ENHANCEMENT OF ANTIFUNGAL EFFECT ON DENTURE BASE RESIN THROUGH MICROCAPSULES DRUG DELIVERY TECHNOLOGY USING TEA TREE OIL AND ITS CHARACTERISATION

Ariffin Z[1], Jaafar M[2], Johari Y[1], Abdul Hamid ZA[2], Mohamad S[1], and Mahadi NNSJ[1].
[1]School of Dental Science, Health Campus, Universiti Sains Malaysia
[2]School of Materials & Minerals Resource Engineering, Engineering Campus, Universiti Sains Malaysia

Correspondence:
Zaihan Ariffin,
School of Dental Science,
Health Campus,Universiti Sains Malaysia,
16150 Kubang Kerian, Kelantan, Malaysia
Email: zaihan@usm.my

Abstract
The introduction of organic materials into polymethyl methacrylate (PMMA) resin has been implemented to reinforce the antimicrobial properties in oral denture bases. A new drug delivery technique using polylactic acid (PLA) microcapsules infuses with organic ingredients such as tea tree oil (TTO). When incorporated into PMMA resin, these microcapsules will provide an effective antifungal effect on the denture surface. The objective is to investigate the antifungal interaction of PLA/TTO microcapsules in polymerised PMMA denture base resin against *Candida albicans.* An advanced encapsulation technique was performed to synthesise PLA microcapsules containing TTO. The TTO/PLA microcapsules were characterised using Ultraviolet-visible (UV-Vis), Fourier-transform Infrared (FTIR), Gas chromatography-mass spectrometry (GCMS) and scanning electron microscope (SEM). Three different concentrations of prepared microcapsules (TTO: PLA (% w/w)), 10%, 50% and 100% were incorporated into polymerised PMMA denture resin. The tested materials were subsequently evaluated for antifungal activity for several weeks at intervals of 7, 14 and 30 days using the well diffusion test. Further observation through SEM was included to assess the attachment of oral microorganisms to the surface of the modified denture base. From the spectroscopic analysis, the microcapsules were successfully synthesised using a new drug delivery encapsulation method. The antifungal activity of polymerised PMMA denture incorporated with TTO/PLA microcapsules demonstrated a significant effect ($p<0.05$) against *C. albicans,* where the inhibition area increased as the concentration increased. The study demonstrated that organic TTO can be encapsulated with PLA, potentially serving as a drug delivery vehicle for controlling drug release. Incorporating TTO/PLA microcapsules into PMMA denture base can significantly enhance the antifungal effect on the denture surface, offering potential usefulness for denture users in the future.

Keywords: Polylactic Acid Microcapsules, Tea Tree Oil, Denture Base Resin, Antifungal Activity, Zone of Inhibition

Introduction

The problem related to dentures is still considered high because of the high demand for patients wearing dentures and other oral prosthetics. However, denture hygiene issues for the aged population require high levels of care due to limited skills to handle regular oral hygiene regimens (1). Antibiotics or antifungal medication can be prescribed for patients with denture hygiene issues; however, concerns remain relating to healthcare costs and microbial antibiotic resistance (2). It would be preferable to choose a restorative material with antibacterial properties that prevent bacterial growth around the restoration (3). Denture base material fabricated by polymerised polymethyl methacrylate (PMMA) resin is commonly used due to its good physical properties, low cost, and ease of use (4). It remains a preferable material for removable complete and partial prostheses. Many researchers have studied the influence of denture cleansers and disinfectant solutions on oral microbial adhesion to denture base materials (5, 6). Recent approaches have been developed, such as

incorporating antimicrobial agents into denture materials. Cinnamon, Neem tree extract, and *Melaleuca alternifolia* oil (also known as TTO) have been established as potent antimicrobial agents that can be used safely (7). Tea tree essential oil was found to affect *C. albicans* significantly. According to Pachava et al. (8), adding tea tree oil to denture soft liners markedly showed the growth of *C. albicans* (8). Another study from Dalwai et al. (2) reported that tea tree oil had a substantial antifungal effect comparable to chlorhexidine gluconate 2%, indicating that *C. albicans* was suppressed up to the 14th day. Similarly, Al-Mashhadane discovered that immersing the heat-polymerised denture base resin in tea tree oil effectively decreased the growth of *C. albicans* cells (9).

However, the main components in TTO, such as terpien-4-ol, α-limonene, and carvacrol, are volatile and cannot be directly used in high concentration for actual situations of oral condition (10). According to a systematic review by An et al. (7), microencapsulation drug delivery technology would be a beneficial approach for medication delivery. Encapsulation technology protects the core (TTO) from external factors such as humidity, pH and temperature (11). One of the polymers commonly used for drug delivery systems is polylactic acid (PLA), which is widely used in the medical field to enhance patient compliance by reducing repeated administrations (12). Because of its biodegradable properties, non-toxicity and biocompatibility, polylactic acid has been successfully employed for drug encapsulation, tissue engineering, medical devices, and biomedical products. The capsules of PLA containing essential oil or the major isolated components promote the controlled release of the active compounds against targeted pathogens. However, because of its molecular structure, merely mixing polylactic acid microcapsules with active ingredients cannot fulfil the performance requirement. Unfortunately, there have been few publications on PLA microcapsules containing essential oils, which presents a tremendous opportunity to investigate the behaviour of this form of microcarrier (13).

