THE EFFECT OF CHOLINE BASED IONIC LIQUIDS ON ANTIFUNGAL, PHYSICAL AND MECHANICAL PROPERTIES OF SILICONE DENTURE LINERS

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By

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LIST OF ABBREVIATIONS

FTIR	Fourier Transformed Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance
VH	Vickers Hardness
SBS	Shear Bond Strength
CB	Choline Borate
CS	Choline Salicylate
PMMA	Poly Methyl Methacrylate
SBDSL	Silicone Denture Base Soft Liners
HIV	Human Immunodeficiency Virus
CILs	Choline-Based Ionic Liquids
IMM	Imidazolium
PY	Pyridinium
IL	Ionic Liquids
PVC	Polyvinylchloride
NMRS	Nuclear Magnetic Resonance Spectroscopy
CYP 450	Cytochrome P 450
AgNps	Silver Nanoparticles
MIC	Minimum Inhibitory Concentration
QA-PEI	Quaternary Ammonium Derived Polyethylene
QAC	Quaternary Ammonium Compounds
CA	Candida Albicans
PDMS	Polydimethylsiloxane

SL	Silicone Liners
TBS	Tensile Bond Strength
Ag	Silver
Np	Nanoparticles
TiO2 NPs	Titanium Dioxide Nanoparticles
Nano-SiO2	Silicone Dioxide
Mol B	Moloplast B
ESKAPE	Enterococcus faecium Staphylococcus aureus Klebsiella pneumonia Acinetobacter baumannii
TNFa	Tumor Necrosis Factor Alpha
RBDCs	Resin Based Dental Composites
CpTi	Commercially Pure Titanium
S. mutans	Streptococcus Mutans
BMIM.BF4	1-n-butyl-3-methylimidazolium tetrafluoroborate
E. faecalis	Enterococcus. Faecalis
CAGE	choline and geranic acid (CAGE)
IDEA	Iongel of a Deep Eutectic Antimicrobial
NSAID	non-steroidal anti-inflammatory medicines
IRCBM	Interdisciplinary Research Centre in the Division of Biomaterials
JEPeM – USM	Jawatankuasa Etika Penyelidikan Manusia Universiti Sains Malaysia
D2O	deuterium oxide

H-NMR	Proton NMR
Ppm	Parts per million
ASTM	American Society for Testing and Materials
ISO	International standard organization
MPA	Megapascals
PTFE	Polytetrafluoroethylene
OD	Optical density
SPSS	Statistical package for the social sciences
SD	Standard deviation
ANOVA	Analysis of Variance
[CnMIM]+[X]	1-alklyl-3-methyl imidazolium ionic liquids
BPCL	Butyl pyridiniumchloride
OCTL	Octyl pyridiniumchloride
[Cho][AA]s)	Choline amino acid ionic liquids

LIST OF SYMBOLS

C=O	Carbon oxygen double bond
C-H	Carbon-hydrogen
OH	Hydroxyl
N-H	Nitrogen-Hydrogen
C=C	Carbon Carbon double bond
CH3	Methyl
B-O	Boron-Oxygen
F	Force
D	Median thickness
A	Area
COO-	Carboxyl
Si-OH	Silanol

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KESAN CECAIR IONIK BERASASKAN KOLINA TERHADAP SIFAT ANTIKULAT, FIZIKAL, DAN MEKANIK PELAPIK DENTUR BERASASKAN SILIKON

ABSTRAK

Kajian in vitro ini bertujuan untuk membangunkan cecair ionik berasaskan kolin yang baharu yang mengandungi pelapik silikon dentur (SDBSLs) dengan potensi antifungus yang lebih baik dan pematuhan dengan kriteria standard untuk pelapik jangka masa yang panjang. Pelapik yang diubahsuai ini kemudiannya dinilai untuk aktiviti antifungus mereka, sifat mekanikal dan fizikal. Cecair ionik salisilat kolina dan borat kolina telah disintesis, dan dicirikan menggunakan spektroskopi inframerah transformasi Fourier dan resonans magnetik nuklear untuk analisis permukaan dan ketulenan masing-masing. Cecair ionik yang disediakan telah dimasukkan ke dalam SDBSL (Moloplast B; DETAX Jerman) pada kepekatan yang berbeza (1%, 2%, dan 5%) untuk membentuk dua kumpulan eksperimen dan enam subkumpulan; CB (CB1, CB2 & CB5); SDBSL yang dimasukkan dengan borat kolina dan CS (CS1, CS2 & CS5); SDBSL yang dimasukkan dengan salisilat kolina. Ubat konvensional dan antifungus; itraconazole (1.25% wt/wt), yang dimasukkan ke dalam pelapik dentur silikon (SDBSL) berfungsi sebagai kawalan negatif dan kawalan positif. Sampel-sampel tersebut telah menjalani ujian mekanikal dengan ujian kekerasan Shore A menggunakan durometer. Sementara itu, kekuatan koyakan dan kekuatan ikatan tegangan dinilai melalui mesin ujian universal. Ujian fizikal dijalankan untuk menilai penyerapan air, kelarutan, dan perubahan berat selepas direndam dalam air suling selama 1 minggu dan 6 minggu dengan penimbangan menggunakan timbangan analitik. Data diproses secara statistik menggunakan SPSS versi 25 dengan ANOVA dan perbandingan berpasangan berganda dengan analisis post hoc ujian Tukey dengan $\alpha = 0.05$ ($p < 0.001$). Keputusan menunjukkan bahawa borat kolina 2% (CB2) mempunyai kesan penghambatan yang ketara terhadap pertumbuhan fungus C. albicans diikuti oleh salisilat kolina 2% (CS2). Salisilat kolina 1% (CS1) menunjukkan kekuatan koyakan maksimum berbanding dengan kawalan positif. Kumpulan eksperimen mempunyai nilai kekerasan Shore A purata yang lebih rendah berbanding dengan kawalan positif dan negatif masing-masing. Kumpulan kawalan negatif mempunyai kekuatan ikatan tegangan tertinggi, diikuti oleh CS1. Selepas 1 minggu rendaman dalam air, kawalan positif menunjukkan penyerapan air tertinggi manakala CS2 menunjukkan penyerapan paling rendah. Sebaliknya, kawalan positif menunjukkan kelarutan yang paling rendah secara konsisten selepas 6 minggu rendaman, sementara kawalan negatif menunjukkan kelarutan tertinggi. Pelapik yang berisi ubat (kawalan positif) memperoleh berat paling banyak dan CS1 memperoleh berat paling sedikit selepas 1 minggu rendaman. Selepas 6 minggu rendaman, semua kumpulan kajian mengalami penambahan berat badan, dengan CB5 memperoleh berat paling banyak dan CS1 memperoleh berat paling sedikit. Kajian ini mendedahkan bahawa penggabungan salisilat kolina dalam pelapik silikon mempunyai kesan yang signifikan terhadap peningkatan potensi antifungus serta pematuhan terhadap sifat mekanikal dan fizikal. Walau bagaimanapun, kajian lanjut yang melibatkan sifat likat kenyal dinamik dan kesitotoksikan perlu disiasat sebelum ujian klinikal.

