivering technology using natural product had been introduced for protection against future colonising of the microorganism and infection for patient wearing denture. Many studies have been interested in incorporating the antimicrobial agents with the dental materials, which focused on incorporating antimicrobial agents into dental materials, such as coating the acrylic surface to improve the antimicrobial activity of the denture base resin (DBR) (Pietrokovski et al., 2016, Bacali C et al., 2019). The modifications of denture base include the addition of nanoparticles of natural products (Ashraf. H et al., 2022), metal coatings (Ferreira et al., 2022) and the incorporation of direct herbal formulation as potent antifungal agents (Dalwai et al., 2014; Abraham, A. Q. & Abdul-Fattah, N., 2017) had been done to improve the antimicrobial potential of denture base. In light of these facts, it is essential to develop new modified biomaterials with the incorporation of encapsulated antimicrobial products to increase the efficiency in treating denture problems. This aims to give better patient compliance where the medication (TTO) could release directly to the oral site through microencapsulation, hence may reduce the side effects on drug interaction within the body.

1.4 Research Questions

1. Is there any potential on the tea tree oil release via biodegradable microcapsules using Ultraviolet-Visible Spectroscopy (UV-Vis), Fourier-Transform Infrared Spectroscopy (FTIR), Gas Chromatography Mass Spectroscopy (GCMS) and Field Emission scanning electron microscope (FESEM)?
2. Is there any antimicrobial effect between commercial PMMA and modified PMMA (in a specified ratio of 10%, 50% and 100% w/w TTO) against *Staphylococcus aureus, Streptococcus mutans* and *Candida albicans* at different leaching days (at intervals of the 7th day, 14th day and 30th day) using well diffusion test?
3. Is there any microbial interaction between commercial PMMA and modified PMMA (in a specified ratio of 10%, 50% and 100%w/w TTO) on *Staphylococcus aureus, Streptococcus mutans* and *Candida albicans* using time killed assay?
4. Is there any morphological changes of the cell for *Staphylococcus aureus*, *Streptococcus mutans* and *Candida albicans* on the surfaces tested PMMA using a field emission scanning electron microscope (FESEM)?

1.5 Research Hypothesis

1. There is potential on the tea tree oil release via biodegradable microcapsules using Ultraviolet-Visible Spectroscopy (UV-Vis), Fourier-Transform Infrared Spectroscopy (FTIR), Gas Chromatography Mass Spectroscopy (GCMS) and Field Emission scanning electron microscope (FESEM).
2. There is a significant antimicrobial effect between commercial PMMA and modified PMMA (in a specified ratio of 10%, 50% and 100%w/w TTO) against *Staphylococcus aureus, Streptococcus mutans* and *Candida albicans* at different leaching days (at intervals of the 7th day, 14th day and 30th day) using well diffusion test.
3. There is a microbial interaction between commercial PMMA and modified PMMA (in a specified ratio of 10%, 50% and 100%w/w TTO) on *Staphylococcus aureus, Streptococcus mutans* and *Candida albicans* using time-killed assay.
4. There are morphological changes of the cell for *Staphylococcus aureus, Streptococcus mutans* and *Candida albicans* on the surfaces tested PMMA using field emission scanning electron microscope (FESEM).

1.6 Research Objectives

1.6.1 General Objective

To compare the antimicrobial activity of new drug delivering technology by incorporating PLA microcapsules comprising tea tree oil (*Melaleuca alternifolia*) into denture base materials against oral microorganisms.

1.6.2 Specific Objectives

1. To characterize the microcapsules PLA incorporated with tea tree oil (*Melaleuca alternifolia*) through new drug delivering technology; using Ultraviolet-Visible Spectroscopy (UV-Vis), Fourier-Transform Infrared Spectroscopy (FTIR), Gas Chromatography Mass Spectroscopy (GCMS) and Field Emission scanning electron microscope (FESEM).
2. To compare the antimicrobial effects between commercial PMMA and modified PMMA (in a specified ratio of 10%, 50% and 100%w/w TTO) against *Staphylococcus aureus, Streptococcus mutans* and *Candida albicans* at different leaching days (at intervals of the 7^{th} day, 14^{th} day and 30^{th} day) using well diffusion test.
3. To compare the microbial interaction between the commercial PMMA and modified PMMA (in a specified ratio of 10%, 50% and 100%w/w TTO) against *Staphylococcus aureus, Streptococcus mutans* and *Candida albicans* using time-killed assay.
4. To assess cell morphology attachment of *Staphylococcus aureus, Streptococcus mutans* and *Candida albicans* on the surfaces of tested PMMA using field emission scanning electron microscope (FESEM).

