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Title

Incorporation of the microencapsulated antimicrobial agent phytoncide into denture base resin.

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TITLE

Incorporation of the microencapsulated antimicrobial agent phytoncide into denture base resin

ABSTRACT

Background: This study aimed to fabricate a denture base resin (DBR) containing phytoncide microcapsules (PTMCs) and determine the mechanical properties of the resin and antifungal activity.

Methods: Fifty-four heat cured rectangular DBR specimens (64 x 10 x 3.3 ± 0.2 mm) containing nine concentrations of PTMC between 0 - 5% (wt/wt) were fabricated and subjected to a three-point bending test. A phytoncide release bioassay was developed using DBR containing 0% and 2.5% PTMCs (wt/wt) in a 24 well-plate assay with incubation of *Porphyromonas gingivalis* at 37°C for 74 h. The antifungal activity of PTMCs against *Candida albicans*, in a pH 5.5 acidic environment was determined in a plate assay.

Results: Flexural strength decreased with increasing PTMC concentration from 97.58 ± 4.79 MPa for the DBR alone to 53.66 ± 2.46 MPa for DBR containing 5.0% PTMC. No release of phytoncide from the PTMCs in the DBR was detected at pH 7.4. The PTMCs had a minimal inhibitory concentration of 2.6 % (wt/vol) against *C. albicans* at pH 5.5.

Conclusions: PTMCs can be added to DBR 2.5% (wt/wt) without adversely affecting flexural strength. PTMCs released the antimicrobial agent at pH 5.5 at concentrations sufficient to inhibit the growth of the *C. albicans*.

Keywords: antimicrobial activity, denture base resin, mechanical property, microencapsulation, phytoncide.

Abbreviations and acronyms: BHI = Brain heart infusion; DBR = denture base resin; ISO = International Organization for Standardization; MIC = Minimum inhibitory concentration; MF = melamine formaldehyde; OD = optical density; PG = *P. gingivalis*; PMMA = polymethyl-methacrylate; PTMCs = phytoncide microcapsules; SEM = scanning electron microscopy; YH = yeast peptone glucose broth + hydrochloric acid; YPG = yeast peptone glucose.

1. INTRODUCTION

There is a decreasing proportion of edentulous people or complete denture wearers in the population, due to increased dental awareness and improvements in treatment options, such as implants (1). However, it is recognised globally that both developed and developing countries are facing an ageing population. The demand for complete and partial dentures is still numerically high because of the rise in life expectancy, and it is not likely to diminish over the next decade (1, 2).

Furthermore, there are denture hygiene issues for the aged population, requiring high levels of care in institutionalised environments. These patients may have limited motor skills due to conditions such as Parkinson's disease, or have the reduced mental capacity for regular oral hygiene regimens (3). Antifungal/antibiotic agents or medication can be prescribed for these patients to treat denture hygiene issues such as denture stomatitis, however, concerns remain, relating to health care costs and microbial resistance to antibiotics (4).

Up to 65% of dental prostheses wearers suffer from chronic inflammation known as denture stomatitis. Denture stomatitis is induced by infections or trauma, angular cheilitis, and denture irritation hyperplasia, amongst other conditions (3, 5-9). Among them, *Candida*-associated denture stomatitis is a very common inflammatory process (5-7, 9, 10). Oral fungi such as *Candida albicans* can colonize the surface of a denture base resin (DBR) and

establish itself as a component of polymicrobial biofilms that can result in inflammation of the oral mucous membrane (3, 11-13).