THE EFFECT OF CHOLINE BASED IONIC LIQUIDS ON ANTIFUNGAL, PHYSICAL AND MECHANICAL PROPERTIES OF SILICONE DENTURE LINERS

ABSTRACT

This *in vitro* study aimed to develop novel choline based ionic liquids incorporated silicone denture liners (SDBSLs) with better antifungal potential and compliance with standard criteria for long term liners. The modified liners were then assessed for their antifungal activity, mechanical and physical properties. Choline salicylate and choline borate ionic liquids were synthesised and characterised with Fourier transform infrared spectroscopy and nuclear magnetic resonance for surface and purity analysis, respectively. The prepared ionic liquids were incorporated into SDBSLs (Moloplast B; DETAX Germany) at varying concentrations (1%, 2%, and 5%) to constitute two experimental groups and six subgroups; CB (CB1, CB2 & CB5); choline borate incorporated SDBSLs and CS (CS1, CS2 & CS5); choline salicylate incorporated SDBSLs. Conventional and antifungal drug; itraconazole (1.25% wt/wt), incorporated silicone denture liners (SDBSL) served as negative control and positive control. The samples were subjected to mechanical testing with Shore A hardness test through durometer. While tear strength and tensile bond strength were evaluated through universal testing machine. Physical testing was conducted to evaluate water sorption, solubility, and weight change following immersion in distilled water for 1 week and 6 weeks with weighing through an analytical balance. Data was statistically processed by SPSS version 25using ANOVA and all pairwise multiple comparison with post hoc analysis of Tukey's

test with $\alpha = 0.05$ ($p < 0.001$). The results revealed that choline borate 2% (CB2) had a considerable inhibitory impact on *C. albicans* fungal growth followed by choline salicylate 2% (CS2). Choline salicylate 1% (CS1) demonstrated the maximum tear strength when compared to the positive control. The experimental groups had significantly lower mean Shore A hardness values than the positive and negative controls respectively. The negative control group had the highest tensile bond strength, followed by CS1. Following 1 week of water immersion, the positive control had the highest water sorption whereas CS2 demonstrated the lowest sorption. In contrast, the positive control displayed consistently the least solubility after 6 weeks of immersion, whereas the negative control showed the highest solubility. The drug-impregnated liners (positive control) gained the most weight and CS1 gained the least weight following 1 week of immersion. After 6 weeks of immersion, all study groups gained weight, with CB5 acquired most weight and CS1 acquired the least. The study reveals that incorporation of choline salicylate in silicone liners has significant effect on enhanced antifungal potential and compliance with mechanical and physical properties. However, further studies including dynamic viscoelastic property and cytotoxicity are needed to be investigated before the clinical trials.

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Oral disease-related health problems are on the rise, posing a significant burden in many developing and underdeveloped countries (Luo et al., 2021). Among the most prevalent and consequential oral diseases globally are dental caries, chronic mouth and facial pain, oral and throat cancer, oral infection and sores, congenital disabilities such as cleft lip and palate, periodontal disease, tooth decay, and tooth loss that affect the mouth and oral cavity and those, that limit an individual's ability (Peres et al., 2019).

Most people globally are impacted by oro-dental illnesses, creating an increasing need for novel therapeutic strategies (Jose et al., 2021). The prevalence of tooth loss and the need for a prosthesis grows with age, raising concerns about denture-related pathologies (Saeed et al., 2020a). Microbial colonization of porous denturesurfaces is an unavoidable consequence (Elawady et al., 2019), as the placement of a removable prosthesis causes substantial changes in the surrounding environment (Gleiznys et al., 2015), which may compromise the integrity of the oral tissues. Mechanical irritation, allergic reactions, plaque accumulation, and fungal or bacterial infections can induce denture-related mucosal lesions (Sterzenbach, 2020). In addition, soft tissues become caught between the denture's hard base and the bone in denture-wearers. Due to the underpinning structure's incapacity to evenly transmit the masticatory force, these traumatized tissues experience pain and discomfort. To overcome this issue, soft lining materials can be used as a cushion on the intaglio surface of dentures to uniformly distribute functional and non-functional loads (Mada & Setyahadi, 2014).