CHAPTER 2

LITERATURE REVIEW

2.1 Denture Base Resin

Removable dental prosthesis (RDP) is a common prosthetic restoration, particularly in partially or completely edentulous patients. Dentures have the surfaces make up of missing teeth as well as soft and hard tissue inadequacies (Hayran Y. et al., 2019). As a result, the difficulties in cleaning and maintaining the prosthesis can happen which may induce the microbial adherence and colonization. RDP users are most likely the older patients who may lack of good oral hygiene practice due to decreased motor sensory capacities, necessitating more efficient denture cleaning (Fernandes. F et al., 2011). There are different types of modified acrylic resin used in dentistry includes reinforced resins, hypoallergenic resins and thermoplastic resins. The denture resins are utilized in the fabrication of dental prosthetic, according to their composition and technique of processing for removable prosthetics to give reasonable high functional stability. Synthetic acrylic polymers are important in dentistry because they have good mechanical and biological properties that make them suitable for wide range of dental applications (Pandey A. et al., 2015).

Among the acrylic polymers, polymethyl methacrylate (PMMA) is the most commonly utilized materials in dental clinics and laboratories for the removable dental prostheses (Zafar M. et al., 2020). It is also used for repairing denture, reline, rebase; as well as fabricating orthodontic retainers, occlusal splints and obturators (Mirizadeh A. et al., 2018). Today, many limitations of poly-methyl methacrylate (PMMA) denture base have been overcome (Vivek R & Soni. R., 2015). Despites the development of different alternative materials, PMMA remains the option because of its physical, mechanical, biocompatibility and aesthetic properties that are satisfying in dental's field and have been clinically accepted for more than seven decades. The acrylic resins can be thermally or chemically cured and have a powder-liquid system (Zafar M. et al., 2020). The powder is made up of PMMA polymer beads, colours, gums, fibres and a few additional ingredients to modify the physical properties. Activators, inhibitors, cross-linkers and methyl methacrylate are present in the liquid components (Chhabra M. et al., 2022). The PMMA continued to appeal in dental applications due to its unique and useful properties, such as the flexibility to be adapted to individual needs.

However, there are several limitations to PMMA denture, such as its poor impact and flexural strength, inadequate surface microhardness and modulus of elasticity (Alhotan A. et al., 2021). Plus, the dental prosthetic surfaces made from PMMA are naturally devoid of any antimicrobial properties. Clinically, the physical features of denture base may act as a substrate for the adhesion of dental plaque, thus promoting the microbial colonization in the denture pores causing the unpleasant smell, denture fracture, discoloration and in some cases, microbial infection (Saxena S. et al., 2017). Numerous factors, including the type of prosthesis, oral microbes, pH levels, and the salivary flow might create circumstances that increase warmth, disrupt the normal salivary flow and impose mechanical compression. These factors may affect the denture biofilm aggregation and promote the production of fungal biofilm which lead to denture stomatitis (Olms. C. et al., 2018).