Polymethyl-methacrylate (PMMA) is the most commonly utilized DBR because it possesses adequate physical, mechanical, and aesthetic properties (14). The antimicrobial activity of PMMA resin has been an area of interest for dental materials researchers and different approaches have been used to incorporate antimicrobial activity, focusing on coating of the acrylic surface (15), copolymerization with antimicrobial monomers such as methacrylic acid (11), quaternary ammonium compounds (7, 16), and glazing techniques (17). Moreover, some researches explored incorporating chlorhexidine diacetate, nystatin and amphotericin (18), miconazole and chlorhexidine digluconate (19) as antifungal agents, as well as the antibacterial agents Zeomic, Bactekiller, and Novaron (20). Similarly, research into the inclusion of silver ion nanoparticles has also been undertaken, aiming to improve the antimicrobial activity of the DBR (3, 8, 21, 22). Another recent approach is to incorporate nano-additives, such as nanoparticles, nanofibers, or nanotubes (23).

Recently, incorporation of natural organic substances such as green tea flavours against *Streptococcus mutans* (24), *Melaleuca alternifolia* (tea tree) essential oil on *Staphylococcus aureus* (25) and in tissue conditioner (13), phytomedicines for *Candida*-associated denture stomatitis (26) and various plant species comprising of peppermint, sage, thyme, oak, marigold, garlic, and aloe for oral cavity inflammation (27) in dental treatments have been studied with varying success.

Microencapsulation technology has been adopted extensively in many industries to provide a controlled delivery of the core material at the desired time and site, by avoiding damage or evaporation of liquid/solid substances during long-term use and under their particular environmental conditions (28-31). However, to date, there have been no attempts to incorporate phytoncide microcapsules (PTMCs) into DBR, despite the potential benefit of improved antimicrobial activity.

The aims of this study therefore were to fabricate a DBR containing PTMC at various concentrations and determine its influence on the mechanical properties of DBR. Then to use a sensitive bioassay to determine the release of the phytoncide from the PTMC-

containing DBR and finally to determine the antifungal activity of the microencapsulated phytoncide against *C. albicans*.

2. MATERIALS AND METHODS

Phytoncide microcapsules (PTMCs, ECOMIST, Go-yang, South Korea) were purchased and used in this study. The composition of the microcapsules is as below:

- Average microcapsule size: 3-20 μm,
- Colour: Yellowish beige colour,
- Microcapsule shell/wall material: Melamine formaldehyde (MF) resin (20-25 wt%),
- Microcapsule core material: Phytoncide (*Chamaecyparis obtusa*) (60-73 wt%),
- Other: Water.

Polymerised PMMA (Heraeus Paladent® 20, PalaXpress®, Heraeus Kulzer Gmb-H, Hanau, Germany) was provided by Durodent Dental Supplies, Melbourne, Australia/Heraeus Kulzer Australia Pty Ltd. All fabrication of the flexural strength specimen was conducted under the International Organization for Standardization (ISO 20795-1:2013) guideline (32).

Porphyromonas gingivalis strain W50 (Melbourne Dental School) was used for the phytoncide release bioassay. *C. albicans* ALC3 (Melbourne Dental School) was used for the antifungal test.

2.1 Preliminary experiment

Five DBR blocks (65 x 40 x 5 ± 1 mm) containing 0% (Control), 0.5%, 1%, 10% and 30% (wt/wt) of PTMCs were fabricated according to the manufacturer's instruction. This test was conducted to determine if the material fulfilled ISO requirements such as surface characteristics, shape capability, colour, translucency and freedom from porosity of DBRs + PTMCs and to determine feasibility of using PTMCs in the heat cure processing method under the visual inspection.

2.2 Fabrication of heat cured DBR specimens

A total of 54 heat cured specimens were fabricated according to the manufacturer's instruction. Initially, two plates (65 x 40 x 5 ± 1 mm) from each group; 0% (Control), 0.5%,

1.0%, 1.5%, 2.0%, 2.5%, 3.0%, 4.0% and 5.0% (wt/wt) that complied to ISO were prepared and each plate was sectioned lengthwise into three slightly oversized strips. The strips were ground and smoothed with a metallographic grinding machine (TegraPol-25, Struers, Copenhagen, Denmark). All strips were measured by a digital calliper (Mitutoyo, Tokyo, Japan) to validate reliable consistent sizes (65 x 10 x 3.3 ± 0.2 mm) for the flexural strength test.

