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A polymeric system for the intra-oral delivery of an anti-fungal agent

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Abstract

Oral candidal infections are often persistent and intractable and thus the aim of this study was to develop a polymeric sustained release device to improve the topical treatment of these infections. A self curing system based on poly(ethyl methacrylate) and tetrahydrofurfuryl methacrylate (PEM/THFM) was used with chlorhexidine diacetate (CX) added at levels between 0 and 12% w/w. Water uptake by the device was assessed gravimetrically and CX release measured by UV spectrometry. Anti candidal activity was established by culturing azole sensitive and resistant strains of *Candida albicans* in the presence of the polymeric delivery device with and without CX. Candidal growth was measured by turbidimetry or surviving colony-forming unit (CFU) formation. There was an initial high release of CX over 24 h followed by a slow diffusion up to 7 days. CX inhibited candidal growth and survival markedly in vitro, with the test samples showing less than 0.5×10^{-7} CFU/ml compared to controls ($3–4 \times 10^{-7}$ CFU/ml). These results indicate the potential of a chlorhexidine containing PEM/THFM polymeric system in the treatment of persistent candidal infections.

Keywords: Intra-oral; Chlorhexidine; PEM/THFM; Polymeric delivery system; Candida

1. Introduction

Candida albicans, the commonest oral fungal organism, has a greater level of pathogenicity and adherence than other *Candida* species and is an oral commensal in up to 65% of healthy adult mouths [1,2]. It can act as an opportunistic pathogen causing acute pseudomembranous candidiasis, chronic hyperplastic candidiasis or denture-induced stomatitis on the mucosal surface beneath dentures [1,2]. In particular, it can cause persistent or intractable infections in immunocompromised patients or in patients who have had a local disturbance in their oral flora [1,2].

Oral candidal infections may be treated systemically with azole drugs such as fluconazole and ketoconazole or topically with nystatin, amphotericin and chlorhexidine [2]. In the last few years, however, azole-resistant strains of *Candida albicans* have emerged [3] and this has created difficulty in clinical management [4]. The efficacy of chlorhexidine as an antifungal agent in a mouthrinse is well established [5,6] and in vitro studies have provided evidence of a remarkable activity against candidal species [7,8]. Importantly, however, the emergence of strains resistant to chlorhexidine has not been observed in clinical studies [9].

The aim of the present study was to establish whether a polymeric delivery device for the sustained topical delivery of chlorhexidine could be developed and to demonstrate that this had a significant killing action against *Candida albicans*. The polymeric system selected for this purpose was a room-temperature polymerising system comprising poly(ethyl methacrylate) powder and tetrahydrofurfuryl methacrylate monomer (PEM/THFM) [10]. This gives a rigid polymeric material consisting of a partial inter-penetrating network, with a lower glass transition temperature and lower polymerisation exotherm than the poly(methyl methacrylate) (PMMA)-based systems widely used in dentistry. It is a ductile material whose physico-chemical properties are well documented [11,12], which has superior biocompatibility than PMMA [13], promotes bone and

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cartilage growth [14] and has proved to be an effective system for the release of gentamicin and bone morphogenetic protein [15,16].

2. Materials and methods

2.1. Drug release device (DRD)

2.1.1. Materials

Poly(ethyl methacrylate) powder (PEM) was kindly supplied by Bonar Polymers Ltd., Newton Aycliffe Co., Durham, UK (Ref. TS 1364). The powder contained 0.6% residual benzoyl peroxide. Tetrahydrofurfuryl methacrylate (THFM) was obtained from Röhm Chemie (Darmstadt, Germany) and *N*,*N*-dimethyl-*p*-toluidine was obtained from Aldrich, UK. Chlorhexidine diacetate was obtained from Sigma, UK.

2.1.2. Specimen preparation

Chlorhexidine diacetate was blended with the PEM powder at levels of 0 (control), 4.5, 6, 9 and 12% w/w. This powder was then added to the monomer liquid (THFM) containing 2.5% (v/v) *N*,*N*-dimethyl-*p*-toluidine, in the ratio of 1 g powder to 0.6 ml liquid; control specimens used PEM with no additive. After mixing, the material was placed in a polythene mould, sandwiched between two pre-coated microscope slides, and cured under 2 atm pressure, to produce duplicate rectangular samples nominally $\sim 42 \times 10 \times 1$ mm.

2.1.3. Water uptake

Water uptake was measured gravimetrically as described previously [17].