In this work, the synthesis and characterisation of the polylactic acid-containing tea tree oil (PLA/TTO) microcapsules utilising the double emulsion and solvent evaporation technique, which is the most extensively used for manufacturing PLA microcapsules (10). The performance of the antifungal effect was further explored after being incorporated with PMMA denture resin to determine the effectiveness of TTO release from PLA microcapsules to inhibit the growth of *Candida* using a well diffusion test and was inspected under SEM as well.

Methodology

Materials

Materials used in this experiment were PLA pellets; Dichloromethane (DCM); Polyvinyl alcohol (PVA) powder; *Melaleuca alternifolia* (tea tree oil; TTO) from Thursday`s Plantation, Australia; polymerised PMMA acrylic resin type II, class 1; Sabouraud dextrose (SD) agar and broth powder from Oxoid; *Candida albicans* (*C. albicans*) from ATCC 10231.

Preparation and synthesis of microcapsules

The PLA/TTO microcapsules were synthesised using the emulsion and solvent evaporation (ESE) technique based on a study by our local researchers but with some modifications (12). PLA pellets were dissolved in DCM, followed by dispersion of 1 ml tea tree oil (TTO). This solution is subjected to vigorous homogenisation to yield the primary emulsion. The primary emulsion was immediately emulsified into PVA solution and stirred at 1,250 rpm for 5 minutes at room temperature to form a secondary emulsion. Then, the speed of the stirrer was decreased to 250 rpm overnight to allow solvent evaporation. The PLA/TTO mixture formed at the bottom of the flask was filtered, washed, and dried overnight at room temperature before further use. Figure 1 shows the flow in the synthesis of PLA/TTO microcapsules.

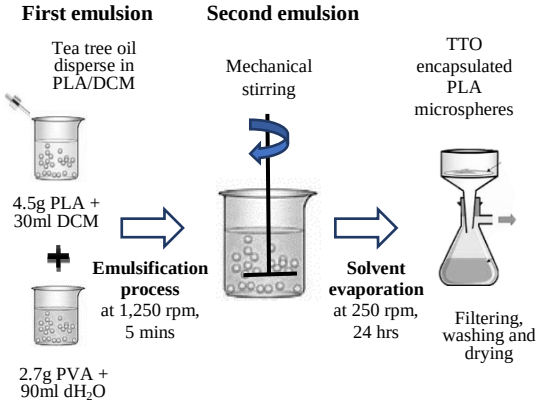

Figure 1: Schematic of double emulsion and solvent evaporation (ESE) technique to encapsulate tea tree oil (TTO) in polylactic acid (PLA) polymer.

Characterisation of PLA/TTO microcapsules

i) Ultraviolet-visible (UV-Vis) spectroscopy

The encapsulation content of PLA/TTO microcapsules was determined by an ultraviolet visible (UV-Vis) spectrophotometer (LAMBDA 365). The wavelength for TTO UV-Vis analysis is performed at 268nm.

ii) Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared (FTIR) spectroscopy with ATR analyses of PLA/TTO microcapsules and the raw TTO were carried out using the transmission method using a Perkin-Elmer spectrophotometer (Spectrum 400). Spectra were obtained at 4 cm-1 resolution between 400-4000 cm-1 of wavenumbers.

iii) Gas-chromatography Mass spectroscopy (GCMS)

The chemical components of PLA/TTO microcapsules were identified through gas chromatography-mass spectrometry (GCMS) machine. Elite 5 columns with hydrogen gas as a carrier were used for separation and analysis.

iv) Scanning Electron Microscope (SEM)

The microscopic morphology of PLA/TTO microcapsules was observed under a field emission scanning electron microscope (FESEM). The sample was coated with gold-palladium, and the microcapsules' morphology was observed at 5.00kV at x2000 magnification.

Incorporation of PLA/TTO microcapsules into PMMA denture resin

The acrylic resin material of PMMA cold-cured powder (Interdent; Slovenia) was mixed according to the manufacturer's recommended polymer: monomer ratio. Three different concentrations of prepared microcapsules (PLA: TTO (% w/w)), 10%, 50%, and 100% were incorporated into the PMMA powder and liquid monomer. The mixture was then packed into a constructed disc-shaped steel mould. The finished samples (n=15): PMMA-PLA/TTO discs were stored in a labelled container containing distilled water and kept at 37°C for four weeks. At intervals of the 7th day, 14th day and 30th day of soaking modified PMMA, the solutions (elutes) will be checked for leaching behaviour.

