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Study of the elution of fluconazole from a self-polymerizing acrylic resin and its activity against resistant *Candida albicans*

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Abstract The study aimed, firstly, to monitor the release of an antifungal drug, fluconazole, from a self-polymerizing poly(methyl methacrylate) (PMMA) denture base resin in artificial saliva and comparing it with the release in water; and secondly, to investigate the effect of the released drug on the growth of resistant and standard strains of *Candida albicans*. A high-performance liquid chromatography–ultra-violet (HPLC–UV) method was used in the analysis of the released eluates into distilled water from self-polymerized PMMA discs doped with the 10% fluconazole antifungal drug. The efficacy of the released drug against resistant and standard strains of *C. albicans* was monitored, using agar diffusion method. The results showed that fluconazole, can be successfully incorporated with the self-polymerized PMMA. The findings suggest that the drug leaches steadily out of the PMMA resin into artificial saliva and distilled water at mouth temperature and that sustained drug release continued throughout the 28 days test period. It was shown that the released drug demonstrated antifungal activity against both standard and resistant *C. albicans.* The findings of this investigation have a clinical value in terms of their significant contribution to the treatment of fungal infections of the oral cavity. The sustained release of antifungal drug from the PMMA resin clearly constitutes a new dosage form of the drug via the poly(methyl methacrylate) delivery system.

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1 Introduction

The *Candida*-associated denture stomatitis is a known denture-related mucosal lesion. It is characterized by inflammation of the palatal mucosa covered by the denture [1, 2]. The condition is usually harmless and is associated with a quantitative increase of yeasts, particularly *Candida albicans*, on the mucosa and the denture's fit surface [2]. Invasive infection of the oral mucosa occurs since the yeast cells are usually present within the denture plaque and on the surface of the mucosa covered by dentures [3]. The lesion may heal after topical antifungal treatment [4, 5] but the incidence of relapse is, however, very high [6–8].

The existing methods of delivering drugs for the treatment of the oral mucosal lesions are highly inefficient. This is mainly due to difficulties in placing an adequate amount of the drug at the intended site and is then being able to maintain the agent in the mouth sufficiently long for its maximum therapeutic potential to be utilized.

Presently, the delivery of drugs is achieved by non-specific periodic application of the agent to the organism, either topically or systemically. This method can lead to undesired side-effects, either at the target site or in the environment around the target, due to fluctuation in drug levels. Incorporating antifungal drugs within tissue

conditioners, temporary chair-side treatment soft liners, has been attempted [9–20] but with limited success. It was reported that medicated soft liners such as a nystatin-containing tissue conditioner affected a decrease in salivary yeast count for only 7 days, after which period the count increased [20].

The tissue conditioners are heavily plasticized gels intended to cover the entire impression surface of the denture including its borders and fold over to the denture's polished surface. These gels quickly become rigid as their plasticizers leach out inside the mouth or on exposure to a simulated intraoral environment leaving behind a hard, unyielding and traumatic liner. Recurrence of fungal infection as early as 3 days following application of medicated tissue conditioners was reported [19].

The search has continued for a better drug delivery system that maintains the ideal therapeutic level of the drug over the required period. The principle of controlled drug release has been reported [8] and aimed to produce this effect by releasing the drug at a predetermined rate over specific time period from a protected reservoir. Application of this principle in the treatment of oral lesions had been attempted and promising in vitro results were claimed [21, 22]. In these claims Chlorhexidine diacetate was used as an antifungal drug and the drug-releasing reservoir used was a self-curing poly(ethyl methacrylate) and tetrahydro-furfuryl methacrylate, a denture relining polymer.

