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# Thermosensitive hydrogel as an *in situ* gelling antimicrobial ocular dressing

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ABSTRACT

Microbial keratitis is a severe ocular condition and one of the most prevalent causes of corneal scarring and associated blindness worldwide. Risk factors include contact lens use, ocular trauma, ocular surface disease and immunosuppression. Initial clinical management mandates intensive (hourly or more frequent) topical administration of broad spectrum antimicrobial therapy for at least 48 h, which may require hospital admission, followed by tailored therapy based on microbiological investigation and the institution of strategies to reduce inflammation and promote healing. In this work we report an ocular wound dressing which can encapsulate and give sustained release of different antibiotics. The use of this dressing would allow patients to have eye drops on a 4 hourly basis, thereby facilitating treatment compliance and reducing hospital admissions.

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## 1. Introduction

A healthy and intact ocular surface is critical for maintaining the transparency of the cornea and enabling clarity of vision. Infectious or non-infectious disease processes which compromise the ocular surface may lead to abnormalities in corneal structure, with loss of integrity of the normal parallel alignment of collagen fibrils resulting in severe visual impairment. Corneal infection (microbial keratitis) rarely occurs in the healthy human eye due to complex host defence mechanism [1]. However, interruption of the corneal surface epithelium and/or an abnormal ocular tear film allows the introduction of micro-organisms, such as bacteria, fungi and protozoa, into the corneal stroma, where they are able to proliferate and cause inflammation and toxin release that contributes to destruction of the corneal substance [2]. Microbial keratitis is a serious ocular condition, and presents a significant disease burden that may result in severe visual disability [3]. Bacterial infection is the most common cause of infection with contact lens wear the biggest predisposing factor to corneal infection, although other risk factors are also associated, including recent ocular surgery, trauma, ocular surface disease, lid margin abnormalities, impaired corneal sensation, chronic use of topical steroids and systemic conditions such as diabetes mellitus, vitamin A deficiency and immunocompromised states [3]. Bacterial keratitis requires prompt treatment with intensive antimicrobial therapy to prevent sight threatening complications, such as scarring, corneal perforation and endophthalmitis [2]. Aims of treatment include initial rapid sterilisation of the infective pathogen, followed by reduction in the inflammatory response, prevention of corneal scarring, and facilitation of epithelial healing [4]. The avascular corneal tissue precludes effective local response from systemic administration of antimicrobial agents, and thus the initial sterilisation phase of treatment typically consists of frequent (hourly or more frequent) topical antibiotics [5]; hospital admission may be necessary where treatment compliance is unlikely or assistance is required with intensive topical therapy. Intensity of topical administration can then be tapered according to clinical response. As laboratory isolation of the infective pathogen may take some time, initial broad spectrum empirical antibiotic therapy is instituted to cover both Gram positive and Gram negative organisms. Topical fluoroquinolones (*e.g.* ofloxacin, levofloxacin and moxifloxacin) are both well tolerated and effective as monotherapy. Alternatively, dual antimicrobial therapy with fortified cephalosporin, such as cefuroxime 5%, and aminoglycoside (*e.g.* gentamicin 1.5%) are also effective [6]. Moreover, prolonged use of aminoglycoside may delay epithelial repair or cause local ocular surface to fluoroquinolone monotherapy may be more effective than dual therapy and is better tolerated [7,8,3].

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Moxifloxacin is a fourth generation fluoroquinolone antibiotic, with broad-spectrum activity against Gram negative and Gram positive bacteria. The mechanism of action of moxifloxacin involves inhibition of DNA gyrase and topoisomerase IV enzymes required for the replication, transcription and repair of bacterial DNA [9,10,11]. In *S. pneumoniae*, it has been shown that moxifloxacin is a poor substrate for active efflux, hence its efflux from bacterial cells is reduced [12], therefore increasing its antibacterial potency, it has also been shown to have lower MIC than fortified antibiotics against ocular pathogens [12]. Gentamicin is an aminoglycoside which inhibits bacterial growth by targeting the bacterial ribosome. It is a broad spectrum antibiotic and is especially effective against Gram positive *Staphylococcus* and Gram negative bacteria. Although effective, one of the major challenges in topically administered drugs is that they are cleaved and washed away rapidly, that is they remain on the cornea for less than two minutes, thus reducing their efficacy and needing frequent administrations by the patient on a daily basis. To enhance the efficacy of moxifloxacin on the corneal surface, various drug delivery systems such as hydrogel based gels have been evaluated. Gellan, alginate, karageenan and chitosan for example have shown to tailor the release of moxifloxacin over a longer time period and therefore reducing the need for frequent administration of the drug.