Well diffusion test

The antifungal test of modified PMMA (containing PLA/TTO microcapsules) will be evaluated using the well-diffusion method. The turbidity of *Candida* inoculum was prepared and compared with 0.5 McFarland standard. The inoculum was swabbed aseptically on the entire surface of the agar. Four wells were made on the plate (about 4 mm diameter), and an aliquot of 20 µL of molten SD agar and 50 µL of elute sample (leaching solution) from each test group were placed into each well accordingly. The plates were incubated at 37°C for 24 hours. All tests were done at five times replicate (14). The negative control used is the unmodified PMMA, while fluconazole is the positive control. All the procedures were repeated for another week to evaluate the results.

The antifungal activity was expressed by measuring the area of the zone of inhibition (mm^2) using the Digimizer software application. All the data were collected, and statistical analysis of two-way ANOVA was performed using SPSS software.

Attachment of C. albicans on the surface of modified PMMA denture

One randomly selected disc for each concentration (n=5) was used to observe the attachment of *C. albicans* on the surface of PMMA denture. The treated PMMA denture (cultured in *Candida*'s broth) discs were gold sputter-coated after mounting on the metallic stub. The specimens were inspected under the FESEM instrument at 5.00kV at x2000 magnification.

Results

Characterisation of PLA/TTO Microcapsules

i) Ultraviolet-visible (UV-Vis) spectroscopy

UV-Vis spectroscopy was used to determine the successful synthesis of the microcapsules. Table 1 shows the encapsulation results of TTO in PLA microspheres. The encapsulation process revealed that about 70.91% of TTO can be released from 1g of microcapsules (Table 1).

Table 1: The UV-Vis analysis of PLA/TTO microcapsules to determine the efficiency release of tea tree oil from the PLA microcapsules.

PLA/TTO microcapsules (mg)	Drug encapsulated (mg)	Drug loading (%)	Drug release (%)
1000	0.00936	3.74	70.91

ii) Fourier-transform Infrared (FTIR) spectroscopy

The infrared (IR) spectrum of TTO is displayed in Figure 2. According to the picture, the free TTO has vibrations at ca. 650-1000 cm-1 is caused by C-O-C aromatic rings. In addition, it possessed distinct characteristic vibrations such as C-O stretching (ca. 1100 cm-1), C=C stretching (ca. 1400 cm-1), C-H stretching (ca. 2900 cm-1), and broad O-H stretching (ca. 3400 cm-1).

The IR spectrum of PLA/TTO microcapsules revealed substantial bands at around ca. 3300 cm-1 (O-H stretching), ca. 2900 cm-1 (C-H stretching), ca. 1100 cm-1 (C-O stretching), and ca. 1000 cm-1 (C-O-C stretching vibration). The stretching vibration peak of the wall material PLA, C=O was observed at 1750 cm− 1. Meanwhile, the stretching vibration peak of C–O–C in the wall material PLA was 1093 cm− 1. Moreover, the stretching and bending vibration peaks of –CH3 in PLA were found around 1460 cm− 1.

iii) Gas Chromatography Mass Spectrometry (GCMS)

The characteristics of major components of TTO (Terpinen-4-ol, Limonene) were found at an RT (retention time) peak of 9.06 (Figure 3).

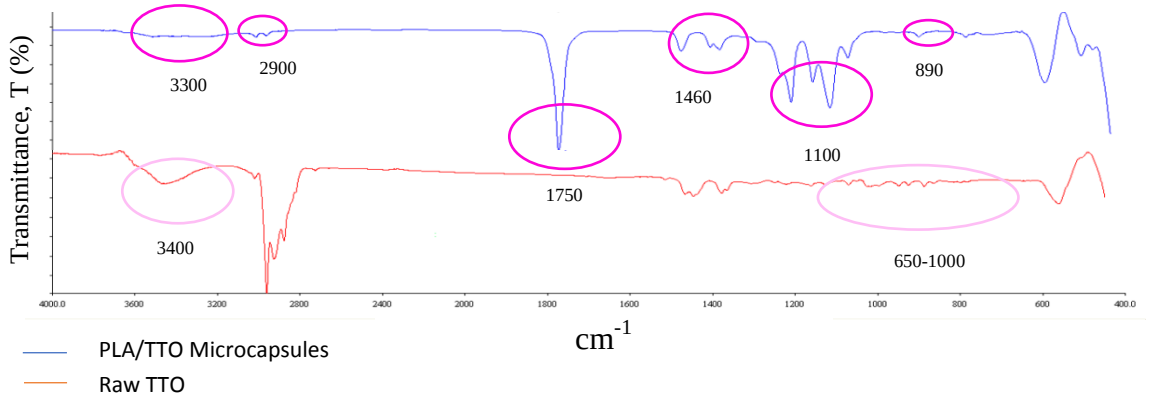

Figure 2: Infrared (IR) spectra of PLA/TTO microcapsules and tea tree oil (TTO) raw solution. The highlighted (circle) part demonstrated the crucial transmittance bands among the tested samples.