The objective of the present study was to establish whether a polymeric delivery device based on the conventional hard self-polymerizing poly(methyl methacrylate) permanent denture relining system could be developed for the sustained delivery of fluconazole antifungal drug. The liner is intended to have a dual action, firstly, an initial therapeutic function confined to the denture/tissue interface at the fit surface of the denture, which is an air-tight sealed area, where the doped liner contacts the denture bearing mucosa only without extending to the peripheries. Secondly, improving the fit of the denture and relieving the supporting tissues from occlusal trauma. The self-polymerized resin was chosen because of its abundance, ease of handling, cost effectiveness and being a room-temperature polymerizing resin, its curing heat is not harmful to the antifungal drug.

2 Materials and methods

2.1 Sample preparation

A self-polymerized poly-PMMA resin, a product of (Paladur: powder batch no. 32, liquid batch no. 012290) was employed. The mixing ratio and the conditions for processing and polymerization recommended by the manufacturer were strictly followed. These were 5 g/3 ml powder/liquid ratio for mixing. A disc shaped specimen of PMMA was impregnated with 10% w/w fluconazole powder (Fig. 1). This amount of drug was reported of having not detrimental effect on the properties of the polymer [21, 22]. The drug was added in the specified ratio to the acrylic resin powder then the mixture of the powders with the liquid monomer were stirred for 15 s and left standing for 4 min until plastic dough was formed. It was then packed in a specially constructed disc-shaped steel mould to produce a disc specimen (3.8 mm diameter and 1.0 mm thickness). After packing the mould, it was allowed to stand for 13 min then placed in a pressure curing unit and cured at 55°C and 2 bar pressure for 15 min.

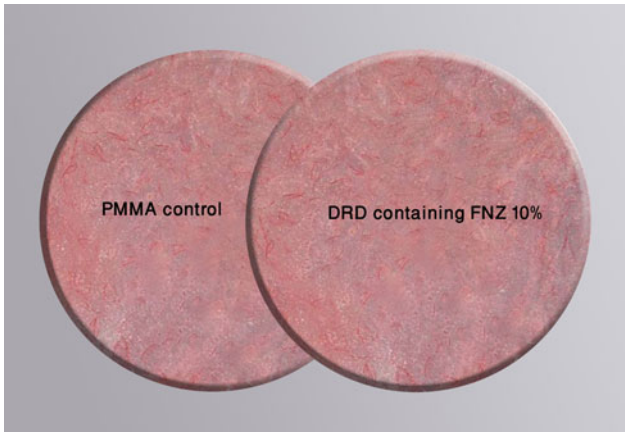

Fig. 1 Discs of the two specimen groups showing the drug release device (DRD) impregnated with 10% fluconazole and the control PMMA disc

2.2 Preparation of saliva

Artificial saliva simulating pooled human saliva in constituents and pH was prepared by dissolving the following chemicals (in the assigned amount for each) in 1 l of double distilled water [23]: ammonium chloride (233 mg), magnesium chloride hexahydrate (43 mg), potassium chloride (1162 mg), potassium thiocyanate (222 mg), calcium chloride dehydrate (210), potassium dihydrogen orthophosphate (354 mg), sodium citrate (13 mg), sodium hydrogen carbonate (535 mg), and disodium hydrogen orthophosphate (375 mg) which are all of analytical grade were purchased from BDH (England).

2.3 High-performance liquid chromatography (HPLC)

2.3.1 Chemicals, reagents and standards

Acetonitril of HPLC-quality, hydrochloric acid and sodium citrate of analytical grade were all purchased from GCC

(England). The standard material of the drug (fluconazole) was purchased from Cadilla pharmaceuticals (Ahmad-Abad, India). The internal standard *p*-methyl phenol was purchased from ACROS (Geel, Belgium).

2.3.2 HPLC-apparatus and working conditions

The analysis was carried out on an isocratic HPLC-apparatus which consists of the following parts: HPLC pump type GBC (Australia), model LC 1110; HPLC injector type Rheodyne 7125 (USA); HPLC–UV–vis detector type GBC (Australia), model LC 1205; Integrator type Spectra Physics model LC 4290.