2.1.4. Chlorhexidine release

The amount of chlorhexidine released from the polymer systems was measured by UV spectrometry. Samples with or without chlorhexidine were immersed in 100 ml sterile distilled water and 3 ml aliquots were removed at intervals up to 14 days. Chlorhexidine release was quantified by comparison to a standard curve and the analysis performed using a spreadsheet package (Microsoft Excel).

3. Microbiology

3.1. Materials

Two strains of *Candida albicans* (132A and 132ACR) were used in the study. 132ACR is a spontaneous mutant of 132A and is resistant to ketaconazole, miconazole and fluconazole, whereas 132A is susceptible to all three compounds [18]. The organisms were grown and maintained aerobically at 37°C on Columbia agar (Life Technologies, Paisley, UK) with 5% (v/v) defibrinated horse blood (TCS Microbiology, Buckinghamshire, UK). Candidal cells for use in the experiments were prepared by transferring a single colony from the agar plates into yeast dextrose peptone broth (DIFCO Laboratories, Detroit, USA) and incubated aerobically overnight at 37°C. The cultures were then centrifuged at 6000 rpm for 6 min and the cells resuspended in the culture broth prior to use in the experiments.

3.2. Effect of DRD on candidal growth

The effect of the DRD on candidal growth was assessed in two ways:

(i) by turbidimetric measurement (at 600 nm E600s) of growth after 24 h co-exposure to DRD's with and without chlorhexidine, and results expressed as a percentage of control cultures;
(ii) by simultaneous co-incubation of the candidal cultures with the device and subsequent determination of the number of surviving colony forming units (CFUs) after exposure for up to 5 h. CFUs were discrete colonies 1–2 mm in diameter, creamy white in colour and were formed when *C. albicans* was placed onto blood agar and incubated aerobically overnight at 37°C. The results were expressed as the number of CFU formed/ml of culture medium (CFU/ml).

DRD's containing 9 and 12% chlorhexidine diacetate w/w PEM were tested and controls containing organisms or DRD only (no drug) were included in each experiment and all were performed in duplicate. All the DRD's were sterilised by exposure to UV light for 60 s before use in the experiments.

4. Results

4.1. Polymeric delivery system

PEM/THFM polymerised to form a glassy polymer and the inclusion of up to 12% w/w chlorhexidine diacetate in the polymer had no discernible effect on the preparation of the samples. This is in accord with detailed studies on the effect of CX on the polymerisation of the PEM/THFM system [19].

Fig. 1 plots percentage water uptake against $t^{1/2}$ for the DRD's in the presence and absence of chlorhexidine. The water uptake of the PEM/THFM system increased as the amount of chlorhexidine in the system increased, with the control (PEM/THFM only) absorbing $\sim 12\%$ water and the DRD's containing 12% drug absorbing up to 22% water at ~ 6 months.

Chlorhexidine release from the DRD was characterised by high initial release followed by a slower

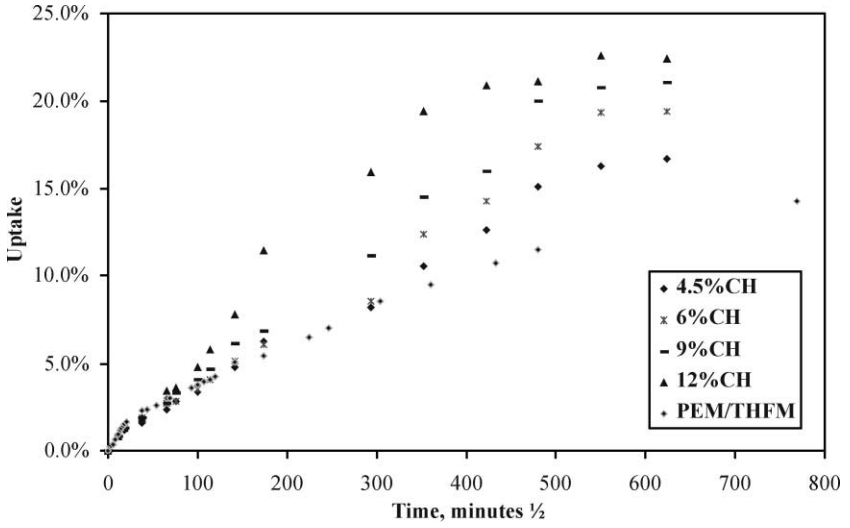

Fig. 1. Water uptake of PEM/THFM DRD containing different concentrations of chlorhexidine diacetate.