Denture stomatitis (DS) is distinguished by the inflammation and erythema of the oral mucosa beneath the denture-bearing areas (Lee M. J. et al. 2020). According to Neppelenbroek et al. (2008), oral infection like denture stomatitis has been closely linked to poor hygiene and prolong denture use, which facilitates the production of denture plaque where *Candida albicans* can be routinely identified. Biofilms of candida in mucosal and inert surfaces of dentures may alter the susceptibility to antifungal agents, which may lead to treatment failure (Pachava et al., 2015). In addition, microbes including *Streptococcus mutans*, *Candida albicans* and *Staphylococcus aureus* species, have been known to colonize the denture surface. This has been linked to systemic infections like pneumonia, chronic inflammatory responses in the oral mucosa and oral disease (Paleari A. G. et al, 2011). Brushing and the use of denture cleanser tablets being the most prevalent method to clean the removable dentures. For example, Polident© cleanser tablets help to keep the removable dentures clean, fresh and hygienic. It also helps to remove plaque and stains following the manufacturer's recommended immersion time for 15 minutes. However, frequent denture cleaning is ineffective against common denture plaque germs, and it may cause future problem to acrylic resin dentures. For now, the systemic or local antibiotic have been administered to treat harmful oral microbe species however, unregulated use of systemic medication can lead to antimicrobial resistance (Bueno M. G. et al., 2015).

Therefore, some researchers come up with few methods to enhance the antimicrobial properties of denture base resins (DBRs) that will be beneficial. Alternatively, in recent years, there has been an increase in the study interest into chemically copolymerizing the antimicrobial compounds with PMMA denture resin. These antimicrobial compounds are consistently dispersed throughout the bulk of the material and offer long-lasting antibacterial action without leaking out from the host material (Rao S. et al., 2021). Studies have shown that copolymerizing antimicrobial monomers, such as Quaternary Ammonium Monomer (QAM), 12-Methacryloyloxy -Dodecyl -Pyridinium -Bromide (MDPB), 2-Tert-Butyl Amino Ethyl Methacrylate

(TBAEMA), Methacryloyloxy Undecyl Pyridinium Bromide (MUPB), Dimethyl Amino Dodecyl Methacrylate (DMADDM), and Acryl Amide Monomer (AAM) into denture base have been found to be effective in preventing the microbial growth (S. Imazato et al., 2012). Furthermore, few strategies on how to incorporate antimicrobial agents into denture base resin and their impact on the prevention and reduction of the growth of the microorganism were also identified (An et al., 2020).

There have been several ways offered to improve the mechanical properties of acrylic application and reduce the risk of tooth cavities during prosthodontic treatment. Recent development through PMMA reinforcement by a few types of fillers has been reported by previous works to produce a significant improvement of PMMA composite properties (Bahador et al., 2016). Surface irregularities and imperfections in polymerized PMMA dentures may enhance the possibility of microbial colonization on them. When the surfaces are exposed to the fluids flushing action, there will be higher chance for successful colonization of microorganisms to the surfaces. One crucial stage in the formation of infection is the microbial adhesion. The denture's porosity may also provide a pathway for microbial permeation and contribute to surface irregularity which may give rise to infections. Denture base with antimicrobial properties can significantly reduce the potential formation of infection. A strong antimicrobial activity against the cariogenic bacteria was established by Bahador et al., (2016), which involved the incorporation of TiO_2/SiO_2 nanoparticles into polymerized polymethyl methacrylate (PMMA) denture base resins.

Although various methods are suggested for the treatment of oral infections, there are few major approaches, including an effective dentures cleaning with the uses of disinfectant solution focusing the treatment on denture base using antifungal agents and making new dentures or using denture lining to encounter the problems. In elderly patients with limited dexterity, it should be expected that they will not be able to perform appropriate denture cleaning using mechanical way. Treating the oral infections like denture stomatitis and other oral cases involves maintaining good oral and denture hygiene, treating the denture defects, thoroughly cleaning and disinfecting dentures, and using topical and systemic antifungals drugs such as azoles, nystatin and amphotericin B (Amanlou et al., 2006). For systemic antifungal treatment, the azoles group is offered, while nystatin and amphotericin B medication are administered topically and occasionally applied to the denture fitting surface prior to its utilization. Topical antifungal drugs are often ineffective owing to dilution and quick removal by saliva flushing, which can lower the antifungal medications to subtherapeutic concentrations (da Silva et al., 2017). Additionally, taking these medications in different dosage may result in certain restrictions on pharmacological toxicity and drug interactions. Moreover, the widespread use of systemic medicines has resulted in the evolution of fungal resistance (Amanlou et al., 2006). Regarding oral hygiene care, the microbiological efficiency of mechanical cleaning using the toothbrush is quite poor. For these reasons, it is frequently suggested for patients to store the denture in chemical solutions, as they provide a technique to facilitate the denture cleaning. However, the immersion of denture in cleansing agents have shown some disadvantages, primarily concerning the damage that can cause changes to physical and mechanical properties of acrylic resin (Costa R. T. F. et al., 2021).