The HPLC-apparatus was operated under the following working conditions: Eluent: acetonitril/0.01 M phosphate buffer (25:75%); Eluent flow rate: 1.0 ml/min; Injection volume: 20 µl; Column: BDS-C18 (25 cm × 4.6 mm, particle size 5 µm); Detector: UV–vis spectrophotometer (λ: 210 nm, range: 1.0); integrator chart speed: 0.5 cm/min, attenuation: 8.

2.3.3 Working standard solution

The working standard solution for fluconazole was prepared by mixing 20 µg/ml of the drug with 50 µg/ml of the internal standard *p*-methyl phenol in HPLC–water.

2.3.4 Leaching behavior

Two discs 6.27 g each which contained 10% w/w of the fluconazole antifungal drug were soaked in 60 ml distilled water. The same procedure was repeated using artificial saliva. The containers which accommodated the samples were left in a thermostatically controlled revolving water bath at 37°C.

The intervals of analyses were 1, 2, 4, 8, 12 h, 1, 2, 4 days 1, 2, 3 and 4 weeks. A small known amount of the test solution of each sample group was taken at each test interval and mixed with aqueous solution of the internal standard of fluconazole, so that its concentration in the final volume (5 ml) becomes 50 µg/ml. Twenty microliters of this mixture solution was injected onto the HPLC-column under the above mentioned conditions.

2.3.5 Qualitative and quantitative analysis

A representative chromatogram of a standard mixture of fluconazole with internal standard is shown in Fig. 2 The qualitative identification of fluconazole peak was performed through comparing the relative retention time (RRT) of the drug, with respect to the internal standard *p*-methyl phenol in the real sample to those (RRT) in the chromatogram of the standard mixture of the drug, which in this case 3.668 min The quantitative determination was performed by using the relative peak areas (RPA) and the relative concentrations (RC).

2.4 Microbiological investigation

2.4.1 Strains and media

A strain of *C. albicans* resistant to Polyenes (Amphotericin B and Nystatin) was isolated from an in-patient hospitalized at the Jordan University Hospital and was confirmed by biochemical tests. Until testing, the yeast was kept frozen in Brain–Heart broth (Difco Laboratories, Detroit,

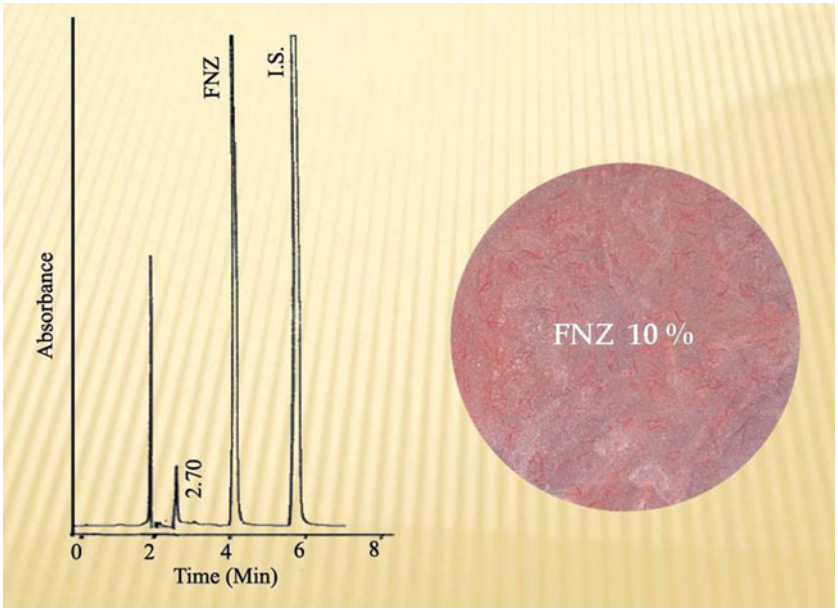

Fig. 2 A representative chromatogram of a standard mixture of fluconazole with the internal standard

MI, USA) with 5% glycerol. For each experiment, the strain was sub-cultured twice on Saborauds agar (Difco) for 24 h at 35°C to ensure viability and purity. The inoculum suspension was prepared by picking five colonies of at least 1 mm in diameter and suspending them in 5 ml of sterile saline solution (0.85%).