2.3 Flexural strength test

The ultimate flexural strength test was conducted using a three-point bending in a universal testing machine (INSTRON-5544, Instron Pty Ltd., Bayswater, Melbourne, Australia). The specimens were stored in purified water at 37 ± 1°C for 50 ± 2 h prior to the flexural test in accordance with ISO. Specimen strips were symmetrically placed on the supports of the flexural test rig. The distance between centres of the supports was 50 ± 0.1 mm, and the loading plunger was midway between the supports to within 0.1 mm. The loading force was then applied to the specimens with a recommended crosshead speed of 5 ± 1 mm/min until the specimen fractured (32). The equation for flexural strength, σ in MPa was applied as outlined below.

$$\sigma = \frac{3Fl}{2bh^2}$$

Where *F* was the maximum load in Newtons exerted on the specimen to failure and l was the distance in mm between the supports, b was the width of specimen and h was the height/thickness of the specimen prior to water storage. The statistical analysis of the ultimate flexural strength test was conducted by Minitab® 17 (Sydney, Australia). The flexural strength data was analysed by One-way ANOVA, $F_{8, 45}$ =165.99; P<0.001 with significance levels (α = 0.05). Each of the nine test groups has six strips to comply with ISO.

2.4 Scanning electronic microscopy

Fractured surfaces of the flexural strength tested specimens were imaged by scanning electron microscopy (SEM) using a FEI Quanta FEG ESEM (FEI, Brno, Czech Republic). The observation groups were selected in four different concentrations, 0%, 2.5%, 3% and 5%. The 2.5% PTMC concentration was chosen based on the result of the flexure strength test.

Three percent (3%) was also included in the SEM study as it had a similar strength to that of the 2.5% PTMC containing DBR. Zero percent (0%) and 5% were included to evaluate both morphological structures of two extreme values for comparison.

2.5 Phytoncide release bioassay

A total of 30 disc-shaped specimens (Ø 10 mm x 3 ± 1 mm) were fabricated for the release of phytoncide from the microcapsules in DBR as a bio-test. The control (n = 15) contained no PTMCs and the experimental group (n = 15) contained 2.5% PTMCs as the result of the flexural strength test (the maximum possible concentration to comply with ISO). Five specimens from each group that complied with ISO were selected at random and sterilized by the ethylene oxide gas (Steritech, Melbourne, Australia).

P. gingivalis strain W50 was selected for use in the bioassay because of its high sensitivity to phytoncide, with a minimum inhibitory concentration (MIC) of 0.008% (33). *P. gingivalis* was grown in brain heart infusion (BHI) broth to an optical density (OD) at a wavelength of 650 nm of 0.6 which equates to a cell density of 1 x 10^9 cells/mL. An inoculum of 25 µL was added to a final volume of 1 mL in each of the 24 wells per plate to give a cell density of 2.5 x 10^7 cells per well. Six groups were developed; 1) uninoculated BHI as a negative control, 2) BHI + PG (*P. gingivalis*) as a positive control, 3) BHI + PG + 0% PTMCs natural surface (NS: simulating denture fitting surface) resin disc, 4) BHI + PG + 2.5% PTMCs NS resin disc, 5) BHI + PG + 2.5% PTMCs lapping surface (LS: simulating denture polished surface) resin disc, and 6) BHI + PG + PTMCs (no resin disc). Sterile forceps were used to place the resin discs containing PTMCs into each well in two 24 well-plates, before filling with the culture medium. After being sealed with an adhesive seal, the plates were then incubated at 37°C in an anaerobic chamber for 74 h after which time bacterial growth was determined spectrophotometrically. The test was conducted with six biological replicates.