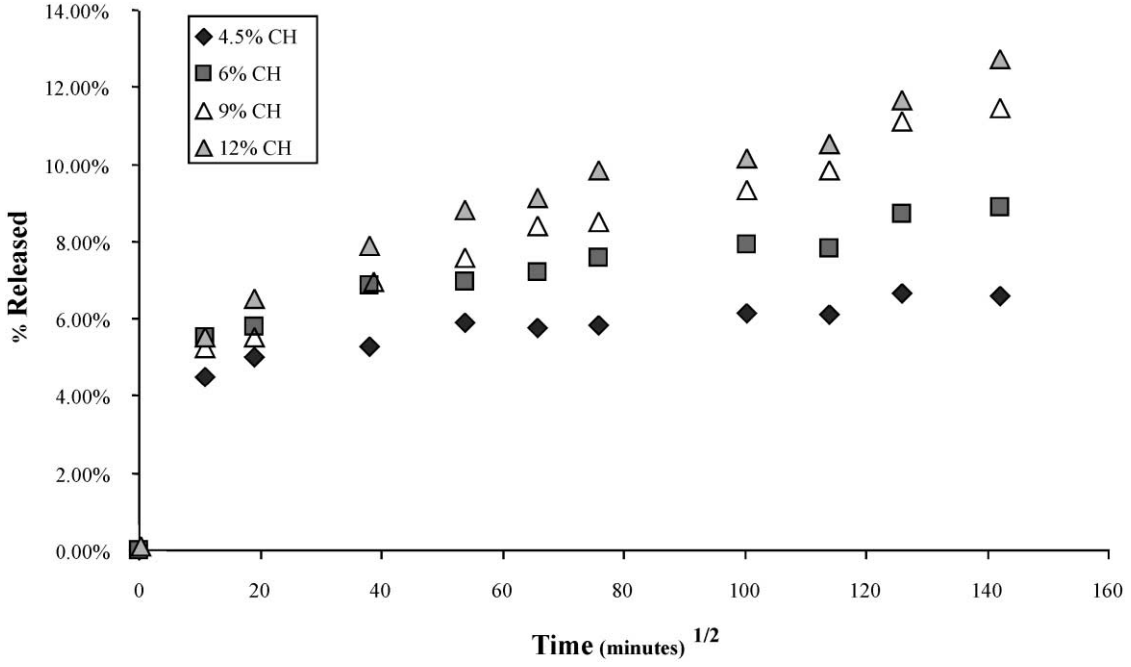

Fig. 2. Release of chlorhexidine from PEM/THFM DRD.

diffusion-controlled process as shown by the linear $t^{1/2}$ plot (Fig. 2) up to 14 days. There was also a dose-dependent difference, with more chlorhexidine being released from DRD's containing the higher concentrations of the drug (Fig. 2). Thus, although 6–12% of the incorporated CX was released over 14 days, between 50 and 80% of the total amount of CX released appeared in the first 24 h.

4.2. Microbiology

Co-exposure of the DRD containing 12% chlorhexidine with *C. albicans* (strain 132A) caused almost total inhibition of growth of the organism compared to controls (Fig. 3).

The effect of short-term exposure to the DRD containing 0, 9 and 12% chlorhexidine diacetate on the growth

of *Candida albicans* strain 132A is shown in Fig. 4. The control DRD (no chlorhexidine) had no inhibitory effect on the number of CFU/ml up to 5 h, whereas DRD's containing 9 and 12% chlorhexidine caused a dose-dependent decrease in the number of surviving CFU/ml (Fig. 4).

The candidal strains 132A and 132ACR revealed similar growth characteristics over the 5 h period with a steady growth up to 4 h followed by a rapid increase in CFU density during the final hour (Fig. 5). However, the growth of both strains was almost totally inhibited by the 12% chlorhexidine containing samples, with the number of CFU/ml dropping several orders of magnitude over the 5 h period (Fig. 5).