2.4.2 The well diffusion test

This test was performed using Saborauds agar. The inoculum used was prepared using the yeast from a 24-h culture on Saborauds agar, a suspension was made in a sterile saline solution (0.85%). The turbidity of the suspension was adjusted with a spectrophotometer at 530 nm to obtain a final concentration to match that of a 0.5 McFarland standard ($0.5–2.5 \times 10^3$). The inoculated agar was poured into the assay plate (9 cm in diameter), and allowed to cool down on a leveled surface. Once the medium had solidified "wells" each 4 mm in diameter, were cut out of the agar and 20 µl of the eluates from fluconazole (10%), were placed into a well in separate assay plates. Control cups containing the drug release device (DRD) alone were also included in each assay plate. The plates were incubated at 35°C for 72 h.

The absence of growth of *C. albicans,* demonstrated by the occurrence of growth inhibition zone around the wells that contained the DRD supplemented with the drug was interpreted as antifungal activity of the drug. The antifungal efficacy of fluconazole present in the immersion fluid (water or saliva) was evaluated at each time interval; the immersion fluid was regularly changed at each test interval. The fungicidal efficacy of the drug was expressed by measurement of the diameter of the inhibition zone present around the well using PBI Readbiotic (PBI International, Milano, Italy) measuring device.

Each experiment was carried out three times for the antifungal drug doped-DRD and was correlated against the control (DRD without the drug).

3 Results

3.1 Drug release device (DRD)

At the outset, the incorporation with the self polymerized PMMA of the antifungal drug did not have any untoward effect on the polymerization reaction of the PMMA or on the preparation of the test sample.

3.2 HPLC analysis of the drug release

Fluconazole demonstrated an initial high rate of elution from the PMMA reservoir during the first 3-4 days

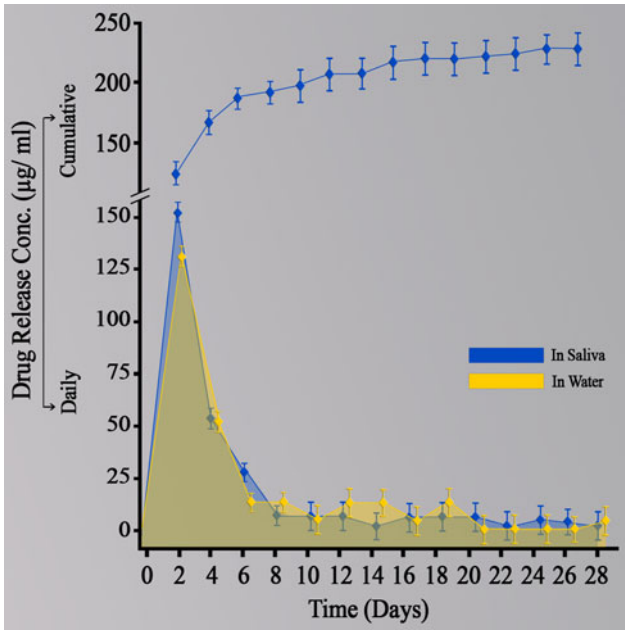

Fig. 3 A plot of drug release concentration against time. The bottom part of the graph demonstrates the concentration of the daily released drug into water or saliva, the two media were changed every test interval. The top part of the graph demonstrates a hypothetical simulation of the clinical situation displaying what would be the cumulative concentration of fluconazole released into the salivary film at the denture/tissue interface and eventually absorbed by the tissues provided that the patient would wear the upper denture continually for 4 weeks. Error bars represent the distribution of data around the mean value of three repeats

followed by a controlled-elution process of sustained release that continued throughout the 28 days test period (Fig. 3). In general fluconazole displayed a higher release rate in synthetic saliva than in water attaining a concentration of up to 207 µg/ml after 4 days compared to 187 µg/ml in water during the same period. The drug release from the samples continue to increase throughout the test attaining a concentration of 277 µg/ml after 28 days in synthetic saliva compared to 249 µg/ml in water during the same period.