2.6 Robustness of microcapsules

The robustness (durability) test of the microcapsules was conducted to determine if the PTMCs were dissolving or becoming deformed, and whether the phytoncide would escape through the capsules in an acidic environment at pH 5.5. Four different conditions; crushed capsules as a control, ethanol 100% (vol%), distilled water, and pH 5.5 citric acid were

developed. 100% ethanol, distilled water and pH 5.5 citric acid solution (0.0032 mM) were adjusted at 2.5% PTMCs concentration by mixing 0.5 g of PTMC in 20 mL of ethanol (wt/vol), distilled water and the pH 5.5 citric acid solution and placed in petri dishes. They were mixed on a vibrator and stored in an incubator (S.E.M. Pty Ltd., Adelaide, Australia) at 37°C for 18.5 h. The observations of the microcapsule morphology were conducted at 1 h and 18.5 h, using an optical microscope (Leica DFC 320, Leica Microsystems Ltd., Heerbrugg, Germany).

2.7 Antifungal test with *C. albicans*

Based on the outcomes of the microcapsule robustness test, the antifungal test was conducted. The aim of the test was to determine if the phytoncide essential oils were released through the microcapsule shell material and whether there were antifungal effects against *C. albicans*. The experiment was performed in an acidic environment of pH 5.5 in a 96 well plate at 37°C. The turbidity method was utilised using a spectrophotometer (VICTOR 3, PerkinElmer, Massachusetts, USA).

2.7.1 *Preparation of materials and solutions*

To conduct this test, PTMCs were sterilised with UV light overnight. YH solution (Yeast peptone glucose broth + hydrochloric acid) was prepared by adding 0.1 M hydrochloric acid to yeast peptone glucose (YPG) growth medium to lower the pH to 5.5 (34). For PMYH solution (10% concentration of phytoncide microcapsules in YH solution), 2 g of UV sterilized PTMCs was added to 20 mL of filtered YH solution to achieve a 10% concentration of PTMCs. *C. albicans* ALC3 was grown in YPG broth for 24 h at 37°C. The cells were harvested by centrifugation (12,000 g, 4 min), washed and resuspended in YH solution. The OD was adjusted to 0.5 at 600 nm, using a spectrophotometer (NanoDrop 2000c, Thermo, K.I. Scientific, Langeneselbold, Germany), which equated to a cell density of 2.3×10^6 cells/mL.

2.7.2 *Distribution of solutions*

The solutions were distributed into a 96 well-plate under sterile conditions. Two groups were developed using a modified serial dilution method of *C. albicans* to PMYH solution (Tab. 1). The plate was incubated for 24 h at 37°C and the OD at 620 nm determined

spectrophotometrically. Comparison of the OD of the paired groups (0% A, F; 5.0% B, G; 2.5% C, H; 1.0% D, I and 0.5% E, J) was used to determine the growth of *C. albicans*. The test was conducted in 6 replicates.

3. RESULTS

3.1 Preliminary experiment

As the concentration increased, clear speckling of yellowish PTMCs was shown, and the colour of the resin changed to an orange colour reflecting the original yellowish PTMCs colour at 10% in DBR infusion of the polymer resin (Fig. 1). At 30% concentration, with a scattered aggregation speckling of yellowish PTMCs, the colour of the resin changed from a pink to a dark orange colour (Tab. 2).

3.2 Heat cured DBR specimens

Each sample group (n=9) was polymerised during the heat cure processing and all specimen strips passed the ISO requirement. However, samples began to show a slight speckling of yellowish spots on the surface from the 1% concentration of PTMC. This became more noticeable as the concentration of PTMC increased and resulted in minor scattered speckling at 5%.

3.3 Flexural strength test

All values of the flexural strength test gradually decreased from 0% to 5% concentration as more PTMCs were added to the DBR powder (Fig. 2). The values steadily dropped from 97.58 MPa (0%, control) to 53.66 MPa (5%), but the decrease was nonlinear, as the change in the rate of flexural strength (MPa) became smaller with each percentage change.