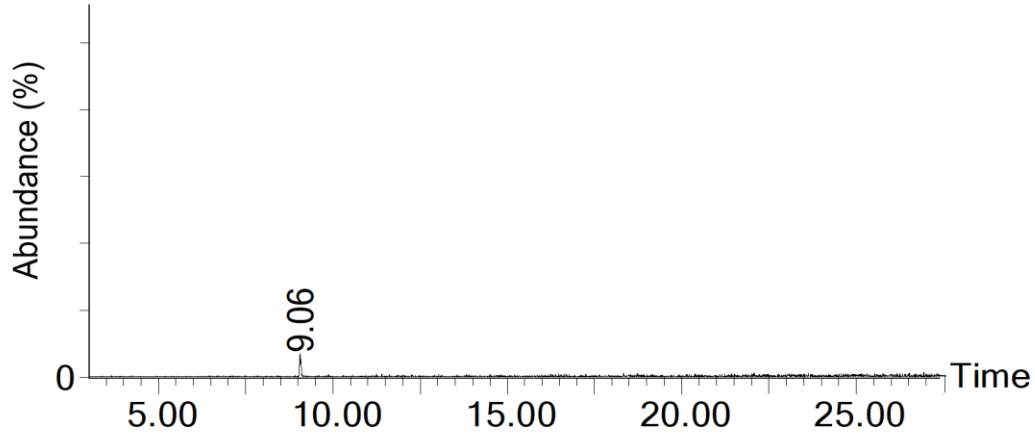

Figure 3: Gas chromatography mass spectrometry (GCMS) of PLA/TTO microcapsules, where the terpinene-4-ol and limonene components are found at the 9.06 peak retention time.

iv) Field Emission Scanning Electron Microscope (FESEM)

PLA/TTO microcapsules were uniformly (Figure 4) and normally distributed, with a diameter of 53.23 ± 18.13 µm.

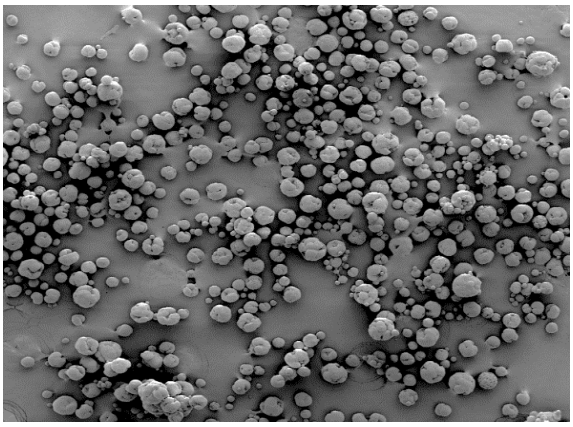

Figure 4: Scanning electron microscope of PLA/TTO microcapsules (freshly prepared) images at x2000 magnification with average size of 53.23 ± 18.13 µm.

Well-diffusion test

Antifungal activity was evaluated by measuring the area zone of inhibition (Table 2). The mean zone of inhibition was compared using a pairwise comparison of two-way ANOVA. Table 2 also showed that the zone of inhibition of 10% w/w concentration of TTO reached a maximum antifungal effect on day 14. Meanwhile, 50% w/w of TTO reached the maximum antifungal effect on day 7 and decreased on days 14 and 30. 100% w/w of TTO showed the highest antifungal effect at day 7. All concentrations give a good antifungal effect on day 14, possibly due to the controlled release of tea tree oil from the core of microspheres.

Table 2: The mean zone of inhibition of modified PMMA denture base resin incorporated with PLA/TTO microcapsules in different concentrations.

	Zone of inhibition (mm^2) (Mean ± SD)		
	10%	**50%**	**100%**
Day 7	21.17 ± 3.49	43.03 ± 3.89	88.28 ± 5.27
Day 14	24.22 ± 3.89	37.57 ± 2.63	58.24 ± 3.15
Day 30	13.50 ± 4.80	23.06 ± 5.12	58.75 ± 4.38

The post hoc test was applied through the Bonferroni procedure to obtain which pair is significantly different. Results from the post hoc tests indicate significant differences amongst the three tested materials on days 7, 14 and 30. From the results in Table 3, it was observed that there was a significant effect of antifungal activity among three different concentrations (10%, 50%, and 100% w/w) at $p<0.05$, [$F(3,54) = 175.889$, $p = 0.000$]. In comparison between the days, there was a significant effect of antifungal activity among three different time intervals (day 7, 14, 30) at $p<0.05$, [$F(2,54) = 1052.4$, $p = 0.000$].

