Acta Biomaterialia 6 (2010) 2554–2561

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

# Antibacterial polyurethane nanocomposites using chlorhexidine diacetate as an organic modifier

N. Fong, A. Simmons, L.A. Poole-Warren *

*Graduate School of Biomedical Engineering, University of New South Wales, NSW 2052, Australia*

ARTICLE INFO

*Article history:*
Received 2 October 2009
Received in revised form 10 December 2009
Accepted 7 January 2010
Available online 13 January 2010

*Keywords:*
Polyurethane
Nanocomposite
Chlorhexidine diacetate
Antibacterial

ABSTRACT

Polymer nanocomposites (NCs) are hypothesised to have enhanced barrier properties compared with pristine polymer, allowing more sustained drug release from the materials. In these NC systems active agents are typically incorporated into the polymer matrix and the release kinetics are theoretically perturbed by well dispersed nanoparticle inclusions. An alternative approach is to exploit active agent interactions with the nanoinclusion. In the proposed NC system, the driving hypothesis is that active agents can have dual functionality, acting as both drug and dispersant. Polyurethane-montmorillonite (PEU-MMT) NCs were prepared in which the antimicrobial agent chlorhexidine diacetate (CHX) was evaluated as an organic modifier for silicate dispersion. CHX was incorporated at various concentrations through organic modification of MMT or within the bulk polymer. X-ray diffraction and transmission electron microscopy analysis suggested that intercalated and partially exfoliated NCs were achieved, with better dispersion occurring in the presence of free CHX within the bulk. Tensile testing results showed that variations in the level of organic modification and nanoparticle loading modulated the mechanical properties. Material stiffness increased with nanoparticle loading relative to pristine PEU, and the ultimate properties decreased with nanoparticle and free CHX incorporation. Antibacterial activity against *Staphylococcus epidermidis* was significant in materials with higher exchanged MMT and NCs containing free CHX, for which 2-log reductions in adherent bacteria were found after 24 h. CHX was successfully used to modulate the material properties in its dual role as a dispersant and antimicrobial agent, suggesting that alternative biocides of similar structure may behave comparably within PEU-MMT NC systems.


## 1. Introduction

Nosocomial infections affect more than 6% of all hospitalised patients in the USA, leading to approximately 99,000 infection-related deaths [1,2], increased hospital stays and a total cost of US$ 4.5–5.7 billion [3]. The majority of these infections are associated with the use of invasive medical devices such as urinary catheters, central venous catheters and endotracheal tubes [4], with significantly higher incidence rates among patients requiring long-term usage. In recent years *Staphylococcus epidermidis* has been recognised as the most important microorganism in device-related nosocomial infections [5].

A major virulence factor of *S. epidermidis* is the propensity to produce copious extracellular polysaccharides. The resulting biofilms are difficult to eradicate as they provide protection from the immune system and antimicrobial therapies [6]. Antibiotic therapy is often administered during the use of invasive devices, however, this practice is controversial as it contributes to high costs, adverse drug reactions and, of critical importance, has led to increasing resistance of microorganisms to antibiotics [7]. Microbial resistance against non-specific biocides, which encompass antiseptics, disinfectants and preservatives, is thought to be less likely than antibiotics due to their differing mechanisms of action [8].

Some current infection prevention strategies include the use of antibiotic or antiseptic coatings on device surfaces [9,10] and impregnation of device components with antibacterial silver [11]. However, these approaches are controversial, particularly in long-term applications. Coatings are usually only effective for short-term applications, given the high diffusivity of the matrix polymer [12], antibiotics are associated with microbial resistance [13] and silver-based antibacterial coatings have been reported to elicit cytotoxicity [14]. Thus further research into more appropriate antibacterial biomaterials for long-term solutions is still required. This study investigated a nanocomposite approach to achieving antibacterial functionality.

Nanocomposites (NCs) are defined as composite materials with the filler component having at least one dimension on the nanoscale. The potential benefits of NCs as biomaterials include enhanced mechanical [15,16] and barrier properties, the latter allowing more controlled or sustained drug release compared with

* Corresponding author. Tel.: +61 2 93853905; fax: +61 2 96632108.
*E-mail address:* l.poolewarren@unsw.edu.au (L.A. Poole-Warren).

1742-7061/$ - see front matter © 2010 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2010.01.005

other materials [17,18]. The NC system selected in this study was based on the silicate montmorillonite (MMT) as the nanoparticle within a poly(ether)urethane (PEU) matrix. Organic modifiers (OMs) are typically required in polymer NC systems to disperse inorganic nanoparticles, as well as compatibilise them with the organic polymer matrix. The resulting dispersion of the nanoparticles achieved following organic modification is illustrated in the schematic in Fig. 1.

Cationic surfactants such as quaternary ammonium compounds (QACs) are usually chosen for dispersion of silicates, since their charged head groups can interact with the silicate particles while their hydrophobic hydrocarbon tails facilitate silicate swelling and polymer intercalation. Due to these favourable structural characteristics, much work has been conducted to investigate QACs within polymer NC systems [19,20]. Styan et al. investigated the use of the QAC methyl tallow bis-2-hydroxyethyl ammonium chloride as an OM to disperse MMT throughout a PEU matrix. The resulting materials were found to have dispersed particles, with silicate spacing increasing from ~1.3 to ~3.7 nm [21]. Assessment of *S. epidermidis* adhesion on NCs studied by Styan et al. showed that the QACs also conferred antibacterial properties to the materials [22].