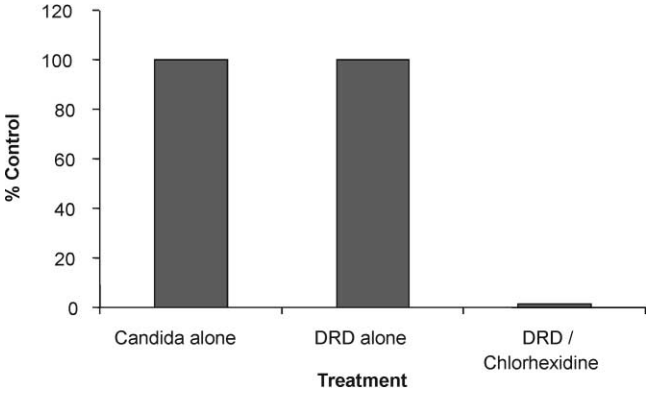

Fig. 3. Candidal growth in the presence of DRD with and without incorporated chlorhexidine.

5. Discussion

Chlorhexidine has been widely used in dentistry and is recognised as the agent of choice in plaque inhibition and gingivitis studies [20]. Its use as an anti-fungal agent has also been well established [5,8] and the results of this study support its action as an effective anti-candidal agent. Chlorhexidine was also able to kill the azole-resistant strain suggesting that it may be a useful alternative to fluconazole or miconazole where the development of resistance may become a problem [4]. The effectiveness of PEM/THFM containing chlorhexidine, also confirms that polymerisation of acrylic resins does not adversely affect the efficacy of the drug [21].

PEM/THFM absorbs up to 30% water depending on the osmolarity of the external solution [22] or the formulation of the DRD [23,24]. This process consists of two stages, a rapid Fickian process followed by the development of discrete clusters of water at, as yet, unidentified osmotically active sites [25]. In the presence of CX, the initial burst probably reflects surface release. The subsequent slow diffusion-controlled release may be the result of complex processes involving water cluster formation around chlorhexidine diacetate particles and the interaction of these clusters with the water uptake process of the polymer per se. These release processes are different to those involved in the release of hydrocortisone from PMMA, which was reported to occur through crazes, formed in the brittle PMMA by the osmotic forces consequent on the hydrocortisone inclusion [26]. Hence, PEM/THFM has superior drug release

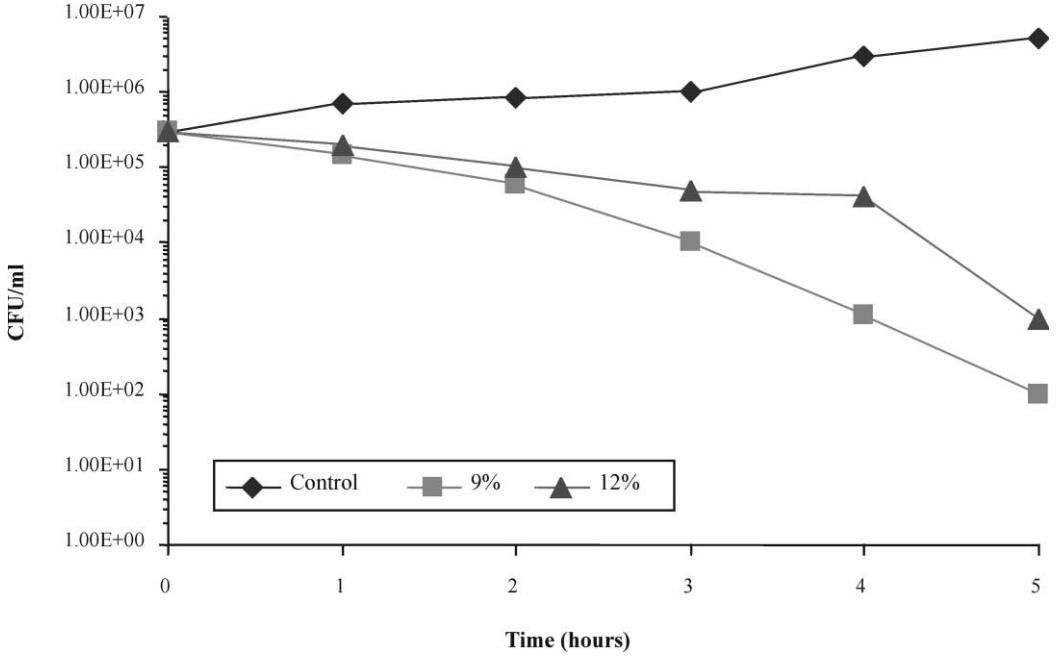

Fig. 4. Effect of DRD containing chlorhexidine on azole-sensitive (132A) and azole-resistant (132ACR) strains of *C. albicans*.