Chitosan is an aminopolysaccharide derived from the partial depolymerisation and deacetylation of chitin, a component found in the exoskeletons of crustacean shells [13]. Chitosan has been proven to be advantageous for medical applications due to its biocompatibility, biodegradability and low cytotoxicity, and has received much attention as the basis of a drug delivery system [14]. In 2000, Chenite et al. developed an injectable, thermosensitive, pH-dependent solution based on the neutralisation of chitosan by addition of β-glycerolphosphate [15]. The resulting solution is a reversibly thermosensitive polymer which is a liquid at room temperature, but a gel at 37 °C [16]. Thermoresponsive materials have gained increasing importance in potential treatments. These are usually formed from co-block polymers [17,18]. These materials are then used to incorporate therapeutics which can be released *in situ* [19,20]. Further developments in this field have moved towards utilising these polymer systems as smart materials where they can be tuned to respond to multiple stimuli or can act as a reporting device *in situ* [21,22]. Loh et al. have previously reported the use of a thermogelling system to deliver natamycin to the eye using poly(F127/PTHF urethane) hydrogels [23]. The thermosensitivity of the chitosan β-glycerolphosphate system is biomedically relevant in wound healing as the liquid can be applied to the front of the eye and flow over the complex topography of the wounded eye before the transforming into a protective, transparent, gel wound dressing [24].

The aim of this work is to develop a flowable wound dressing for the eye which can be administered as a liquid eye drop but forms a firm gel dressing on contact with the cornea. The gel has antibacterial drug, such as moxifloxacin or gentamicin, incorporated to eliminate bacteria which have colonised the wound and also acts as an occlusive dressing to protect the cornea.

## 2. Materials and methods

All materials used in this project were purchased from Sigma (Poole, UK) unless stated otherwise.

### 2.1. Bacterial strains

The bacteria used in this study were clinical isolates from patients at the Queen Elizabeth Hospital, Birmingham, UK;

### 2.2. Hydrogel preparation

This was carried out as previously described by Chenite et al. [15]. Briefly, chitosan was dissolved in (0.1 M) HCl solution using a magnetic stirrer for 3 h. The resulting solution was then chilled at 4 °C for 3 h. β-glycerolphosphate dissolved in deionised water was added drop-wise to the chilled chitosan solution whilst stirring to produce a clear, homogenous liquid solution. The solution was incubated at 37 °C to form a gel. For antibiotic loaded samples, moxifloxacin (100 μL, 0.25 μM) or gentamicin (100 μL, 1% v/v) were added to the Chitosan/β-glycerolphosphate liquid solution, vortexed for 30 s and incubated at 37 °C to form a gel.

### 2.3. Rheological characterisation

A parallel plate rheometer (TA instruments ARES system) was used to characterise the gelation process. The plate diameter was 35 mm and the frequency was 1 Hz. Chitosan (Heppe-Medical Chitosan, Halle, Germany)/β-glycerolphosphate solution was prepared as previously described. Temperature scans were performed on the hydrogel to characterise the thermosensitive gelation process, gelation was indicated by an increase in the storage modulus (G').

### 2.4. Antimicrobial efficacy testing of the hydrogel

The Chitosan/β-glycerolphosphate gels with and without moxifloxacin and gentamicin were prepared as previously described and inoculated with *S. aureus* from an overnight culture in Lennox Broth (LB) broth (10 μL, ~1 × 10 [6] cells). Six gels were prepared per treatment and set in a 24 well plate at 37 °C and LB Broth added to the well. The gels were incubated overnight at 37 °C. The gels were removed from the incubator and the broth removed. The gels were vortexed to break up the structure and serially diluted in sterile phosphate buffered saline (PBS) and then plated out on LB Agar. The plates were incubated overnight at 37 °C and the visible colony forming units counted.

### 2.5. Release of moxifloxacin and gentamicin from chitosan/β-glycerolphosphate gels

Chitosan/β-glycerolphosphate gels were loaded with antibiotics, moxifloxacin (100 μL, 0.25 μM) and gentamicin (100 μL, 1% v/v) and gelled at 37 °C. The gels were suspended in PBS (1 mL). At each time point the PBS was removed and fresh PBS added to the vial. The release of moxifloxacin was measured using absorbance at 293 nm according to literature procedures [25]. The release of gentamicin was measured using absorbance at 202 nm according to literature procedures [26]. The cumulative release was obtained by the summation of the previous absorbance values.

### 2.6. Antibacterial efficacy of released antibiotics

The solutions obtained from the release study were then tested for antimicrobial efficacy. *S. aureus* was grown overnight in LB Broth. The solutions were then diluted in LB Broth (1:1) and inoculated with *S. aureus* followed by incubation overnight at 37 °C. The solutions were serially diluted with sterile water and plated out on LB agar and the plates incubated at 37 °C overnight. The number of colonies on the plate was counted.

### 2.7. Cytotoxicity of gels to primary corneal fibroblast cells

Primary human corneal fibroblast cells were isolated from donor human corneas under ethics (08/H1206/165). The cells were cultured in DMEM substituted with fetal calf serum (10% *v*/v) and penicillin/streptomycin (1% *v*/v). Cells were harvested at confluency by removal of the media and the cell monolayer washed three times with PBS (5 mL) and then incubated in trypsin:EDTA (2 mL) for 5 min at 37 °C. Once the cells were detached from the culture flask the trypsin:EDTA was blocked using cell media and the cells seeded into a 24 well plate at a density of 50,000 cells/well and 6 wells/treatment group. The cells

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