3.4 Scanning electronic microscopy

SEM imaging of the fractured surface morphology indicated that the PTMCs were uniformly dispersed in the DBR. As the concentration of PTMCs rose from 0% to 5%, a greater number of PTMCs were observed (Fig. 3). The more phytoncide capsules added into the resin, the

more agglomeration occurred, particularly at the concentration of 5% (Fig. 4, D). The general size of PTMC spheres was measured between 5 and 50 µm.

After the polymerisation processing, the structure of the PTMCs mostly appeared to maintain the spherical shape, although some were partially deformed, fractured, or displayed a spherical void. Although the capsules were embedded in the resin, there was a thin barrier/gap between the resin and capsule shell material (Fig. 5, B and C: red arrows).

3.5 Phytoncide release

No release of phytoncide from the PTMCs incorporated into the DBR was detected in the bioassay using *P. gingivalis* in a 24 well-plate assay at pH 7.4. Each well was marked with a *P. gingivalis* growth score out of 3, with the positive control (BHI + PG) at 3 and the negative control (BHI alone) at 0. Plate 1 showed no change in *P. gingivalis* growth between the three groups (BHI + PG, BHI + PG + 0% PTMCs NS resin disc, and BHI + PG + 2.5% PTMCs NS resin disc) with the score of each well all at "3". Similar results were also present in plate 2, showing no turbidity change through the three different groups (BHI + PG, BHI + PG + 2.5% PTMCs LS resin disc, and BHI + PG + only PTMCs) with "3" as a result for each well.

3.6 Robustness of microcapsules

Crushing the capsules as a control showed complete deformation of the PTMCs (Fig. 6A). Most of the capsules expanded and some had dented areas on the shell surfaces in the 100% ethanol treated group (Fig. 6B). The distilled water treated group C showed similar patterns to the 100% ethanol treated group (Fig. 6C). The microcapsules in pH 5.5 citric acid, appeared expanded and exhibited multiple deformation areas in different parts of the shell. Some parts of the capsule also appeared to have dissolved (Fig. 6D).

3.7 *C. albicans* antifungal test

A 96 well-plate test was conducted to determine the effect of PTMCs on *C. albicans* growth at pH 5.5. *C. albicans* growth decreased in a dose dependent manner as the PTMC concentration increased, indicating that the phytoncide plant essential oil was released from PTMCs at pH 5.5 (Fig. 7). An MIC of 2.6% (wt/vol) PTMC against *C. albicans* was

determined by fitting a straight line through the three concentrations that showed incomplete inhibition of growth.

4. DISCUSSION

Development of antimicrobial activities for DBR has been studied by many researchers, however, limited research has investigated the incorporation of chemical or artificial antimicrobial solutions. Preliminary results in this study suggested that a PTMC concentration of less than 10% in the polymer could be a feasible mixture of PTMCs and DBR powder for fabricating heat cured samples. The concentration of PTMC tested was further restricted to 5% or below due to concerns about the effects on the flexural strength of the material.

In the heat cured samples, a speckling of white/creamy spots on the surface of DBR + PTMCs appeared. It was assumed that the half broken or deformed microcapsules were presenting micro indentations with a yellowish phytoncide colour throughout 0.5% to 5%. In terms of the clinical acceptability, as shown Fig. 1, the yellowish PTMCs were hardly shown up to 1% PTMC concentration in the DBR from the preliminary test. Therefore, if they complied with the mechanical test requirement, they were regarded as clinically acceptable.