Alternative medicines have been proven to be more popular over the past decades. In recent years, natural products have gained popularity as an option to artificial additives or pharmacologically relevant drugs. In order to include antimicrobial agents utilizing natural products, researchers have explored the antibacterial activity of polymerized resin in dental materials. They have employed various ways and technique such as, coating the denture materials with neem extracts, essential oils and other substituents. Natural remedies are becoming more popular, with some considered excellent replacements for medications in cases of minor or moderate health issues (Carvalho C. et al., 2019). Essential oils have grown in popularity in fields such as biomedicine, pharmaceuticals, cosmetics, cuisine, agriculture, and veterinary medicine. Asides from being natural alternatives to synthetic preparations, these oils have sparked attention in the phyto therapeutic sector for disease prevention and treatment (Firenzuoli F. et al., 2014). Therefore, incorporating natural medications in dental field may work very well in the treatment of oral infection.

2.2 Antimicrobial agents

In the diverse microbial ecology of the oral cavity, bacteria like *Streptococcus mutans*, and *Staphylococcus aureus* species can readily colonize the denture base resins and create a plaque biofilm (Pasricha et al., 2014). The colonization can lead to, denture stomatitis, dental caries and other oral mucosal disorders, therefore the performance limits caused the PMMA dentures from being an ideal denture base (Paleari et al., 2011). However, not much focus has been placed on the denture cleansing agents' ability to remove *Candida*-associated mature biofilm, whose cells are known to be more resistant to chemical and antimicrobial cleaning agents (Nikawa H. et al., 1999). Although there are few approaches to the effect of herbal medicine to fight the viability of oral bacteria, especially on acrylic denture resins, it is crucial to remark that there is an easy-to-find and plentiful native species of some nations (Alavarce et al, 2015). According to review by Inacio Silveira et al., (2021) natural products were reported to have efficacy in the treatment of oral candidiasis; denture stomatitis. Topical antifungal treatments, dental care, hygiene procedures and denture cleaning, as well as fixing of anatomical defects in the denture base, are all necessary for denture stomatitis treatment. Several antifungal drugs are used including nystatin, amphotericin B, fluconazole, clotrimazole, miconazole and ketoconazole (Bueno M.G. et al., 2015). However, a significant recurrence rate has been reported in conventional topical and systemic treatments due to the difficulties in maintaining the contact between the contaminated acrylic denture base (Yarborough A. et al., 2016). Plus, it is important to take into account the potential for these therapies which can cause allergic responses, hepatoxicity, nephrotoxicity and drug-drug interaction (Lombardi T. et al., 1993). Although the currently available medication release at the site of infection can reduces the effects, the use of natural-based medicine as an alternative to antifungal or antimicrobial treatment for dentures has been recently recommended.

Many researches have indicated that a wide range of plant extracts exhibit antifungal action against oral microbes, suggesting their potential in denture stomatitis treatment. Some natural treatments such as Propolis used as gel form, *Punica granatum* gel and *Pelargonium graveolens* olive oil in mouthwash (Pinelli L.A et al., 2013) have proven efficacy as conventional antifungals used in treating patients who have a denture stomatitis. As a result, it would be appropriate to examine the use of natural items in both prevention and treatment of dentures. As mentioned earlier, essential oils (EOs) have gained great popularity in various applications. Essential oils are concentrated compounds derived from plants as secondary metabolites often have a strong odour. They can be found in a variety of plant parts, including seeds, buds, leaves, flowers, fruits, stem and roots. However, the essential oils are usually kept in specialized secretory cells of the plant. Because of their lipophilic characteristics, they may penetrate easily through the stratum corneum, mucous membrane and the microbial cell membranes. Therefore, essential oils have antibacterial properties because of their lipophilicity (Wiatrak K. et al., 2021). Terpenes and terpenoids are usually found in essential oils, which have the strong antimicrobial action (Burt S. A. et al., 2003). The loss of cellular membrane integrity or function was reported to be the primary cause of microorganism cell death because of the presence of terpenes in essential oil. Essential oils have multidirectional antifungal effects, particularly against *Candida* species, by altering the morphology of fungal cell wall, inhibiting the enzymes involved in cell wall synthesis, inhibiting the ergosterol synthesis, modify the permeability of the cell membrane, and producing reactive oxygen species (ROS) (Sing S. et al., 2016). In dentistry, the acrylic base of partial dentures covers significant surface area of oral mucosa, which provide an ideal environment for the accumulation of fungi and bacteria that will eventually lead to denture plaque formation (Khasawneh S. et al., 2002). Therefore, the denture wearers need to have a strict treatment against antifungal and antibacterial activities.