It is well-established that relining an ill-fitting denture improves the patient's ability to chew and speak, reduces discomfort and soreness, and increases psychological comfort (Stavreva, 2021). Resilient liners are an excellent auxiliary in alleviating denture pain. During cyclic masticatory stresses, its intrinsic viscoelasticity aids in providing cushioning and equal load distribution over the denture-bearing mucosa (Pisani et al., 2012). Based on their intended use, liners are divided into two categories: short-term liners and long-term liners. Short-term liners, commonly known as tissue conditioners, are temporary liners used to treat and condition denture-bearing tissues that have become inflamed. In addition, they are used in repairing ill-fitting dentures, functional impressions, and immediate dentures. The presence of tissue conditioners creates a cushion thatcomforts the underlying mucosa and thus provides a favorable environment for healing. Polyethymethacrylate resins are the main component of tissue conditioners, and they have other additions like plasticisers and alcohol to make them workable. Plasticiser and alcohol leaching cause rapid hardening of the material, which might take anywhere from a few days to a week or two. Therefore, it is recommended that the tissue conditioner be replaced every 3 to 4 days (Dorocka-bobkowska et al., 2017).

Long-term soft liners are divided into two types: plasticised acrylic denture base soft liners and silicone denture base soft liners. They are mainly used to treat individuals with certain oral morphological and physical characteristics, such as sharp, thin, or severely resorbed ridges, severe bone undercuts, and congenital or acquired palate deformities (Verma et al., 2018). They function well in absorbing masticatory loads and distributing loading force during function, hence enhancing the patient's comfort and acceptance of the prosthesis. However, because they are also made of

Polyethylmethacrylate and also contain additive materials such as monomers and plasticisers (Babu et al., 2019), they inherit the same weaknesses as tissue conditioners, which leach out plasticisers that cause the surface of the liner to become rough and hard, thereby promoting calculus formation and food debris accumulation (Nagay et al., 2020).

Another form of a long-term soft liner, a Silicone-based soft liner, has gained popularity over plasticized acrylic liners due to their lower glass transition temperature advantages, which maintain the soft gel state for a longer time, hence, lasting viscoelasticity (Dorocka-bobkowska et al., 2017). Despite their widespread use since the 1950s, they continue to exhibit poor adhesion to acrylic denture bases and develop surface roughness and porosity with time. These conditions provide a suitable environment for plaque formation. These plaque deposits are difficult to remove, and any vigorous removal attempts may result in liner distortion. Additionally, the porous nature of the liner permits Candida albicans and other microbes to flourish on and within it, which results in a condition called denture stomatitis (Rodrigues & Shenoy, 2013).

Candida-associated denture stomatitis, also known as denture-induced stomatitis, is a common complication of denture-wearing (Budtz-jørgensen & Budtz-jørgensen, 2009). It is a multifactorial disease that has been linked to Candida albicans (Gleiznys et al., 2015). Denture-induced stomatitis affects 60-65% of regular denture wearers (Aydoğdu, 2016), and it is frequently found in conjunction with angular cheilitis and glossitis. For such patients, the condition is a constant source of concern, necessitating the use of antifungal medication. There are other factors that can contribute to denture-induced candidiasis, like mucosal trauma, allergic reaction, and dietary.

Systemic antifungal medication, oral hygiene care, denture cleaning, and disinfection procedures, denture replacement, eliminating anatomical defects, restoring non-traumatic occlusion, and nutritional restitution are also widely used in managing cases of denture-induced candidiasis (Sánchez-Aliaga et al., 2016). It is known that intraoral antifungal therapy has a number of disadvantages, including the cleansing effect of saliva and muscle in the oral cavity, which reduces the drug's potency below the appropriate therapeutic concentration, as well as the issue of patient compliance (Ellepola & Samaranayake, 2000; Watamoto et al., 2009). Therefore, attracts the interest of global researchers seeking the most effective intraoral antifungal therapeutic applications.

Common intraoral antifungals used to treat, denture-induced candidiasis include azoles pyrrole ring antifungal drugs, polyene antifungal drugs, propenyl amine drugs, and second-generation triazole drugs (J. Fang et al., 2021). Pyrrole ring drugs include imidazoles and triazoles which inhibit ergosterol synthesis in fungi to demonstrate antifungal effect. Imidazoles include clotrimazole, ketoconazole, and miconazole (Lalla & Dongari-Bagtzoglou, 2014). Polyene antifungal drugs include nystatin and amphotericin B. The mechanism of action is cell membrane changes, cell lysis, and necrosis. These drugs are prescribed with a lipid body agent to reduce toxic side effects (Hamill, 2013). Itraconazole and fluconazole are second-generation triazole drugs and widely used antifungal medications to treat oral candidiasis (J. Fang et al., 2021). At a minimum inhibitory dose, itraconazole has considerable fungicidal activity and a high rate of mycological cure (Chow, 1999). It predominantly inhibits fungal ergosterol synthesis as well as the activity of specific enzymes, resulting in suppression of the enzyme cytochrome P-450 14-alpha-demethylase (protein enzyme in hepatocytes) and oxidative damage of microbial cell (Sánchez-Aliaga et al., 2016; Santawisuk et al., 2013).

A sustained drug delivery system for Candida infection intraoral drug therapy has been developed by incorporating potential pharmaceuticals into acrylic resin or silicone-based soft liners (Chow, 1999; Turon et al., 2017), where Nystatin, Fluconazole, and Itraconazole have been advocated (Sánchez-Aliaga et al., 2016). In addition, there is a growing tendency towards the use of phytotherapeutic/herbal substances, nanoparticles, and metal coatings as reactive components for denture soft liners (Nam, 2011; S. Sharma & Hegde, 2014). All of these research initiatives aim to enhance the antifungal properties of denture liners. However, many of the procedures used thus far have been shown to affect silicone soft liners' physical or mechanical properties, either through decreased hardness, lower viscoelasticity, increased solubility in oral fluids, or poor adherence to the acrylic denture base (Kanjanamekanant et al., 2017).