The ultimate flexural strength was determined, because the flexural strength is a critical factor in prosthodontics, as dental prostheses are required to withstand repeated loading during daily use. Midline fracture of complete dentures is a common maintenance issue for removable prostheses (3, 35). From the results, the ultimate flexural strength values of DBR tested in this study decreased as the concentration of PTMCs was increased from 0% to 5.0%. It was concluded that 2.5% inclusion of PTMCs in the polymer was the most that could safely be incorporated, without affecting flexural strength as the mean value of the ultimate flexural strength was 65.72 MPa. This still enabled the material to conform to the ISO requirement of 65 MPa. The incorporation of PTMCs may modify the properties of the denture base materials because the incorporation of antimicrobial agents into methacrylates can impair the mechanical properties. Supporting this finding, Addy and Handley (36) investigated the physical properties of polymerized and plasticized acrylic resin

incorporating chlorhexidine acetate and found that the inclusion of the antimicrobial agent altered the physical property of the polymerised resin.

The PTMCs dispersed fairly homogeneously throughout the denture base resin, as determined by SEM but there seemed to be no coupling between the two materials. This led to a reduction of the strength of the DBR as greater PTMC concentrations were included, they would likely act as voids within the DBR structure. Another possible reason for the decrease in ultimate flexural strength could have been the incomplete polymerisation processing of the denture base resin due to the incorporation of PTMCs. This would result in a significant amount of residual monomer which would cause a loss in mechanical properties of the resin (35) possibly due to plasticization.

Based on the flexural strength test result and DBR ISO limitation, the maximum percentage incorporation of PTMCs was determined. This 2.5% PTMC concentration was then used to determine if phytoncide was released by the PTMCs embedded in the DBR. If all the phytoncide in the PTMCs diffused out of the DBR disc supplemented with 2.5% PTMCs a concentration of 18.54 mg/mL would have resulted in this assay, based on 70% of the weight of the PTMCs being phytoncide. To determine release of phytoncide at neutral pH from the PTMCs, we used a bioassay that relied on the exquisite sensitivity of the Gram-negative bacterium *P. gingivalis* to phytoncide. *P. gingivalis* has a reported MIC of 0.08 mg/mL which is approximately 232 times lower than the concentration that would have resulted if all the phytoncide was released. Hence this was a sensitive assay for phytoncide release, however, no *P. gingivalis* growth inhibition was detected. The phytoncides were not released either through the melamine formaldehyde (MF) capsule material or through the DBR resin or were potentially inactivated during the heat curing process. Most likely physical force or chemical action is needed to rupture the microcapsules and release the phytoncide. It was assumed that the phytoncide would not release efficiently due the unique characteristic of the MF resin such as water insolubility, greater durability and mechanical resistance, the temperature and pressure resistance (37). However, it was required to withstand the heat cure processing and to endure and 100°C, pressure between 200 - 400 kPa and a dry environment when mixing with powder form of PMMA.

Based on the information of phytoncide release test, we investigated the microcapsule robustness to verify whether the PTMCs were dissolving or becoming deformed, and whether the phytoncide would escape through the capsules in an acidic environment at pH 5.5. Because the strength and deformability of microcapsules are dependent on their formulation and processing conditions, and the shell formation is related to the composition of components as well as pH, temperature, type and the amount of emulsifier used (38). Alič *et al*. (28) found that the general thickness of shells in the microcapsules was between 300-500 nm, and MF capsules could be deformed during the formation of the capsules under differing conditions of acidity, time and temperature.

Through the robustness test, it was found that treatment of the PTMCs with 100% ethanol (vol%) had no significant effect, whereas both mechanical force and treatment with a pH 5.5 citric acid solution caused significant disruption of the PTMCs. This disruption would enable the release of the antimicrobial phytoncides.

Oral bacteria such as *S. mutans* and *Lactobacillus* species and oral fungi such as *C. albicans* produce high levels of lactic acid as a result of anaerobic fermentation of dietary sugars (39). This can result in plaque pH falling to around pH 5 for extended periods of time. Furthermore, ingesting acidic food or drink can create acidic conditions in the oral cavity. von Fraunhofer and Rogers (40) reported that most soft drinks contained one or more food acidulants such as citric, phosphoric, malic, tartaric, and other organic acids in addition to fermentable carbohydrates, mainly sucrose. This results in even lower plaque pH for longer periods of time.