Other studies into the antibacterial activity of polymer NCs are still relatively scarce in the literature, however, such research has currently shown much promise in polymer NCs for antimicrobial applications. Wang et al. [23] studied the bactericidal activity of chitosan/organic rectorite NCs, in which the chitosan matrix was proposed to have an antibacterial effect, which improved with better silicate dispersion. Enhanced barrier properties of the NC materials were reported, as well as antibacterial efficacy against Gram-negative and Gram-positive bacteria. Another study by Zhao et al. [24] investigated the antibacterial properties of a polydimethyloxane (PDMS)/clay–silver–chitosan NC system against a range of urinary pathogens. Intercalated and exfoliated NCs were achieved demonstrating excellent antimicrobial properties in ring tests.

Other research groups have reported delivery of drugs from NC systems in which the drug was added to the polymer matrix [25,26] or antibacterial nanoparticles, such as silver, were incorporated within polymer matrices [27,28]. The present study focuses on a novel polymer NC approach whereby the antibacterial agent was selected to act as the OM. It was hypothesised that appropriate biocides may exhibit dual functionality, acting as both a dispersant and an active agent. The high surface area of the dispersed silicate theoretically supports sequestration of substantial concentrations of the antibacterial OM without having an impact on the polymer matrix properties. In fact, improvements in dispersion should directly affect the mechanical and barrier properties leading to polymers with better properties without the need for chemical modification of the matrix polymer. In addition, since the antibacterial is non-covalently bound to the silicate, its release from the polymer matrix will be possible.

The main objectives of this research were to study the effectiveness of the antimicrobial *N*,*N*″-bis(4-chlorophenyl)-3,12-diimino-2,4,11,13-tetraazatetradecane-diimidamide diacetate (chlorhexidine diacetate, CHX) as an OM and to evaluate the antibacterial activity of the resulting NC. CHX has been widely reported as an effective antibacterial compound and is believed to be less cytotoxic than QACs [29], as well as being less susceptible to microbial resistance than antibiotics [8]. Specifically, nanoparticle dispersion, mechanical properties and antibacterial properties of a series of NCs incorporating a range of CHX-modified nanoparticles and free, unbound CHX were investigated.

## 2. Materials and methods

### 2.1. Materials

An elastomeric PEU with 35 wt.% hard segment concentration was used, prepared from 4,4-diphenylmethane diisocyanate (MDI), the macrodiol poly(tetramethylene oxide) polyol (PTMO) (1000 g mol$^{-1}$) and the chain extender 1,4-butanediol (BDO) in the ratio 7.5:100:46.3, with 0.003 dibutyltin dilaurate added as catalyst [21]. A 5 wt.% polymer suspension was made using PEU pellets (Urethane Compounds, Melbourne, Australia) that had been washed in Milli-Q water and dried overnight in a 60 °C oven. The pellets were allowed to dissolve in dimethylacetamide (DMAc) (Sigma–Aldrich) for a period of 7 days, continuously stirred on a low heat.

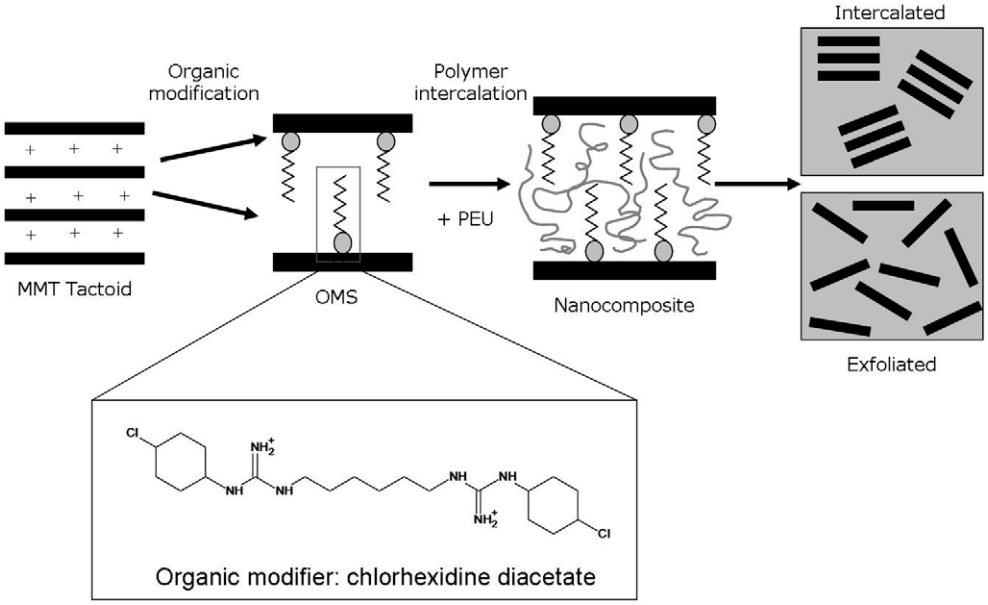


**Fig. 1.** Organic modification of layered silicates is often required to compatibilise the hydrophilic nanoparticle with the hydrophobic polymer, allowing polymer intercalation and hence, NC formation. The organic modifier used in this study was chlorhexidine diacetate, a dicationic molecule with a hydrophobic hexamethylene chain linking the cationic groups.

| ID | Title | Pages |
|---|---|---|
| 2432 | Antibacterial polyurethane nanocomposites using chlorhexidine diacetate as an organic modifier | 8 |