Kanjanamekanant and colleagues demonstrated that Imidazolium-based ionic liquids and Nystatin incorporating tissue conditioners showed superior antifungal activity and claimed to have the stability of the tissue conditioner material (Kanjanamekanant et al., 2017). This finding paves the way for future research to optimise intraoral antifungal therapeutic approaches. Ionic liquids are inorganic salts with low melting points that exhibit antibacterial activity. They are composed of large cations such as imidazolium (IM) and pyridinium (Py), as well as anion compounds such as chloride, bromide, and nitrate (Bystrzanowska et al., 2019). They have been recognized by the scientific community for decades (Macfarlane et al., 2016), but there is a growing interest in ionic liquids as energetic materials (Gadilohar & Shankarling, 2017), lubricants (Zhou et al., 2009), disinfectants (Pendleton & Gilmore, 2015), and surfactants (Łuczak et al., 2008). They are referred to as task-specific tuned liquids because their physicochemical properties can be altered independently by modifying anions and cations. Their structural adaptability creates a one-of-a-kind architectural platform for independently modifying the properties of the cation and anion. This enables researchers to develop innovative functional materials while keeping the ionic liquid's desirable properties (Gindri et al., 2015; Pendleton & Gilmore, 2015).

Ionic liquids such as quaternary ammonium compounds (Garcia et al., 2020b), choline-based ionic liquids (Azevedo et al., 2017), and imidazolium ring-based ionic liquids have been used in dentistry related research due to their sustained antifungal and antibacterial activity, cytocompatibility, and improved physicochemical properties (Nikfarjam et al., 2021). Ionic liquid’s cationic structure contributes to its antibacterial action (Gindri et al., 2014). The positive charges on IL interact with the negative charges on acidic phospholipids in the microbial cell membrane, causing the hydrophobic tail of ILs to be integrated into the hydrophobic microbial membrane core. At higher concentrations, ionic liquid damages the cell membrane by solubilizing hydrophobic cell membrane components, forming mixed aggregates. This cascade of events denatures structural proteins, results in intracellular component leakage, and ultimately results in cell death (Kanjanamekanant et al., 2017; Nikfarjam et al., 2021; Zheng et al., 2017).

Various dental polymers, including polymethyl methacrylate (PMMA) and polyvinyl chloride (PVC), include ionic liquids in their composition as plasticizers. These plasticizers reduce the glass transition temperature and viscosity of polymers, increasing the flexibility and processability of polymer products (Bodaghi, 2020). Ionic liquids have been used in dental polymers as a solvent, plasticizer, surfactant, and disinfectant (Chang et al., 2017; Gholami et al., 2022b; Hodyna et al., 2018).

New aspects of ionic liquid application areas are being revealed and developed. For example, Ionic liquids' positive charges and high affinity for negatively charged surfaces, such as bacterial membranes, are used in bioapplications to combat infections (Nikfarjam et al., 2021). Antibacterial orthodontic adhesive based on ionic liquids, 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl), affirmed potent antibacterial activity against Streptococcus mutans (Martini Garcia et al., 2019).

Another dental application of ionic liquid is in coating titanium dental implants; where the dicationic imidazolium-based ionic liquids were used for wear protection of the implant and anti-inflammatory coating layer (K. T. Kim et al., 2019; Ramburrun et al., 2021; Wheelis et al., 2020a).

1.2 Problem statement and justification of the study

The modification of denture liners was highlighted as the primary focus of current research in this area. In an effort to improve the antifungal potential of denture base liners, the composition of denture soft liners has been modified to include nanoparticles (N. Ahmad et al., 2020), metal coatings (rd et al., 2021; A. Ferreira et al., 2022), and antifungal drug delivery systems incorporating therapeutic agents (A. Ferreira et al., 2022).

However, almost all methods have been observed to alter the compliance of silicone soft liners in terms of physical and mechanical properties. The altered compliance has been reported either in the form of decreased hardness, decreased viscoelasticity, elevated solubility in oral fluids, or poor adhesion to the acrylic denture base'' (Kanjanamekanant et al., 2017). In addition, denture-induced candidiasis is a mixed biofilm infection that poses a number of therapeutic problems. In light of these facts, it isessential to develop novel silicone denture liners with maintained compliance and enhanced antifungal characteristics (Salim et al., 2012).

Ionic liquids have also been added as solvents or plasticizers in dental polymers like polymethyl methacrylate (PMMA) and polyvinyl chloride (PVC). They improve materials' flexibility, durability, and antimicrobial potential (Kanjanamekanant et al., 2017). Recently, choline-based ionic liquids have received special attention in the development of environmentally friendly ionic liquids. Investigations have established that choline-based ionic liquids have a broad spectrum of antimicrobial activity, particularly against Gram-positive and Gram-negative bacteria as well as Candida albicans (H. Ahmad et al., 2019; Ibsen et al., 2018a). Ionic liquid impregnated silicone denture soft liners (SDBSLs) can pave the way towards the development of antifungal denture liners with sustained antimicrobial activity without compromising the mechanical strength and stability of oral fluids.

1.3 General objectives of the study

The study's general aim was to synthesize and characterize ionic liquids before using them for the fabrication of novel silicone denture base liners, incorporating choline based ionic liquids. These ionic liquids-impregnated silicone denture base liners were further investigated for their antifungal properties as well as physical and mechanical properties to ensure the compliance.

1.4 Specific Objectives of the study

1.4.1 Objective 1

To characterize the structure of synthesize choline borate and choline salicylate ionic liquids using Fourier-Transform Infrared spectroscopy and Nuclear magnetic resonance spectroscopy.

1.4.2 Objective 2

To determine the antifungal potential of the ionic liquid impregnated silicone denture base liners and control groups against candida species employing direct contact test via spectrophotometry.

1.4.3 Objective 3

To determine the water sorption, solubility, and percent mass change of the ionic liquid impregnated silicone denture base liners and control groups using short term immersion (1 week) and long term immersion (6 weeks).

1.4.4 Objective 4

To determine the tear strength, tensile bond strength and hardness of the antifungal drug and ionic liquid impregnated silicone denture base liners and control groups with universal testing machine and durometer.

1.5 Research questions

1. Does the impregnation of choline based ionic liquids enhance the antifungal potential of silicone denture base soft liners?