As previously described, *Candida*-associated denture stomatitis is a very common inflammatory condition. Oral fungi such as *C. albicans* can colonize the surface of DBR and establish themselves as a component of polymicrobial biofilms that can result in inflammation of the oral mucous membrane (3, 11-13). Therefore, the robustness test provided a justification for conducting the antifungal test of the PTMCs in an acidic environment at pH 5.5 against *C. albicans*. *C. albicans* growth decreased in a dose dependent manner as the PTMC concentration increased. This is clearly indicating that the MF microcapsules were disrupted at pH 5.5, releasing the phytoncide. Previous studies have shown that phytoncides are active against *C. albicans* at neutral pH (41), therefore release

of the phytoncides at pH 5.5 would have a continuing effect on fungal growth even if the pH increased.

Since MF is a synthetic material and is regarded as possibly a toxic and harmful component to humans (42), it is suggested to use alternative kinds of natural biodegradable shell materials, such as gelatine-carboxymethylcellulose (CMC) or gelatine-arabic gum materials, which has proven to release core materials with no interaction between the shell and active ingredient (37, 43). This overcomes the drawback of potentially toxic materials and accomplishes an easier release of phytoncide from the capsule, which has already been used in the microcapsule industry (43).

5. CONCLUSIONS

Phytoncide contained within microencapsulates (PTMC) can be added to denture base resin (DBR). Increasing PTMC concentration in DBR reduced the integrity of the DBR but at concentrations less than 2.5% the DBR meets international standard requirements. At neutral pH phytoncide was not released from PTMCs embedded in DBR. However, at acidic phytoncide was released from the PTMCs in sufficient concentrations to inhibit the growth of *Candida albicans*. This study demonstrates that incorporation of suitable microcapsules containing antimicrobial agents could be a good strategy to combat denture stomatitis.

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Tables

Table 1. The antifungal test protocol.

	Concentration	Well	*C. albicans* inoculum	YH† solution	10 % PMYH§ solution
Group 1	0.0%	A	100 µL	100 µL	0 µL
	5.0%	B	100 µL	0 µL	100 µL
	2.5%	C	100 µL	50 µL	50 µL
	1.0%	D	100 µL	80 µL	20 µL
	0.5%	E	100 µL	90 µL	10 µL
Group 2	0.0%	F	0 µL	200 µL	0 µL
	5.0%	G	0 µL	100 µL	100 µL
	2.5%	I	0 µL	150 µL	50 µL
	1.0%	J	0 µL	180 µL	20 µL
	0.5%	K	0 µL	190 µL	10 µL

† YH solution: YPG‡ broth + hydrochloric acid

‡ YPG: Yeast peptone glucose

§ PMYH solution: 10% concentration of phytoncide microcapsules in YH solution

Table 2 . Preliminary test results according to the percentage of phytoncide microcapsules (PTMCs).

Phytoncide %	Surface characteristics/ Shape capability	Freedom of porosity	Colour/Translucency
0% (Control)	Smooth, glossy surface	No porosity/voids	No change
0.5%	Smooth, glossy surface	No porosity/voids	No change
1.0%	Smooth, but a little speckling of yellowish PTMCs	No porosity/voids	Very minor change, passed translucency
10.0%	Scattered speckling of yellowish PTMCs	No porosity/voids	Orange colour, passed translucency
30.0%	Scattered aggregation speckling of yellowish PTMCs	No porosity/voids	Dark organge colour, passed translucency

FIGURE LEGENDS

Figure 1. Preliminary test samples containing 0%, 0.5%, 1%, 10% and 30% PTMCs from left to right and top to bottom.

Figure 2. Fexural strength test of nine phytoncide concentration in the denture base resin (n = 9 for each concentration). Mean values are displayed and error bars show standard deviations (SD). Red line indicates the International Organization for Standardization (ISO) requirement (65 MPa).