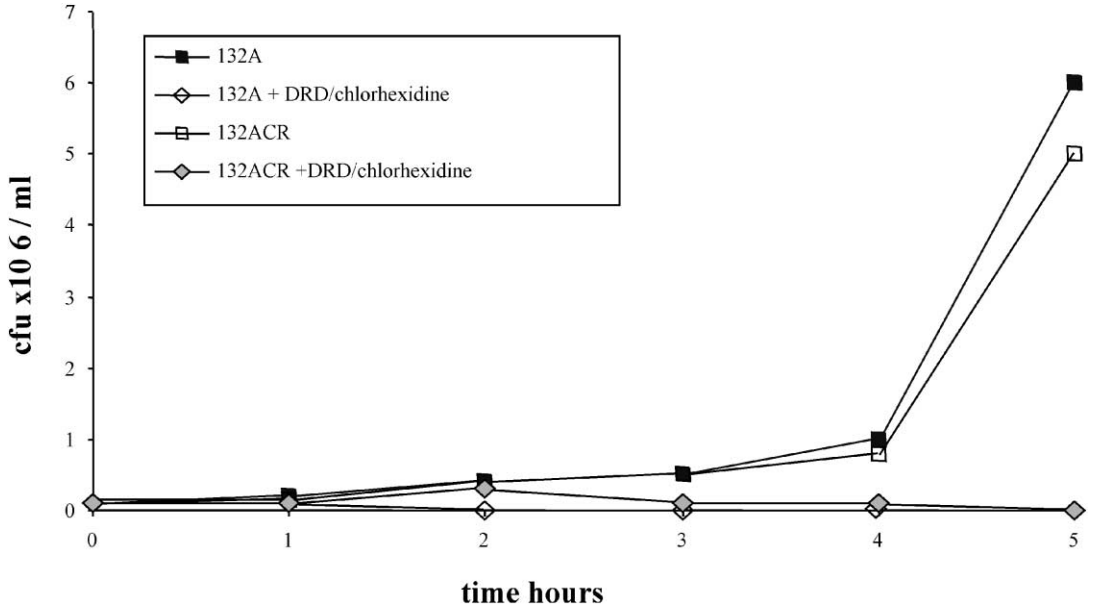

Fig. 5. Effect of DRD containing different doses of chlorhexidine on *C. albicans* 132A.

characteristics compared with both methyl methacrylate and *n*-butyl methacrylate-based systems [27,28]. The incorporation and subsequent release of chlorhexidine diacetate from PEM/THFM, is also in keeping with earlier findings on other drugs using this polymer system [15,16].

The mechanism by which CX affects candidal species is not clearly understood, although some studies have suggested that it may disrupt the fungal cell wall by binding to glucan moieties [29], inhibiting cell replication [6] or by preventing adhesion of candidal blastospores to mucosal epithelial cells or acrylic denture base plates [9,30]. This latter mechanism is important in the development of denture-associated candidal infections and has led to the use of CX as a treatment for these lesions [31]. Alternative delivery systems have been explored including the use of coatings applied to the denture surface [32–34] as well as incorporation of CX in denture base plates based on cold- or heat-cured PMMA polymeric systems [35–38]. Although these studies demonstrated release of CX and inhibition or killing of candidal species, drug incorporation within the polymers occurred either by adsorption after soaking in CX solutions or by the addition of methanol or water as a solvent with the polymer [35–38]. The use of a solvent may adversely affect the physical properties of the dentures [38], probably by the induction of crazing in the brittle structure of PMMA [26]. The incorporation of CX in PEM/THFM, however, was achieved by the simple inclusion of the diacetate salt with PEM powder. In addition, studies on the PEM/THFM system as a vehicle for fluoride ion release have demonstrated that even with over 100% water uptake, the loss of mechanical properties of the material is no more than that experienced with the parent PEM/THFM system (Patel et al., in preparation).

In conclusion, this study has demonstrated that chlorhexidine may be successfully incorporated into PEM/THFM and that the amount of drug released was sufficient to inhibit and kill both azole-sensitive and azole-resistant strains of *Candida albicans*. Its anomalous water uptake aids sustained drug release, which makes the polymer well suited for use as a tooth-borne appliance, denture lining or implant. PEM/THFM containing chlorhexidine may, therefore, provide a useful adjunct for the treatment of intractable or persistent candidal infections.

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