Table 3: The statistical analysis of antifungal effect of tested materials where there is a significant difference among concentrations and days ($p<0.05$).

Source	df	Mean square	F	p-value
Concentration	3	11647.5	175.889	0.000
Day	2	1052.4	15.893	0.000
Error	54	66.221		

Attachment of C. albicans on the surface of modified PMMA

From the microscopic view in Figure 5, *C. albicans* at the lowest concentration (10% w/w) disc is highly attached to the denture surface. In comparison, at 50% and 100% w/w concentration, the attachment of *Candida* was less distributed and loosely attached to the denture surface.

Discussion

Different therapeutic strategies have been implemented to prevent and treat denture problems, ranging from using denture disinfectants and cleansers to administering oral and systemic antifungal medications (15). The incorporation of organic materials as antifungal agents has been reported in previous studies (10, 16, 17). Findings from Zhu et al. (10) revealed that antifungal agent such as TTO can be encapsulated with PLA microspheres by using ocetanyl succinic anhydride (OSA) modified chitosan to modify the polylactic acid where TTO is used as the core materials (10). A different study by Pérez-Limiñana et al. (16) noted that using natural polymers as an alternative way of shell-forming materials is now popular. Their work advocated the use of gelatin-based microcapsules as an emerging technology with the TTO microcapsules synthesised by complex coacervation process using biodegradable shell-forming polymers (type B gelatine and carboxylmethyl cellulose; CMC).