3.3 Microbiology

The percolate from the DRD that contained 10% fluconazole showed a clear antifungal activity. This was demonstrated by the occurrence of a zone of inhibition of *C. albicans* growth around the well that contained the DRDs with fluconazole compared with the control, i.e., DRD alone (Fig. 4).

Fluconazole contained in the DRDs continued to display the antifungal potential throughout the 28 days test period. This was demonstrated by the plot of the width of the inhibition zone of *Candida albicans* growth versus time (Fig. 5).

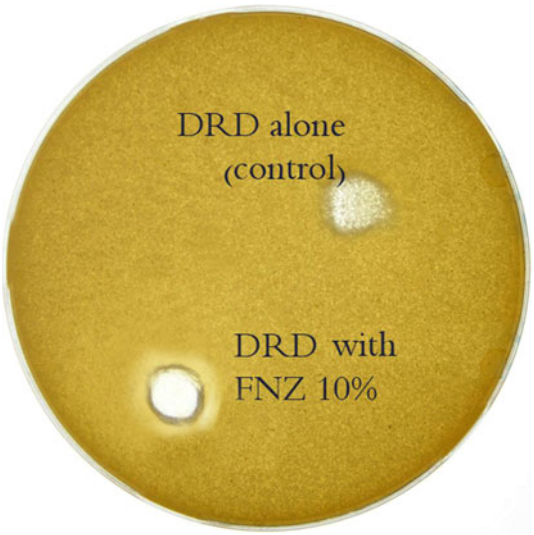

Fig. 4 Demonstrating the zone of inhibition of the *Candida albicans* growth around the well that contained the DRD with 10% fluconazole antifungal drug. Note the absence of an inhibition zone around the well that contained the control, i.e., the PMMA alone

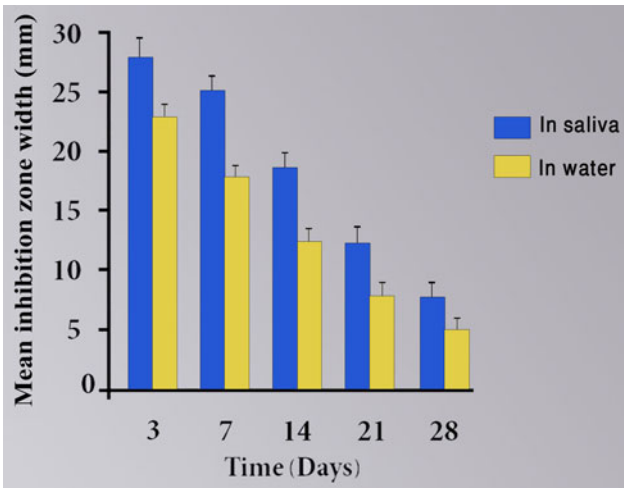

Fig. 5 A histogram plotting the width of the inhibition zones of the *Candidal* growth affected by fluconazole release into water and saliva, the two media were changed at each interval over the 4 weeks test period. Error bars represent the distribution of data around the mean value of three repeats

4 Discussion

The release of fluconazole from the PMMA DRDs to distilled water and artificial saliva indicated that polymerization of the PMMA did not adversely affect the antifungal drug nor did doping the PMMA acrylic resin with fluconazole alter the diffusion characteristics of the resin. This finding is in agreement with previously reported studies that employed polymers for delivering antifungal drugs [21, 22, 24].

Elution of fluconazole from self-polymerized PMMA demonstrated an initial release into distilled water reaching a magnitude of 0.5% which is equivalent to 3.6 mg of the drug during the first 4 days. Following the initial high elution rate, the release of the fluconazole demonstrated a slower and steadier diffusion process for up to 28 days. The change in the rate of drug release is attributed to the fact that the leaching behavior of fluconazole into water is governed by a concentration dependent diffusion process [22].