2. Does the impregnation of choline based ionic liquids affect the water sorption of silicone denture base soft liners?

3. Does the impregnation of choline based ionic liquids affect the tear resistance, tensile bond strength to denture base acrylic resins and hardness of the silicone denturebase

soft liners?

1.6 Research hypothesis

1. The impregnation of choline-based ionic liquids effectively enhances the antifungal potential of silicone denture base soft liners (SDBSLs).
2. The impregnation of choline-based ionic liquids effectively enhances the antifungal potential of silicone denture base soft liners (SDBSLs).
3. The impregnation of choline-based ionic liquids does not affect the tear strength, tensile bond strength, and hardness of SDBSLs.

CHAPTER 2

LITERATURE REVIEW

2.1 Denture soft liners

Denture soft liners are a critical component in removable prosthodontics, providing comfort and improved fit for denture wearers (Pesun, 2019). Their primary role is to act as a cushion between the rigid denture base and the delicate oral mucosa, thus distributing masticatory forces more evenly and accommodating any irregularities in the mucosal surface. Among the various materials used for soft liners, silicone-based optionshave gained prominence due to their superior properties and performance (Kreve & Dos Reis, 2019).

The advent of denture soft liners marked a significant advancement in improving the quality of life for denture wearers. Initially, the materials used were often short-term solutions, such as temporary soft acrylics. However, the need for more durable, long-lasting, and biocompatible materials led to the development of silicone-based soft liners (Abdelnabi & Swelem, 2020). These liners are distinguished by their longevity, resistance to wear and tear, and their ability to maintain resilience (Pesun, 2019) and cushioning effect over extended periods. Silicone's inherent properties, such as being non-irritating to oral tissues and less porous than other materials, make it a preferred choice for many patients and clinicians (Dorocka-bobkowska et al., 2017; Jacob et al., 2021).

Denture soft liners are primarily used to enhance the comfort and fit of dentures (figure 2.1) for individuals with various oral conditions (Białożyt-Bujak et al., 2021). They act as a cushion between the hard denture base and the delicate oral mucosa,making dentures more tolerable and functional for patients (Elawady et al., 2021). Oral problems and conditions that might require the use of denture soft liners include resorbed and flabby ridges, bony prominences or undercuts, denture stomatitis, xerostomia (dry mouth), mucosal atrophy, surgical rehabilitation, implant overdentures and neurological conditions (Ogawa et al., 2016). Patients with significant bone resorption often have less stable dentures due to a lack of adequate ridge height and firmness. Soft liners can help distribute masticatory forces more evenly across the mucosa, reducing pressure points and enhancing stability (Ibraheem & ElGabry, 2021).

Figure 2. 1: Maxillary and mandibular complete dentures mounted on casts.

Rigid dentures may induce pain and discomfort in individuals with sharp bony prominences or undercuts in the oral cavity. Soft liners provide a cushioning layer that can adapt to these irregularities, preventing sore spots and trauma (Elawady et al., 2021). A frequent inflammatory disease of the tissues underlying a denture is denture stomatitis. It's commonly attributed to candida infections, persistent denture wear, and inappropriate denture hygiene. A soft liner can help improve hygiene, reduce irritation, and when impregnated with antifungal agents, can also help treat the fungal component of stomatitis (Abuhajar et al., 2023; John & Palaniappan, 2023).

Individuals with dry mouth, often due to medications, systemic conditions, or radiation therapy, may find dentures particularly uncomfortable as the lack of saliva can lead to increased friction and irritation. Soft liners can mitigate xerostomia byproviding a more forgiving, lubricated surface (Elawady et al., 2021).

Patients with thin, atrophic mucosa, often due to age or long-term denture wearing, may experience discomfort and pain with standard hard dentures. Soft liners distribute forces more gently across the mucosa, protecting it from excessive pressure and trauma (Khalaf et al., 2020). Soft liners facilitate surgical rehabilitation following oral surgery or in cases of oral trauma. They can be used temporarily to protect the healing tissues from the hard denture base. They can also be used as a medium for gradual tissue conditioning and adaptation during the healing process (Singh et al., 2014).

Soft liners serve as a shock-absorbing layer in implants supported over dentures, shielding the osseointegrated implants. They further protect the surrounding tissues from excessive occlusal stresses and potentially prolong the serviceability of the prosthesis and the implants (Khalaf et al., 2020)

Neurological conditions that cause involuntary orofacial movements, such as Parkinson's disease or certain types of cerebral palsy, may benefit from soft liners that help cushion and distribute these dynamic forces more evenly, reducing the risk of mucosal injury (Al, 2021). Common indications of denture liners are enlisted in table 2.1.

Table 2. 1: Common indications of denture liners

Local predisposing factors		Systemic predisposing factors	
Short-term	**Long-term**	**Short-term**	**Long-term**
Poor oral hygiene	Trauma (ill-fitting denture)	Broad-spectrum antibiotics	Physiological factors (age, pregnancy)
Topical steroids	Smoking	Dietary factors (deficiency state)	Dietary factors (deficiency state)
Dietary factors (high carbohydrate intake)	Radiation		Immune defect (AIDS)
	Xerostomia		Malignancy (leukemia)
	Local mucosal lesions and dental problems		Endocrine(diabetes mellitus)

2.2 Fungal infections in denture wearers

Dentures, while providing a functional and aesthetic solution for individuals with missing teeth, also present unique challenges related to oral health. One prominent concern among denture wearers is the susceptibility to fungal infections, primarily due to the close contact between denture materials and the oral mucosa. This comprehensive review aims to explore the prevalence, causative factors, and preventive measures related to fungal infections in denture wearers (Manikandan et al., 2022).

Fungal infections, particularly candidiasis, represent a common issue in individuals wearing dentures. Candida species, especially Candida albicans, are frequently implicated in denture-related fungal infections (M. M. Gad & Fouda, 2020). A Study have reported varying prevalence rates, with some indicating that up to 65% of denture wearers may experience candidiasis at some point. The warm and moist environment under dentures provides an ideal breeding ground for fungi, contributing to the high incidence of these infections(Manikandan et al., 2022).