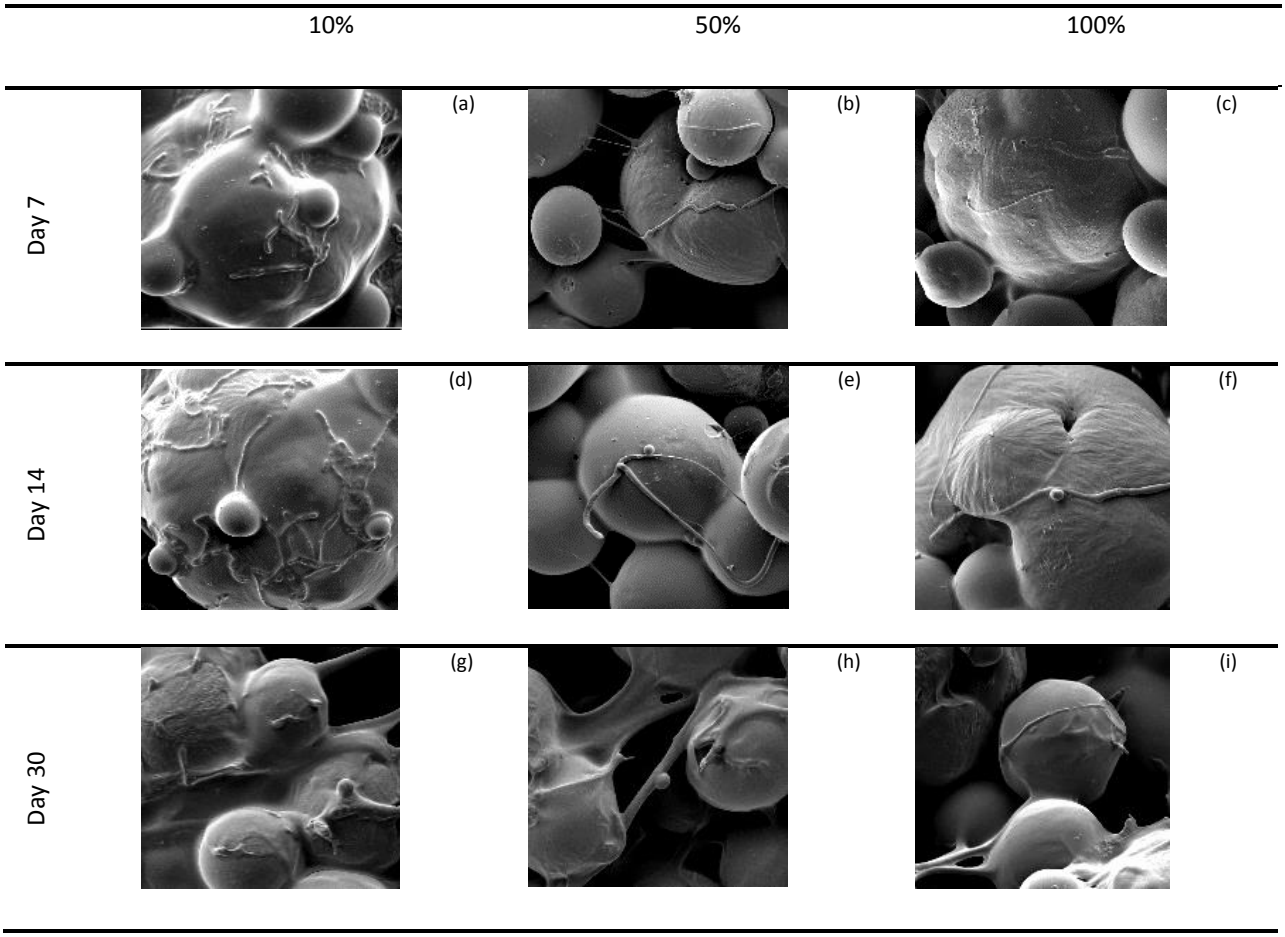

Figure 5: Scanning electron microscope of cell attachment of *C. albicans* on denture base at different concentrations of 10%, 50% and 100% at days 7 ((a), (b), (c)), days 14 ((d), (e), (f)), and days 30 ((g), (h), (i)) respectively. All images were observed under 5000x magnification.

Both papers have proved that the antimicrobial activity of encapsulated TTO gives a controlled release via the rupture of the microcapsules (10, 16). A recent research review by Hosseini and Jafari (18) established that the performance of encapsulated bioactive compounds over their free form and the significance of encapsulation techniques are listed where the type of encapsulants used are highly influence the release mechanism of the bioactive compound (18). Another study that used three different essential oils: peppermint, tea tree and thyme with oregano that were encapsulated in cellulose nanocrystal chitosan reinforced films, is reviewed in this paper. The encapsulation technique was found to increase the stability of the EOs while also having antifungal efficacy and a gradual release of active compounds (17, 18). However, this study only used polylactic acid polymer to stabilise the emulsion phase during the encapsulation, and polyvinyl alcohol was used as an emulsifying agent (12).

Emulsion and solvent evaporation (ESE) is a well-known method used for manufacturing drug-encapsulated microcapsules. Microencapsulation using ESE process is chosen to manufacture PLA microsphere because it provides a drug carrier that may be released in a regulated manner within the therapeutic range for an extended period. Furthermore, ESE-produced microcapsules had a regular surface with no ruptures. This is because using an emulsifier with an ideal PVA concentration (3% w/v) stabilised the emulsion, reducing the surface and interfacial tension and preventing coalescence and agglomeration (12). ESE-prepared microcapsules have particle sizes ranging from 5 µm

to 100 µm, making them ideal for drug delivery and limiting the size distribution (19).

The successful synthesis of microcapsules was determined by characterisation and morphologic analysis. Firstly, UV-Vis was done to determine the drug encapsulation, drug loading and the release profile of the drugs. The UV-Vis detection showed that 1g of microcapsules can release about 70.91% of tea tree oil from the PLA microspheres within a week, which can achieve the desired long-term release (10). Hence, the efficiency of the tea tree oil being encapsulated can be guaranteed. The determination of the entrapment efficiency of microspheres also could be improved by using FTIR (20). The graph shows that the IR spectrum of PLA/TTO resembles a few important bands as the raw tea tree oil. Our study observed several vibration peaks shifted from those of the standard TO due to the encapsulation process.

The differences among the spectra of raw TTO and PLA/TTO microcapsules had more transmittance (%) at ca. 1700 cm^{-1} (C=O stretching), 1460 cm^{-1} (C=C stretching), and ca. 1100 cm^{-1} (C-O stretching) than the raw TTO. The discrepancies between the spectra of raw TTO and TTO/PLA microcapsules verified that TTO was effectively coated inside the shell of PLA (21). Likewise, the FTIR results from a previous study by Beşen (20) on the application of TTO in textiles showed that TTO could be encapsulated with polymeric wall materials (20).

Analysis of gas chromatography (GCMS) was done to strengthen the evidence of the successful encapsulation process. GCMS helps detect the main component more precisely with the compound name present in the samples. The results found that the RT peak resembles the previous studies from Tranchida et al. (22) and Gafner and Dowell (23), which also showed that the major component of TTO, terpinene-4-ol and limonene, can be found at an RT peak from 9.06. Apart from that, the efficacy of a microencapsulation procedure may be directly evaluated by analysing the shape of the microcapsules since the structures with fractures or flaws might limit the carrying and protection of the microencapsulated active components (24). For the morphology analysis, the distribution of the microcapsules was observed using a scanning electron microscope (FESEM). The microcapsules were found to be regular in shape. It was discovered that the average size of microcapsules ranged from 40-60 µm as previous studies of microencapsulation from Du et al. (25) and Dong et al. (26), which this size gives 70% to 90% efficiencies for drug-loaded microcapsules (25-27). Another study from Kong et al. (28) showed that the TTO microcapsules with 40% drug-loaded surpassed the encapsulation efficiency (28). Thus, they conclude that this size range has the potential for controlling drug release.