Similar elution profile was reported for residual unpolymerized methyl methacrylate monomer from the same self-polymerizing PMMA resin when exposed to both distilled water and artificial saliva at mouth temperature [25].

It has been clearly established that methacrylate-based polymers absorb up to 30% water depending on the osmolarity of the external solution [21] or the formulation of the particular polymer [21, 22, 24]. The mechanism of elution seemed to consist of two phases, a rapid linear behavior obeying Fick's law, followed by the development of discrete clusters of the immersion liquid of an unidentified osmotic activity [22].

In the presence of fluconazole, the rapid elution phase probably indicates surface release process. The subsequent slow phase of sustained release may be the result of complex processes involving formation of fluid clusters around the drug molecules and the interaction of these clusters with the mechanism of fluid absorption of the acrylic resin. Similar behavior has been reported for the release into distilled water but of a different drug from a methacrylate-based polymeric system [21].

The elution behavior of fluconazole may also be enhanced by crazes and surface porosity, formed in the brittle PMMA by the osmotic forces consequent on the inclusion of the antifungal drug. This is consistent with previously reported finding of a study that used the same polymeric system for delivery of hydrocortisone [26].

Having established that a fluconazole-supplemented polymeric device do release the antifungal drug in controlled concentration for up to 4 weeks, it became essential to investigate whether the concentration of the released drugs was high enough to affect an antifungal activity upon the growth of *C. albicans*. This entailed the microbiological investigation conducted in this study which ascertained that the released concentration of the antifungal drug did induce antifungal effect against *C. albicans* by inhibiting its growth in Saborauds culture for the entire 4-weeks period. These findings confirmed those of an earlier study [27] which also showed that antifungal drugs diffuse out of a self-polymerized acrylic resin in fungicidal concentrations over a period of at least 3 weeks.

In the clinical context, the cumulative nature of fluconazole release into the surrounding fluid media helps saturate the salivary film which bathe the tissue surface of a denture base with a continuous release bouts of the antifungal drug.

5 Conclusions

The findings of the present study showed that fluconazole, can be successfully incorporated with self-polymerizing PMMA and that the drug leaches out of the polymer into the surrounding fluid medium in an environment similar to that of the oral cavity with respect to humidity and temperature.

The sustained incremental elution of the antifungal drug from the doped polymeric denture base is particularly interesting phenomenon since it could be considered a potential for the use of the doped acrylic resin reliner as a vehicle for delivering antifungal drugs to the affected denture bearing tissues. Such a polymer would have a dual function, a relining layer to improve the fit of the denture and a reservoir for delivering an antifungal drug at the exact site of pathology where *Candidal* infection lesions of the tissue bed, usually of the maxillary denture, are found. A patient wearing such a treatment upper denture must be instructed not to take the denture out for the therapeutic period of 4 weeks.

The results of the microbiological investigation confirmed the antifungal efficacy of the drug-supplemented delivery system.

Clinical studies are essential in order to test the efficacy of the suggested drug-delivery system, before implementing the new dosage form of antifungal drug for the treatment of denture associated oral *Candidiasis.*