Several factors including poor denture hygiene, ill-fitting dentures, prolonged denture wear and immunocompromised status contribute to the increased susceptibility of denture wearers to fungal infections. Inadequate denture hygiene is a primary risk factor. Failure to clean dentures regularly allows for the accumulation of microbial biofilms, creating an environment conducive to fungal growth. Effective denture cleaning practices are crucial in preventing infections (Ponde et al., 2021).

Ill-fitting dentures can also lead to microtrauma and tissue irritation, creating entry points for fungi. Continuous friction between ill-fitting dentures and oral tissues exacerbates the risk of developing fungal infections (Thilakumara et al., 2017).

Extended periods of denture use, especially when wearing dentures overnight, contribute to the accumulation of oral secretions and reduced salivary flow, fostering fungal overgrowth. Denture wearers are advised to give their oral tissues a rest to mitigate this risk (Abuhajar et al., 2023).

Individuals with compromised immune systems, such as those with diabetes or undergoing immunosuppressive therapy, are more susceptible to fungal infections. Denture wearers with underlying health conditions require vigilant monitoring and preventive measures (Manikandan et al., 2022).

Numerous studies have investigated the prevalence and risk factors associated with fungal infections in denture wearers. A study by Manikandan (Manikandan et al., 2022) examined the oral mycobiota of denture wearers and found a higher prevalence of Candida species compared to non-denture wearers (Manikandan et al., 2022). The research highlighted the importance of denture hygiene in preventing fungal overgrowth.

In a longitudinal study, (Gacon et al., 2019)), denture hygiene practices were assessed in relation to the incidence of candidiasis. The findings underscored a direct correlation between poor denture hygiene and an increased risk of fungal infections, emphasizing the need for patient education on proper cleaning techniques (Gacon et al., 2019). Research by Tasopoulos et al (Tasopoulos et al., 2023) investigated the impact of denture material on fungal adhesion. The study revealed that certain denture materials exhibited higher fungal adherence, suggesting that material selection plays a role in infection prevention (Tasopoulos et al., 2023).

A systematic review by Hilgert, (Hilgert et al., 2016) analyzed interventions for preventing and managing fungal infections in denture wearers. The review identified antifungal agents, improved denture hygiene, and regular dental check-ups as effective strategies for reducing the prevalence of candidiasis (Hilgert et al., 2016).

Preventing fungal infections in denture wearers involves a multifaceted approach. Dental professionals play a crucial role in educating denture wearers about proper denture hygiene practices, including (Odeh et al., 2012) regular cleaning, removal of dentures at night, and the importance of routine dental check-ups. Ensuring well-fitting dentures is essential to prevent tissue irritation and microtrauma. Regular assessments and adjustments by dental practitioners contribute to patient comfort and reduce the risk of fungal infections (Martins & de Lacerda Gontijo, 2017).

The use of antifungal agents, either incorporated into denture materials or applied topically, has shown promise in preventing fungal infections. Research continues to explore the efficacy and safety of these interventions. Investigations into the influence of denture materials on fungal adherence underscore the importance of material selection. Denture materials with reduced microbial adherence properties may offer an additional layer of protection against fungal infections (Odeh et al., 2012).

2.2.1 Oral candidiasis

Oral candidiasis, also known as oral thrush, is a common mucosal infection caused by the opportunistic fungal pathogen Candida, primarily Candida albicans. The condition is characterized by creamy white lesions, usually on the tongue or inner cheeks, and can spread to the roof of the mouth, gums, tonsils, or the back of the throat (Abuhajar et al., 2023). A detailed literature review on oral candidiasis would cover several key aspects. Most cases are attributed to C. albicans, but other species like C. glabrata and C. tropicalis are also involved. The transition of Candida from a commensal organism to a pathogenic one involves biofilm formation, morphological changes, and the expression of virulence factors (Vila et al., 2020).

Individuals with HIV/AIDS, diabetes, or those undergoing chemotherapy or taking broad-spectrum antibiotics are at higher risk. Poorly fitted dentures or inadequate oral hygiene can create favorable conditions for fungal growth. Age (common in infants and elderly), nutritional deficiencies, and certain genetic predispositions are also vulnerable to oral candidiasis (Lu, 2021). The literature consistently shows that oral candidiasis is a complex condition influenced by a myriad of factors. Effective management requires a comprehensive approach that includes not only the treatment of the condition but also, the modification of risk factors and underlying conditions. Continuous research and development in diagnostics and therapeutics are critical to addressing challenges such as antifungal resistance and recurrent infections (Vila et al., 2020).

Denture-induced candidiasis, commonly known as denture stomatitis, is a prevalent condition primarily among denture wearers and is mostly attributed to the fungus Candida albicans. This condition is characterized by inflammation and redness of the mucosa beneath the denture-bearing areas. The detailed structure of *C. albicans* biofilms was first examined by scanning electron microscopy (SEM) (Hawser and Douglas, 1994). Figure 2.2 shows SEM image of a mature *C. albicans* biofilm. These biofilms are composed of yeast, hyphal, and pseudohyphal elements (Ponde et al., 2021).