The antifungal activity of the leaching solutions was evaluated by comparing the mean zone of inhibition, and it was observed that there was a significant difference among all the concentrations tested. The results show that the release of tea tree oil from the microcapsules began on day 7. At day 14, the antifungal effect of the tested sample showed the highest release of tea tree oil with a greater zone of inhibition. At this moment, it can be assumed that the tea tree oil reached a maximum release. However, the mean zone of inhibition (between day 14 and day 30) showed a decreasing pattern in the antifungal effect. This can be attributed to the decrease in the release of TTO from the microcapsules; hence, there is a reduction of tea tree oil release, shown by the smaller zone of inhibition between the days.

The slow diffusion release is most likely caused by the drug diffusing from the polymer's core via water cluster formation around the drug particles, regulated by concentration-dependent diffusion (12). Tea tree oil's inhibitory action was decreased after microencapsulation when compared to free tea tree oil, which may be due to the microcapsules' wall. The barrier created may regulate the release of the essential oil and its bioactive compounds (24). Among the three tested samples, the highest concentration (100% w/w) gives a higher zone of inhibition, which may indicate that the samples are concentration-dependent. The mean inhibition zones corresponded to the elution of the antifungal agents.

It was demonstrated that the modified PMMA can interact with the *Candida* cells according to different concentrations. Different doses of TTO release cause the disruption of the cell's attachment (27). The effect of TTO was dose-dependent, at which the highest concentration (100% w/w) demonstrated the most prominent reduction in adherent *Candida* on the denture surface. Hence, the distribution of the fungal cells is less. Concentration of 50% w/w also reduced the adherence of the fungal cell as

compared to 10% w/w. These alterations may be due to bioactive compounds in the TTO affecting the fungal cell wall to adhere to the denture (10). Controlled drug delivery systems are currently being investigated to overcome the limitations in conventional dosage forms and improve the potential of the respective drug. However, this study only verifies the experimental performance of the incorporated antifungal agents in dentures. It is suggested that further tests need to be implemented to check the changes in chemical, drug interaction and physical properties of the denture base before clinical use.

Conclusion

The experimental study establishes that the antifungal agent, tea tree oil, can be encapsulated successfully by the simple coacervation of the ESE method using PLA polymer as the shell, potentially serving as a sustainable drug delivery vehicle for controlled release. Incorporating PLA/TTO microcapsules into PMMA denture base resin gives significant antifungal effects on the denture, offering a potential alternative for denture users in the future. Further studies of the effects on denture performance strength are required, including *in vivo* studies.

Acknowledgement

The authors would like to thank the Ministry of Higher Education (MOHE) for the financial support for this work under the Funded Research Grant Scheme (FRGS) (FRGS/1/2021/SKK0/USM/02/31).

Competing interest

The authors declare that there are no competing interests. The authors are responsible for the content and writing of the paper.

Reference

1. Ryalat S, Darwish R, Amin. New form of administering chlorhexidine for treatment of denture-induced stomatitis. Ther Clin Risk Manage. 2011; 14:219-225.
2. Dalwai S, Rodrigues SJ, Baliga S, Shenoy VK, Shetty TB, Pai UY, *et al.* Comparative evaluation of antifungal action of Tea Tree Oil, chlorhexidine gluconate and fluconazole on heat polymerised acrylic denture base resin – an in vitro study. Gerodontology. 2014; 33(3):402-409.
3. Zhang S, Chen J, Yin X, Wang X, Qiu B, Zhu L, *et al*. Microencapsulation of Tea Tree Oil by spray-drying with methyl cellulose as the emulsifier and wall material together with chitosan/alginate. J. Appl. Polym. Sci. 2016; 134(13):1-10.
4. Meng TR, and Latta MA. Physical properties of four acrylic denture base Resisns. J. Contemp. Dent. Pract. 2005; 6(4):93-100.
5. Jovanović M, Obradović R, Pejčić A, Stanišić D, Stošić N, and Popović Ž. The role of *candida albicans* on the development of stomatitis in patients wearing dentures. Sanamed. 2018; 13(2):175-181.
6. Chincholikar S, Sridevi J, Kalavathy N, Singh S, Kapoor A, and Saumya S. Comparative evaluation of two antifungal agents incorporated in auto polymerising denture base resin, heat polymerising denture base resin and permanent silicone soft liner-an in vitro study. J. Clin. Diagn. Res. 2019; 13:49-54.
7. An S, Judge R, Wong R, Arzmi M, Palamara J, and Dashper S. Incorporation of the microencapsulated antimicrobial agent phytoncide into Denture Base Resin. Aust. Dent. J. 2018; 63(3):302-311.
8. Pachava KR. In vitro antifungal evaluation of denture soft liner incorporated with tea tree oil: A new therapeutic approach towards denture stomatitis. J. Clin. Diagn. Res. 2015; 9(6):62-64
9. Al-Mashhadane F. Tea tree oil: A new antifungal agents against *candida albicans* cells on heat–cured acrylic resin denture base material. An in vitro study. Al-Rafidain Dent J. 2007; 7(3):54-57.
10. Zhu Z, Hu J, and Zhong Z. Preparation and characterisation of long-term antibacterial and pH-responsive Polylactic acid/Octenyl succinic anhydride-chitosan @ tea tree oil microcapsules. Int. J. Biol. Macromol. 2022; 220:1318-1328.
11. Martins IM, Barreiro MF, Coelho M, and Rodrigues AE. Microencapsulation of essential oils with biodegradable polymeric carriers for cosmetic applications. Chem. Eng. J. 2014; 245:191-200.
12. Sabee MMSM, Awang MS, Bustami Y, and Hamid ZA. Antimicrobial activity evaluation for gentamicin loaded PLA microspheres. Mater Today: Proc. 2019; 16:2060-2066.
13. Campini PA, Oliveira ÉR, Camani PH, Silva CG, Yudice ED, Oliveira SA, *et al*. Assessing the efficiency of essential oil and active compounds/poly (lactic acid) microcapsules

