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Research paper

Characterization of a polyurethane-based controlled release system for local delivery of chlorhexidine diacetate

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ABSTRACT

Conventional formulations of chlorhexidine usually provide short-term efficiency, requiring repeated applications to maintain antibacterial activity. Therefore, appropriate release system of chlorhexidine controlling local drug delivery would reduce the number of applications and enhance patient compliance.

The aim of this study was to develop a controlled release system based on medical polyurethane for the local delivery of chlorhexidine diacetate (CDA). CDA-loaded polyurethane films (CDA-Films) and CDA-loaded polyurethane sandwiches (CDA-Sandwiches) were obtained by casting and solvent evaporation.

The physico-chemical aspects of CDA-loaded polyurethane systems were investigated, and the crystalline state of CDA in the polymeric system was highlighted. CDA-Films exhibited appropriate mechanical properties for further applications. Drug release was measured in two different media: (i) distilled water and (ii) physiological saline solution to mimic in vivo conditions. Drug release studies were performed up to 11 days on CDA-Films and 29 days for CDA-Sandwiches. Release of CDA depended on drug loading and the structure of the system. In particular, release of CDA from the sandwich system followed zero-order kinetic. The release rate was significantly lower in physiological solution. Antibacterial studies were carried out on CDA-Films against *Staphylococcus aureus* and *Staphylococcus epidermidis* showing 35 days persisting antibacterial activity.

In conclusion, the polyurethane-based system developed in this study is potentially useful as a local delivery system for CDA and could be used not only in surgery but also in dental and clinical applications.

1. Introduction

Chlorhexidine, a bis-biguanide antiseptic, is widely used to treat and prevent skin and mucosal infections with a low topical toxicity. In addition, it has been demonstrated that chlorhexidine has a strong substantivity within the oral cavity, making it efficient for antiplaque activity [1,2]. Since last two decades, chlorhexidine is recognized in dentistry as gold standard against antiplaque and gingivitis [2]. In this field, chlorhexidine is used either as rinse solution between 0.2% and 0.5% (w/v). Furthermore, several formulations containing chlorhexidine are available for topical delivery (e.g. scrub and rubs) and urinary or central venous catheter impregnation [1].

However, due to uncontrolled release, chlorhexidine activity might not be sustained in conventional formulations. Consequently, repeated applications needed to maintain its antibacterial efficacy might result in patient discomfort due to bitter taste, teeth discoloration and occasional mucosal irritations [3]. A controlled release system for antiseptic will prolong antimicrobial activity locally in order to reduce the number of applications or lower dosage form and enhance patient compliance.

Taking into account these potential adverse effects, sustained released delivery system based on ethylcellulose [4,5], cross-linked protein matrix [6], acrylic strip [7] and tooth-bonded chlorhexidine delivery based on poly (ε-caprolactone) [8] were proposed. More recently, innovative systems based on chlorhexidine-loaded chitosan microspheres [9], inclusion of chlorhexidine in cyclodextrin [10,11], poly (ε-caprolactone) nanocapsules (Nanochlorex®) [12,13], ethylene vinyl acetate copolymer containing chlorhexidine [14–16], methacrylate systems [17–19], and thermosensitive vinyl ether-based hydrogel were reported [20].

Polyurethanes are thermosetting polymer products of a step-reaction polymerization process. These synthetic polymers have been found in many applications as biomaterials due to their excellent physical properties and good biocompatibility. It is clinically used in central venous catheters, vascular grafts, mammary

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prostheses, implants and drug delivery systems [21]. Over the past few years, the development of antimicrobial-loaded polyurethane catheters has been reported as an approach for the prevention of nosocomial infections [22,23].

In the present study, polyurethane-based controlled release systems were developed in order to provide prolonged and controlled release of chlorhexidine diacetate for antiseptic activity. Medical polyurethane was evaluated as a drug carrier material to modulate the release profile. The systems prepared by solvent evaporation were characterized for (i) solid state of chlorhexidine and (ii) drug–polymer interaction by Differential Scanning Calorimetry, Hot-stage Microscopy and Attenuated Total Reflection Fourier Transform Infrared Spectroscopy. Complementary analysis of morphology and mechanical properties was conducted by Scanning Electron Microscopy and tensile test. Finally, antibacterial efficiency of chlorhexidine-loaded polyurethane system was studied against two Staphylococci strains and discussed from drug release profile.

2. Materials and methods

2.1. Materials

Chlorhexidine diacetate dihydrate (CDA), sodium chloride and sodium acetate were purchased from Sigma–Aldrich (Lyon, France). Tetrahydrofuran, glacial acetic acid and acetonitrile were supplied from Carlo Erba (Val de Reuil, France) at HPLC grade. Medical grade polyurethane (PU) (3M Unitek, Cergy Pontoise, France) was used as a polymer to incorporate CDA. Polycarbonate filters (0.45 μm) were purchased from Fisher Bioblock (Illkirch, France).