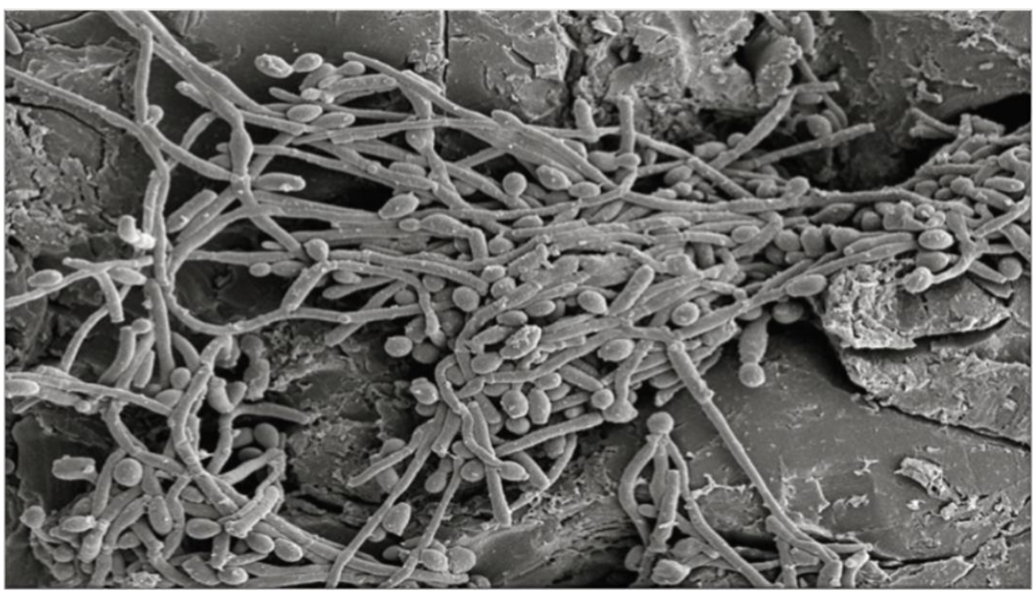

Figure 2. 2: SEM image of a mature *C. albicans* biofilm (48 h) (Haque et al., 2016)

The prevalence of oral candidiasis varies globally but is notably higher in the elderly population and tends to affect more women than men. The pathophysiology involves the adherence of Candida to the denture's irregular surfaces, which often accumulate and form a biofilm. This biofilm is not only resistant to antifungal agents but also to the mechanical cleaning methods typically used for denture care (Gacon et al., 2019; Manikandan et al., 2022).

One of the key findings in research literature is the significant role of poor oral hygiene and ill-fitting dentures in promoting this condition. Dentures provide a unique microenvironment that, when combined with saliva and crevices in the material, can lead to fungal accumulation (Redfern et al., 2022)

Nightly wearing of dentures and infrequent cleaning exacerbate the situation by providing continuous warmth and moisture, ideal for fungal growth. Inflammation ensues due to the mechanical irritation from ill-fitting dentures and the chronic presence of the fungus, leading to the clinical presentation of denture stomatitis ((Delaney et al., 2019).

Studies have also focused on the treatment and prevention of denture-induced candidiasis. Traditional approaches include antifungal medications, emphasizing the importance of proper denture hygiene and regular dental check-ups for denture adjustments (Parul Uppal Malhotra et al., 2020). Another study investigated the potential of biofilm-resistant materials and anti-fungal coatings to reduce denture-related infections (M. M. Gad & Fouda, 2020).

Despite these advancements, the prevention and effective management of denture-induced candidiasis largely rely on patient education about proper denture care, including regular cleaning, overnight soaking in antifungal solutions, and ensuring a proper fit to minimize mucosal irritation. The comprehensive approach to managing this condition underlines the importance of addressing both the microbial aspect and the prosthetic-related factors (Bajunaid, 2022; Elawady et al., 2021).

2.2.2 Comprehensive review of antifungal agents used in dentistry

A comprehensive review of antifungal agents in dentistry provides a critical examination of the drugs used to manage and treat fungal infections in the oral cavity, predominantly caused by species of the Candida, Aspergillus, and Cryptococcus genera, among others (Villar & Dongari-Bagtzoglou, 2021). The necessity of these agents is underscored by the prevalence of fungal infections which can range from mild oral thrush to severe, potentially life-threatening systemic infections, especially in immunocompromised individuals. The development of antifungal medications represents a significant advancement in both medical and dental fields, beginning with the discovery of nystatin in the 1950s, marking a paradigm shift in the management of fungal infections. Over the decades, several classes of antifungal agents have been developed, each with a unique mechanism of action and spectrum of activity (Swamy et al., 2018).

Antifungal agents are typically classified into several major groups: polyenes, azoles, echinocandins, and allylamines (Patel, 2022). Polyenes, such as nystatin and amphotericin B, function by binding to ergosterol in the fungal cell membrane, leading to increased membrane permeability and cell death (J. Fang et al., 2021). They have been a mainstay in treating oral candidiasis but are often associated with significant side effects when used systemically. Azoles, including fluconazole and itraconazole, inhibit the synthesis of ergosterol, a critical component of the fungal cell membrane, effectively leading to fungal cell death (Pristov & Ghannoum, 2019). Due to their broad spectrum andimproved safety profile, azoles have become the most widely used antifungal agents in both systemic and topical oral applications.

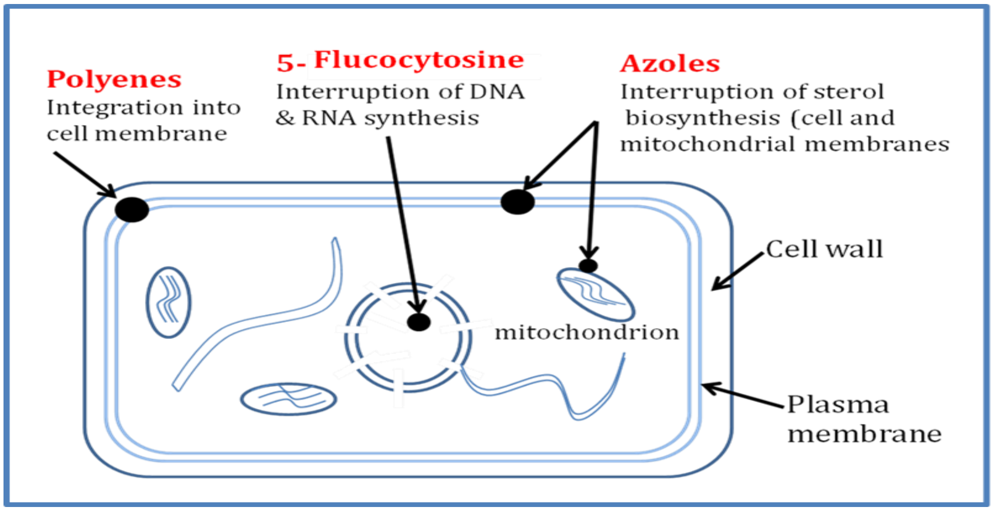

Figure 2. 3: Mechanism of action of antifungal agents (Jana et al., 2023)