against common foodborne pathogens. Int. J. Biol. Macromol. 2021; 186:702-713.
14. Songsang N, Anunmana C, Pudla M, and Eiampongpaiboon T. Effects of litsea cubeba essential oil incorporated into denture soft lining materials. Polymers. 2022; 14(16):1-13.
15. Casaroto AR, and Lara VS. Phytomedicines for Candida-associated denture stomatitis. Fitoterapia. 2010; 81(5):323-328.
16. Pérez-Limiñana MÁ, Payá-Nohales FJ, Arán-Ais F, and Orgilés-Barceló C. Effect of the shell-forming polymer ratio on the encapsulation of Tea Tree Oil by complex coacervation as a natural biocide. J Microencapsul. 2013; 31(2):176-183.
17. Hossain F, Follett P, Salmieri S, Vu KD, Fraschini C, and Lacroix M. Antifungal activities of combined treatments of irradiation and essential oils (EOS) encapsulated chitosan nanocomposite films in vitro and in situ conditions. Int. J. Food Microbiol. 2019; 295:33-40.
18. Hosseini H, and Jafari SM. Introducing Nano/microencapsulated bioactive ingredients for extending the shelf-life of food products. Adv. Colloid Interface Sci. 2020; 282:1-28.
19. Lengyel M, Kállai-Szabó N, Antal V, Laki AJ, and Antal I. Microparticles, microspheres, and microcapsules for Advanced Drug Delivery. Sci Pharm. 2019; 87(3):1-31.
20. Beşen, BS. Production of Disposable Antibacterial Textiles via Application of Tea Tree Oil Encapsulated into Different Wall Materials. Fibers Polym. 2019; 20(12):2587-2593.
21. Zhu KJ, Li Y, Jiang HL, Yasuda H, Ichimaru A, *et al*. Preparation, characterisation and in vitro release properties of ibuprofen-loaded microspheres based on polylactide, poly(epsilon-caprolactone) and their copolymers. Journal of Microencapsulation. 2005; 22(1):25-36.
22. Tranchida PQ, Shellie RA, Purcaro G, Conte LS, Dugo P, Dugo G, *et al.* Analysis of fresh and aged tea tree essential oils by using GCxGC-QMS. J. Chromatogr. Sci. 2010; 48(4):262–266.
23. Gafner, S, Dowell A. Tea tree oil laboratory guidance document. Botanical Adulterants Prevention Bulletin; ABC-AHP-NCNPR Botanical Adulterants Prevention Program: Austin, TX, USA. 2018:1-13.
24. de Melo AM, Barbi RC, Almeida FL, de Souza WF, Cavalcante AM, de Souza HJ, *et al*. Effect of microencapsulation on chemical composition and antimicrobial, antioxidant and cytotoxic properties of lemongrass (cymbopogon flexuosus) essential oil. Food Technol. Biotechnol. 2022; 60(3):386-395.
25. Du W, Li E, and Lin R. Preparation and Characterization of Nano-$CaCO_3$/Ceresine Wax Composite Shell Microcapsules Containing E-44 Epoxy Resin for Self-Healing of Cement-Based Materials. Nanomaterials. 2022; 12(2):1-18.
26. Dong ZJ, Touré A, Jia CS, Zhang XM, and Xu SY. Effect of processing parameters on the formation of spherical multinuclear microcapsules encapsulating peppermint oil by coacervation. J Microencapsul. 2007; 24(7):634-646.
27. Li M, Wang S, Li R, Wang Y, Fan X, Gong W, et *al.* The Mechanical and Antibacterial Properties of Boron Nitride/Silver Nanocomposite Enhanced Polymethyl Methacrylate Resin for Application in Oral Denture Bases. Biomimetics. 2022; 7(3):1-12.
28. Kong P, Abe JP, Masuo S, and Enomae T. Preparation and characterisation of tea tree oil-β-cyclodextrin microcapsules with super-high encapsulation efficiency. J. Bioresour. Bioprod. 2023; 8(3):224-234.