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Montmorillonite–chitosan–chlorhexidine composite films with antibiofilm activity and improved cytotoxicity for wound dressing

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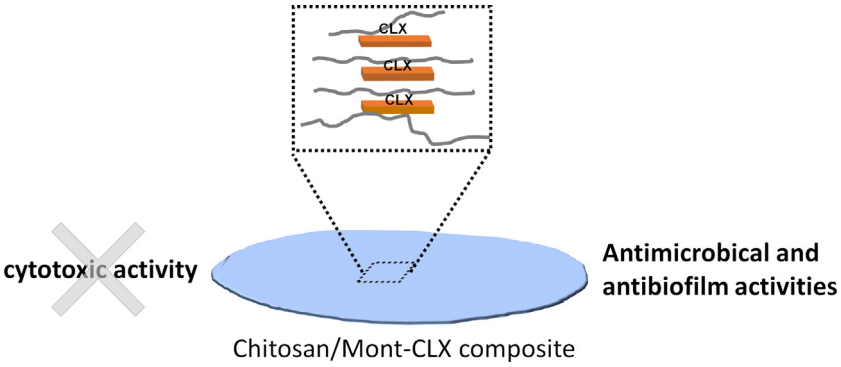
GRAPHICAL ABSTRACT

Chitosan films containing chlorhexidine intercalated into montmorillonite were prepared. Antimicrobial and antibiofilm activities and cytotoxicity were investigated.



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ABSTRACT

Hypothesis: Chlorhexidine (CLX) is a good antimicrobial agent, but its use in treatment of wounds is limited because of its cytotoxicity towards human fibroblasts. A delivery system, able to release CLX in a localized and prolonged manner, could guarantee antimicrobial activity with reduced cytotoxic. Thus in this work the preparation and characterization of chitosan/montmorillonite composite films containing CLX, able to offer a prolonged CLX release, is described. The antimicrobial and antibiofilm activities and cytotoxicity of films were investigated.
Experimental: CLX was intercalated between the layers of montmorillonite (MONT-Na), and the intercalated product (MONT-CLX) was characterized by X-ray powder diffraction (XRPD), thermogravimetric analysis (TGA) and FT-IR spectroscopy. Then chitosan/MONT-CLX films were prepared and characterized. For comparison, films loaded with neat CLX and MONT-Na/CLX were prepared. All prepared films were tested for their antimicrobial and antibiofilm activities. Cytotoxicity towards human skin keratinocytes and human fibroblasts HuDe was evaluated as well.

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Findings: All prepared films showed good antimicrobial and antibiofilm activities. As concerns cytotoxicity the film containing MONT-CLX at 1% CLX concentration resulted no cytotoxic. These results confirm the potential use of chitosan films containing MONT-CLX as a potential wound dressing material to prevent microbial colonization in wounds.

1. Introduction

The term biofilm indicates colonies of bacteria or fungi that are attached to a surface that can be both synthetic and natural. Biofilms adhere to a surface by means of adhesins such as flagellar proteins and the secretion of an extracellular polymeric substance composed of polysaccharides and extracellular DNA. When organized in this structure, microorganisms are protected from the hostile host environment and obtain benefits from the co-operativity as a part of community. Thus, once formed, the biofilm makes it more difficult for the host organism to eradicate the infecting microorganisms [1,2] with consequent risk of leading to chronic infections despite antibiotic treatment [3]. Chronic biofilm infections, such as catheter infections, endocarditis and osteomyelitis, often persist indefinitely unless the infected material is removed and this persistence is also evident in chronic wounds in which biofilm infection results in a delayed healing. In wounds biofilm infections can be reduced by debridement and treatment with systemic antibiotics. Unfortunately, no single strategy has proved consistently effective in suppressing biofilm, thus other tools should be associated such as topical antiseptics. They can penetrate biofilms and cause significant microbial death [4]. Chlorhexidine (CLX) is considered the gold standard antiseptic that is active against Gram-positive and Gram-negative bacteria, molds, yeasts and viruses. It shows antimicrobial properties and is used in topical formulations such as creams for cleansing and antisepsis of skin, burns and dermal wounds. Its use is limited to cleansing with short-term wound treatment because of its toxicity to primary human dermal fibroblasts [5]. A strategy for decreasing cytotoxicity could be a formulation able to release CLX slowly and in a localized manner. At the best of our knowledge only few papers deal on CLX localized and modified release formulations such as chitosan/hyaluronic acid wound dressings [6], polymeric multilayers [7], biodegradable microspheres [8], sodium carboxymethylcellulose composite film containing chlorhexidine–zirconium phosphate nanoparticles [9] and polysaccharide lyophilized wafers [10]. Among them only three report both on antimicrobial activity and reduced cytotoxicity [7–9]. Recently, a paper on CLX chitosan and MONT composite has been prepared and proposed for controlled oral mucosal delivery of CLX. In this composite chitosan and CLX are mainly adsorbed on the MONT surface [11].