An *in vitro* study concluded that preparation of resin surfaces with typical antimicrobial agents significantly decrease the adherence of *Candida* species on acrylic denture resin. Natural organic compounds such as *Melaleuca alternifolia* (tea tree) essential oil have been recently employed for antimicrobial purposes on *Staphylococcus aureus* for antimicrobial purposes (An, S. et al., 2018). Tea tree oil (TTO) has been described as an ideal disinfectant for topical applications since it can easily penetrate the skin and offer antimicrobial activity against a wide range of pathogens without causing irritation (May J. et al, 2000). Tea tree oil was found in variety of products including toothpaste, mouthwash, soaps, facial cleansers, shampoo and moisturiser in different concentrations (Southwell, I. & Lowe, R. 1999). According to Al-Mashhadane (2007), tea tree oil offers a natural alternative and reports indicate that using this agent in dental hygiene products have showed it to be an effective antimicrobial agent. The Myrtaceae family's genera *Leptospermum*, *Melaleuca*, and *Kunzea* are referred as "tea tree" plants. But these plants have no flavour, fragrance or composition in common with the true tea plant (*Camelia sinensis*) (Southwell I. & Lowe, R. 1999). Tea tree oil is one of the valuable essential oils extracted from *Melaleuca alternifolia* leaves. As the plant is indigenous to Australia, the oil is generally known as Australia tea tree oil (Yadav et al., 2016). Australian tea tree oil derived from *Melaleuca alternifolia* is a well-known, extensively researched oil with medical and commercial benefits (Carson C.F. et al., 2001).

According to a study from Wiatrak K. (2017), patients who used toothpaste containing tea tree oil reported improve in hygiene and periodontium health. Another literature documented several reports on the use of *Melaleuca alternifolia* oil against the growth of *Candida* species on the acrylic resin surface (Pachava. K. R., 2015 & Al-Mashhadane., 2007). In addition, study by Polat et al., (2023) observed that, using tea tree oil as a denture cleanser may reduce the surface roughness of the dentures compared to other chemical agents (cleaning solution). One of the studies in evaluating the antimicrobial of tea tree oil, fluconazole and chlorhexidine gluconate on denture acrylic resin, observed that tea tree oil has similar effectiveness against *Candida* as chlorhexidine and superior to fluconazole (Dalwai S. et al., 2016). Moreover, according to *in vivo* study by (Neppelenbroek et al., 2008), the combination of tissue conditioner soft liner and tea tree oil had an effective fungicidal action against *C. albicans*. Another study used resilient soft lining materials combined with tea tree oil and showed *in* vitro antifungal effectiveness at 40% vol/vol concentration at a dosage of 2 ml, suggesting that the denture stomatitis and other potential oral infections may be treated using this essential oil (Vankadara et al., 2017). Therefore, tea tree oil proved to have activity against the *Candida* by inhibiting the adherence of their biofilm, in prevention against denture stomatitis. It is a crucial issue for elderly and immunocompromised patients, to be aware of the potential antimicrobial activity of denture base resins. Further research is required to determine the best ways to incorporate biodegradable microcapsules or nanocarriers incorporating with the organic antimicrobial agents into denture base materials. Hydroxyapatite-based (HA) fillers also have influence toward the properties including some antimicrobial properties to the oral microorganism (Kolmas et al., 2016).