References

1. Budtz-Jørgensen E. The significance of *Candida albicans* in denture stomatitis. Scand J Dent Res. 1974;82:5–51.
2. Budtz-Jørgensen E, Theilade E, Theilade J. Regional variations in viable bacterial and yeast counts of 1-week-old denture plaque in denture stomatitis. Scand J Dent Res. 1983;91:288–95.
3. Pollack B, Buck IF, Kalnius J. An oral syndrome complicating pschopharmacotherapy study II. Am J Psychiatry. 1964;121: 384–6.
4. Budtz-Jørgensen E, Theilade E, Theilade J. Quantitative relationship between yeasts and bacteria in denture-induced stomatitis. Scand J Dent Res. 1983;91:134–42.
5. Bergendal T, Holmberg K, Nord CE. Yeast colonization in the oral cavity and feces in patients with denture stomatitis. Acta Odontol Scand. 1979;37:37–45.
6. Brook IM, van Noort R. Controlled delivery of drugs. Br Dent J. 1984;157:11–5.
7. Garcia CR, Siquerios A, Benet LZ. Oral controlled release preparations. Pharm Acta Helv. 1978;53:99–109.
8. Paul DR. Polymers in controlled release technology. American Chemical Society Symp.; Series No. 33 controlled release polymeric formulations; 1976. p. 1–3.
9. Douglas WH, Walker DM. Nystatin in denture liners—an alternative treatment of denture stomatitis. Br Dent J. 1973; 135(2):55–9.
10. Douglas WH, Clarke DA. Physical and mechanical properties of nystatin containing denture liners. J Prosthet Dent. 1975;34:428–33.
11. Thomas CJ, Nutt GM. The in vitro fungicidal properties of Viscogel, alone and combined with nystatin and amphotericin B. J Oral Rehabit. 1978;5:167–72.
12. McCarthy JA, Moser JB. Tissue conditioning and functional impression materials. In: Dent Clin North Am: removable prosthodontics. Toronto: Saunders; 1984.
13. Quinn DM. The effectiveness, in vitro, of tniconazole and ketoconazole combined with tissue conditioners in inhibiting the growth of *Candida albicans*. J Oral Rehabil. 1985;12:177–82.
14. Okita N, Orstavik D, Orstavik J. In vivo and in vitro studies on soft denture material: microbial adhesion and tests for antibacterial activity. Dent Mater. 1991;7(3):155–60.
15. Schneid TR. An in vitro analysis of a sustained release system for the treatment of denture stomatitis. Spec Care Dentist. 1992;12:245–50.
16. Truhlar IMR, Sahy K, Sohnle P. Use of a new assay technique for quantification of antiflungal activity of nystatin incorporated in denture liners. J Prosihcl Dent. 1994;71:517–24.
17. Nikawa HT, et al. Antifungal effect of zeolite-incorporated tissue conditioner against *Candida albicans* growth and/or acid production. J Oral Rehabil. 1997;24:350–7.
18. Kulak Y, Kazazoglu E. In vivo and in vitro siud of fungal presence and growth on three tissue conditioning materials on implant supported complete denture wearers. J Oral Rehabil. 1998;25:135–8.
19. Chow CKW, Matear DW, Lawrence HP. Efficacy of antifungal agents in tissue conditioners in treating candidiasis. Gerodontology. 1999;16(2):111–8.
20. Geerts GA, Stuhlinger MF, Basson NJ. Effect of antifungal denture liner on the saliva yeast count in patients with denture stomatitis: a pilot study. J Oral Rehabil. 2008;35:664–9.
21. Riggs PD, Braden M, Patel M. Chlorhexidine release from room temperature polymerizing methacrylate systems. Biomaterials. 2000;21:345–51.
22. Patel MP, Cruchley AT, Coleman DC, Swai H, Braden M, Williams DM. A polymeric system for intra-oral delivery of an anti fungal agent. Biomaterials. 2001;22:2319–24.
23. Shellis RP. Synthetic saliva for cultural studies of dental plaque. Arch Oral Biol. 1978;23:485–9.
24. Amin WM. A study of adhesion between soft lining materials and poly(methyl methacrylate). PhD thesis, University of London; 1987.
25. Alawi MA, Amin WM. Effect of aging on monomer elution from poly(methyl methacrylate) resin under simulated intra-oral conditions. FEB. 2007;16:408–14.
26. Brook IM, Van Noort R. Drug release from acrylic polymers via channels and cracks: in vitro studies with hydrocortisone. Biomaterials. 1985;6:281–5.
27. Lamb DJ, Martin MV. An in vitro and in vivo study of the effect of incorporation of Chlorhexidine into autopolymerizing acrylic resin plates upon the growth of *Candida albicans*. Biomaterials. 1983;4:205–9.