Figure 3. Scanning electron microscope (SEM) image of the fractured surfaces of denture base resin (DBR) + phytoncide microcapsule (PTMC) (A: 0%; 200x, B: 2.5%; 200x, C: 3.0%; 200x and D: 5%; 200x).

Figure 4. Scanning electron microscope (SEM) image of the phytoncide microcapsule (PTMC) clusters with agglomeration (A: 0%; 1000x, B: 2.5%; 1000x, C: 3.0%; 1000x and D: 5%; 1000x).

Figure 5. Scanning electron microscope (SEM) image of the integration between the phytoncide microcapsuels (PTMCs) and dentrue base resin (A: 0%; 4000x, B: 2.5%; 4000x, C:

3.0%; 4000x and D: 5%; 4000x). Red arrow indicates a gap between the PTMCs and the resin matrix.

Figure 6. Optical microscopy images of four different groups of microcapsules in different test groups at a magnification of 1000x (Scale: 20 μm). A: Crushed capsules, B: Ethanol 100%, C: Distilled water, and D: Citric acid (pH 5.5).

Figure 7. Effect of phytoncide microcapsuels (PTMCs) concentration on the growth of *C. albicans* calibrated as optical density (OD) at pH 5.5. Note: Minimum inhibitory concentration (MIC) = 2.6%.

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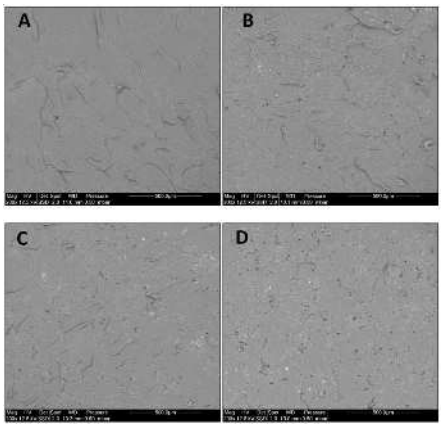

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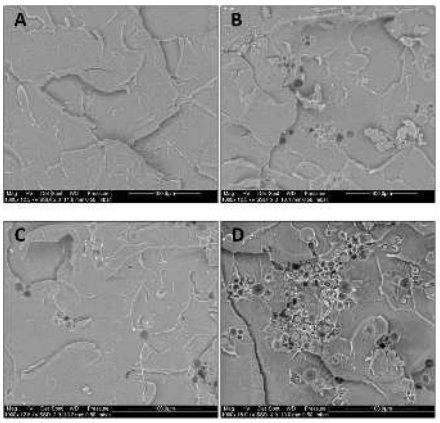

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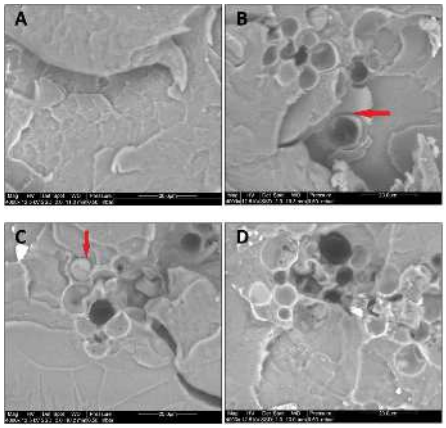

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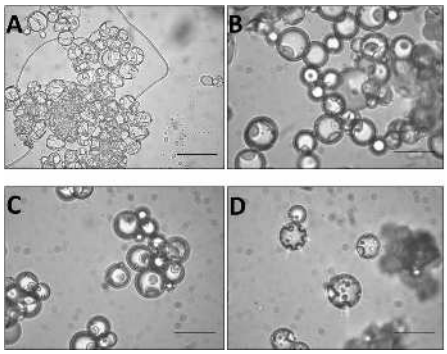

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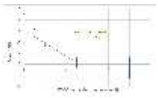

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