Essential oil and plant extracts have long been linked to medicinal benefits and it has been suggested that these natural organic components may be included into dental materials (An et al., 2018). Tea tree oil has showed relatively strong antibacterial activities against oral bacteria and some fungi. According to previous studies from Li W.R. et al. (2016), their findings indicated that tea tree oil can penetrate the cell wall and the cytoplasmic membrane of the tested bacterial and fungal strains, resulting in structural damage and cytoplasmic loss. Thus, the activity demonstrated similar antimicrobial activity against both bacterial and fungal strains. Because of its biological activities, the oil's therapeutic potential is being explored in pharmaceutical and industrial sectors. Tea tree oil has received attention for its antifungal, anti-inflammatory and antibacterial activities. Plus, tea tree oil also has been highlighted in scientific publications due to its obvious inhibitory profile against bacteria and its exceptional efficacy as an antimicrobial agent (Yadav et al., 2017). As per literature findings, the antibacterial action of essential oil may be due to loss of membrane integrity and inhibition after its application. However, tea tree oil may have certain adverse effects such as being hazardous if swallowed in higher doses, since it is a complex mixture of components prone to volatilization and cannot be used in its concentrated condition because it may cause skin irritation and allergic responses (Martins et al., 2011).

According to Vankadara et al., (2017), their study of using 40% *M. alternifolia* (tea tree oil) showed a lesser colonization, which contradicts the finding of Neppelenbroek et al., (2008) who found that using 20% vol/vol of tea tree oil inhibited the *C. albicans* growth. They concluded that the differences may be related to the variation in the strain used for their study or due to the changes in method. However, the dose-dependent effects of tea tree oil can be solved by preserving the essential oil. Many essential oils have strong flavours and are unstable to oxidation, thus, it is necessary to encapsulate these components in a core-shell material to reduce oxidative degradation, control the release rate or even to extend their shelf-life (Martins et al., 2009). According to Chouhan et al, (2017), the microencapsulation method enhances the antimicrobial efficacy of essential oils by allowing controlled or sustained release and close interaction with the microorganisms. The researchers are trying to combine the essential oils with nanomaterials to generate a powerful and potent antimicrobial agent. Therefore, microencapsulation drug delivery technology for essential oil (like tea tree oil) would be a beneficial approach for better drug delivery in pharmaceutical applications (Dalwai et al., 2016).

2.3 Microencapsulation technology

The practical uses of many natural antimicrobial agents were hampered by a number of issues, despite the fact that they have significant benefits over conventional antibiotics. These challenges include the high costs of production and the high instability of some linear peptides due to their sensitivity of fast degradation. To address these challenges, researchers begun working on the development of synthetic analogues or fragments of natural antimicrobial. The ultimate objective of these initiatives is to improve their applicability in microbial biofilm elimination (Gao et al., 2024). Microencapsulation of drugs can be used to modify and extended of controlled release rate within therapeutic range and might even aid to hide its strong flavour or odour (Martins et al., 2011). Microencapsulation is one of the most widely used formulation techniques to elaborate controlled release applications in several fields as pharmaceuticals, essential oils, self-healing coatings, biomacromolecules, adhesives, fragrance, flavours and also used to taste-masking of the bitter taste of drugs (Patil, D.K. et al., 2015). In term of structural design, there are several types of microspheres that are currently available that are used to encapsulate pharmaceutical drugs. Microcapsules are made up of two parts consists of the core and the shell. The core (internal component) contains the active agents (for example, essential oil), while the shell (external component) protects the core from the outside environment (Martins et al., 2014). Taking into the count, the particle size of the microcapsules could give a significance release profile of the drug depending on the fabrication technique to produce the microsphere (Ma & Su, 2013). Several methods have been developed for producing microcapsules that is match to different types of core and shell materials, as well for creating particles of various sizes and thicknesses, hence altering the release rate of the active principle.

The primary objective of microencapsulation is to entrap the substance in a protective matrix/shell that will bestow the good characteristics in terms of resistance, solubility and the controlled release of microcapsules (Martins et al., 2014). Given the limitation of some methods and physicochemical characteristics of essential oils, the