Echinocandins, such as caspofungin, are a newer class of antifungals that inhibit the synthesis of β-1,3-glucan, an essential component of the fungal cell wall. Though less commonly used in oral infections, they represent an important option for systemic infections due to their efficacy and safety profile. Lastly, allylamines, like terbinafine, target squalene epoxidase in the ergosterol synthesis pathway, primarily used in dermatophytic infections (Gintjee et al., 2020; Shirsat et al., 2022).

In the realm of dentistry, antifungal agents are utilized for several indications. They are the cornerstone in the treatment of oral candidiasis, presenting in various forms such as pseudomembranous, erythematous, or hyperplastic candidiasis (Lu, 2021). Denture stomatitis, angular cheilitis, and chronic atrophic candidiasis are among the common fungal conditions encountered in dental practice, particularly affecting individuals wearing dentures, those with poor oral hygiene, or undergoing immunosuppressive therapy.

Furthermore, antifungal prophylaxis is crucial in patients with high-riskprofiles (Pristov & Ghannoum, 2019) including those receiving cancer therapy,undergoing organ transplantation, or living with HIV/AIDS, to prevent the occurrence orrecurrence of oral fungal infections. Antifungals are also used in endodontic treatments to address fungal infections within the root canal system (Manikandan et al., 2022; Swamy et al., 2018).

However, the clinical use of antifungal agents is not without challenges. Resistance to antifungal drugs is a growing concern, with reports of Candida species, particularly Candida albicans, developing resistance to commonly used drugs like fluconazole (A. Ferreira et al., 2022). The mechanisms of resistance are multifaceted, involving alterations in the target enzymes, efflux pump overexpression, and biofilm formation, making infections difficult to treat and manage (Cavalheiro & Teixeira, 2018). This resistance calls for a judicious use of antifungals, adherence to treatment guidelines, and the development of new antifungal agents or combination therapies to overcome resistance. The development and evolution of these agents have been marked by a continual effort to enhance efficacy, safety, and spectrum of activity (Vanreppelen et al., 2023). Despite the challenges posed by drug resistance, the future holds promise with the advent of novel therapies and strategies. As research progresses, it is imperative for dental professionals to stay informed about the latest developments and guidelines in antifungal therapy to effectively prevent and manage oral fungal infections, ensuring the best possible care fortheir patients (Kean & Ramage, 2019; Tits et al., 2020).

The future directions in the field of antifungal therapy are promising, with ongoing research focusing on novel drug targets, drug delivery systems (Şenel et al., 2021), and the exploration of natural and alternative compounds with antifungal properties. The advent of nanotechnology and targeted drug delivery systems offers the potential for more effective and less toxic treatment options. Moreover, understanding the host-microbe interaction and the immune response to fungal infections can provide insights intodeveloping vaccines or immunotherapies against oral fungal infections (Sousa et al., 2020).

2.2.3 Itraconazole: A Preferred Option for Oral Candidiasis

Itraconazole is a synthetic triazole antifungal agent that works by inhibiting the enzyme 14α-demethylase, which is crucial for converting lanosterol to ergosterol, an essential component of the fungal cell membrane. Without ergosterol, the cell membrane cannot maintain its integrity and function, leading to fungal cell death (Xiao et al., 2022).

The pharmacokinetics of itraconazole are characterized by good oral bioavailability, which is enhanced by acidic gastric pH. It is highly protein-bound (>99%) and has a large volume of distribution, indicating extensive tissue penetration. Itraconazole is metabolized in the liver through the cytochrome P450 (CYP) 3A4 enzyme, and its metabolites, along with the parent drug, are excreted mainly through feces. The drug's half-life allows for once-daily dosing in most treatment regimens, improving patient compliance (Beck et al., 2020).

Itraconazole's pharmacodynamics involve a concentration-dependent antifungal effect, with activity against a broad range of fungi, including Candida species. Its efficacy in oral candidiasis is attributed to its ability to achieve and maintain therapeutic concentrations in the oral mucosa and saliva, ensuring effective eradication of the infection (Czyrski et al., 2021).

2.2.4 Clinical Efficacy in Oral Candidiasis

Itraconazole's efficacy in treating oral candidiasis has been well-documented across various patient populations, including those with compromised immune systems. It provides a therapeutic alternative for patients who do not respond to or cannot tolerate other antifungal agents such as fluconazole or nystatin. Studies have shown itraconazole to be highly effective in clearing Candida infections from the oral cavity, with success rates comparable to or exceeding those of alternative treatments (Del Rosso et al., 2023; Jović et al., 2019; Tsai & Tsai, 2019).

One of the key advantages of itraconazole is its ability to maintain effective concentrations in the oral mucosa and saliva long after plasma levels have diminished, ensuring sustained antifungal activity. This property is particularly beneficial for treating oral candidiasis, as it provides continuous suppression of fungal growth in the oral environment (Jović et al., 2019).

2.2.5 Advantages of Itraconazole over Other Antifungals

Itraconazole's broad spectrum of activity includes efficacy against strains of Candida that are resistant to other antifungal agents. This makes it an invaluable tool in the clinician's arsenal, particularly in cases where resistance is suspected or confirmed. Additionally, itraconazole's favorable safety profile and lower potential for drug interactions, especially compared to other systemic antifungals, further justify its preference in many clinical scenarios. The drug's pharmacokinetic properties allow for flexible dosing regimens, which can be adjusted based on the severity of the infection,