2.2. Preparation of chlorhexidine diacetate-loaded polyurethane systems

Samples were prepared by casting and solvent evaporation. Medical grade PU dissolved in tetrahydrofuran [7.5% (w/v)] was added to CDA powder under vortex agitation for one minute. Two types of PU systems were prepared. Firstly, unloaded PU-Film and 5%, 10%, 20%, 40% CDA-Films (w/w) were obtained by casting 2 mL of CDA–PU mixture in tetrahydrofuran into a flat bottom crystallizing dish (∅: 60 mm; 10-mm height). The solvent was allowed to evaporate at about 25 °C for 30 min. Secondly, 20% CDA-Film sandwiched between two unloaded PU layers (named 20% CDA-Sandwiches) was obtained by successively pouring and drying the 3 layers. The first unloaded PU layer was obtained by casting 2 mL of pure PU solution in a flat bottom crystallizing dish (∅: 60 mm; 10-mm height). The solvent was allowed to evaporate at about 25 °C for 30 min. The second layer of CDA-loaded PU was cast onto the PU layer as prepared for 20% CDA-Films described earlier. The third layer was obtained by repeating the casting as for the first PU unloaded layer.

For both systems, after solvent evaporation, crystallizing dishes were left in a hood overnight to complete drying. CDA-Films and sandwiches were checked until the weight remained constant.

2.3. Physico–chemical characterization of chlorhexidine diacetate-loaded polyurethane systems

2.3.1. Macroscopic observation

CDA-Films were thin, flexible and easy to handle. CDA-Sandwiches were thicker but still flexible. The thickness of each sample was measured in five positions by a digital thickness gauge (Mitutoyo, Tokyo, Japan).

2.3.2. Physico-chemical characterization

CDA, PU-Film, 5%, 10%, 20% and 40% CDA-Films were characterized.

2.3.2.1. Differential Scanning Calorimetry. Thermal properties of CDA-Films were characterized by Differential Scanning Calorimetry (DSC). Measurements were carried out on a Mettler Toledo DSC 812e module controlled by STARe software (Mettler-Toledo, Zürich, Switzerland). An aluminum pan was filled with 7.5 ± 0.5 mg of CDA-Films accurately weighed by a microbalance MT 5 (Mettler-Toledo, Zürich, Switzerland) and subsequently hermetically sealed. DSC analysis was performed from 25 °C to 180 °C with a heating rate of 5 °C/min, in inert atmosphere (N_2, flow 100 mL/min). The integral enthalpy of fusion (ΔH) and the normalized enthalpy of fusion (ΔH_f normalized, J/g) were calculated from peak area by STARe software. The normalized enthalpy of fusion was calculated in function of CDA amount in the sample.

2.3.2.2. Hot-Stage Microscopy. Hot-Stage Microscopy (HSM) experiments were carried out using a polarized light microscope OPTIPHOT 2-POL Nikon (Nikon, Tokyo, Japan) equipped with a Linkam HSF 91 hot-stage and a Linkam TP 93 heating system (Linkam Scientific Instruments Ltd, Tadworth, UK). Samples were subjected to a heating program from 25 °C to 160 °C at a rate of 10 °C/min in air flow. Photographs were taken at different temperatures by using a color camera Nikon Coolpix 4500 (magnification 200×).

2.3.2.3. Attenuated Total Reflection Fourier Transformed Infrared Spectroscopy. Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR) spectra of CDA, PU-Film and CDA-Films were recorded on a Thermo Scientific Nicolet iS10™ FT-IR spectrometer equipped with a Smart iTR™ ATR sampling accessory (Thermo Fisher Scientific, Illkirch, France). Samples were scanned from 4000 to 600 cm^{-1} at a resolution of 4 cm^{-1}.

2.3.2.4. Scanning Electron Microscopy. Scanning Electron Microscopy (SEM) was used to examine the surface morphology of PU and CDA-Films at 15 kV (Jeol 6400 apparatus, Jeol, Tokyo, Japan). Samples were fixed on a holder and coated with gold (10-nm thick) by metallization under vacuum.

2.3.3. Mechanical properties

The mechanical properties of films were determined from tensile test measurement, using a universal one-column electronic dynamometer (Acquati, Milan, Italy) at 25 ± 1 °C and relative humidity of 55%. Rectangular samples (45-mm length; 10-mm width) of 5%, 10%, 20% and 40% CDA-Films were tested. Samples were clamped between grips set at initial distance of 30 mm. Stress and strain were recorded during vertical extension at 50 mm/min. Tensile strength (MPa), elasticity modulus (MPa) and elongation at break (%) were determined from three replicates for each film.

2.3.4. Drug release study

Drug release was measured in two different media: (i) distilled water and (ii) 0.9% (w/v) NaCl aqueous solution adjusted to pH 7. All experiments were performed in a shaking bath thermostated at 37 °C by immersing disks (∅: 10 mm) of 5%, 10%, 20%, 40% CDA-Films and of 20% CDA-Sandwich in sealed glass vial containing 10 mL of the media. The experiments were done in triplicate. At fixed intervals, 1 mL of sample was withdrawn and replaced by pre-warmed medium. Samples were filtered through a polycarbonate membrane (0.45 μm) and analyzed by previously developed High Performance Liquid Chromatography (HPLC) [12]. Briefly, analysis was performed on Agilent 1200 series using the