Thus with the aim of decreasing the problem of cytotoxicity, a polymeric composite film containing CLX able to release it in a localized zone and in a prolonged way could be the proper tool. A polymeric composite is made by the combination of a polymer and an inorganic filler which is employed to improve the desired properties of the polymer. Clays are one group of nanofillers which have been widely used for the preparation of polymer nanocomposites. Clay minerals belong the group of silicates with layered structure. The layers are built from tetrahedral sheets in which a silicon atom is surrounded by four oxygen atoms and octahedral sheets in which a metal like aluminum or magnesium is surrounded by eight oxygen atoms. In montmorillonite the isomorphic substitution of some Al with Mg ions make the sheets negatively charged and thus the clay sheets are naturally found stacked on top of each other, with positively charged ions intercalated between the layers. These cations are exchangeable with other ones of larger sizes too, which can be intercalated and released under proper conditions. These properties make montmorillonite a very interesting matrix for pharmaceutical applications [12–21]. One polymer that has attracted attention for composites with pharmaceutical use is chitosan. It is a naturally occurring cationic polymer, composed of glucosamine and N-acetyl glucosamine units linked by β-(1–4)-glycosidic linkages. Under slightly acidic conditions, most of the amino groups of chitosan are protonated and the resulting chitosan contains multiple cationic charges with great affinity with the negative charged layers of montmorillonite [11,22–26]. In fact chitosan is easily able to intercalate into the negatively charged layers of montmorillonite to give composites with good characteristics such as mechanical properties, swelling, water uptake, thermal behavior and bioadhesion. Besides chitosan shows also beneficial properties for application to wound healing [22,23,27–29].

Thus in this paper chitosan was chosen as a polymer for preparation of composite films obtained using CLX intercalated between the layers of montmorillonite. The films were characterized and evaluated for their antimicrobial and antibiofilm activity. Cytotoxic effects against keratinocytes and fibroblasts were evaluated as well.

2. Experimental section: materials and methods

2.1. Materials

Nanofil 116 (cation exchange capacity of 120 mequiv/100 g of clay), used as MONT-Na, and CLX diacetate were kindly furnished by Rockwood Clay Additives GmbH (Moosburg, Germany) and Degussa AG (Hanau-Wolfgang, Germany) respectively. Chitosan medium PM (200–800 cP, 1 wt.% in 1% acetic acid, 25 °C, Brookfield) and glycerol 85% were purchased from Sigma-Aldrich Chemical (Milan, Italy). Deionized water was obtained by a reverse-osmosis process with a Milli-Q system (Millipore, Rome, Italy). Other reagents and solvents were of reagent grade and were used without further purification.

2.2. Experimental techniques

XRPD patterns were recorded using a Philips X'PERT PRO MPD diffractometer operating at 40 kV and 40 mA, with a step size of 0.0170 2θ and a step scan rate of 30 s, using Cu Kα radiation and an X'Celerator detector.

The CLX content of the intercalated compound was determined by coupled thermogravimetric (TGA) and differential thermal (DTA) analyses performed with a Netzsch STA 449 C apparatus in air flow at a heating rate of 10 °C min^{-1}.

FT-IR spectra were recorded in air, at room temperature on a Jasco FT/IR-410, 420 Herschel series (Jasco Corporation Tokyo, Japan) in KBr dispersion. Samples were prepared by gently grounding the powders with KBr. The data region was 4000–400 cm^{-1}.

2.3. CLX intercalation into MONT-Na

MONT-Na (3 g) was dispersed in 90 mL of deionized water by vigorous stirring for 24 h at room temperature. An aqueous solu-