Microencapsulation using glass microcapillary devices of clostridium difficile specific bacteriophages in pH responsive Eudragit® S 100 for colon targeted delivery

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1. INTRODUCTION

Clostridium difficile is a gram positive, spore forming anaerobe with the ability to survive for long periods in the environment. It is responsible for nosocomial diarrhoea and is a major healthcare burden worldwide. Transmission occurs via the faecal-oral route due to poor hygiene or as a consequence of antibiotic treatment in hospital patients. Currently, it is only susceptible to three marketed antibiotics and other treatment options include faecal transplantation and potentially bacteriophages (phages). Possibility of treatment failure and high reoccurrence rate has led to an increased interest in bacteriophage therapy. Bacteriophages are viruses with narrow host ranges which undergo specific phage-bacterial interactions. They are able to lyse C. difficile without subsequent dysbiosis of the colon typical of antibiotic treatment. They are self-amplifying, in that upon infecting C. difficile in the gut they are able to replicate and increase in concentration rapidly thereby effectively killing the infecting host population.

Enteric pathogens such as C. difficile pose a number of challenges for site-specific delivery of phages. The highly acidic environment of the upper GI tract renders phages inactive prior to reaching the site of infection. Therefore, microencapsulation of phages in a pH responsive polymer (Eudragit® S100) using a glass microfluidic device to facilitate encapsulation, for colon targeted delivery is proposed here.
the polymer chains and pH dependent release of the encapsulated phages (Figure 1a). To produce phage-encapsulated Eudragit® S100 microparticles a microfluidic glass capillary device was used to allow precise control over particle size and shell thickness (Figure 1b).

The aim of the study was to investigate long term stability of the device and develop a process for phage encapsulation in core-shell particles. Characterisation of microparticle morphology and evaluation of subsequent phage release kinetics was carried out.

2. EXPERIMENTAL

Core-shell droplets were produced using a two-phase glass capillary device with counter-current flow focusing placed on the stage of an inverted microscope. The orifice size of the collection capillary was 200 µm (Figs. 1c, 1d). The inner phase was an aqueous Eudragit® S100 solution (5% w/v) with phages at 10⁸ PFU ml⁻¹. The outer phase consisted of Miglyol 840 with 2% (w/v) polyglycerol polycricinoleate (PGPR) emulsifier and 1% (w/v) 4-aminobenzoic acid. The collected particles were cured for 24 h to allow adequate exposure to acid in the oil phase. The polymer was precipitated using 4-amino-benzoic acid in the continuous phase. Image J software was used to analyse the droplet sizes. Scanning electron microscopy (SEM) was used to take images of particles. Sorensen’s buffer (NaH₂PO₄ and Na₂HPO₄) 0.2 M at pH 7.2 was used to dissolve polymeric particles and release phage. Phage enumeration was carried out by the double-layer agar method. Briefly, 10 µl was removed at time intervals, serially diluted and spotted on a lawn of C. difficile on an agar plate. After 24 h anaerobic incubation, plaques were counted and recorded as PFU ml⁻¹.

3. RESULTS AND DISCUSSION

Long term study results showed that the droplet generation was stable for a period of 6 h without compromising the monodispersity of the droplets. The droplet diameter remained fairly constant throughout this period with the coefficient of variation (CV) staying below 2%. Initially, the flow of the inner and outer phases (1.2 and 7 ml hr⁻¹ respectively) was adjusted to achieve a dripping regime for stable droplet formation; therefore, stabilization took approximately 45 min before a stable droplet size within a narrow size range was achieved. The average diameter of the droplets was 155 µm with a mean CV = 0.9 %. This is an important parameter in therapeutic formulations as the size of the particles influences the release kinetics of the bioactive agent. Videos were recorded for droplet size analysis; Figure 1c shows a shot of the droplet and/or particle formation in a glass capillary device. Figure 1d shows SEM images of the different sized particles that can be tailored as desired with varying flow rates in a glass capillary device. The spherical nature of the particles demonstrates their ability to retain bacteriophages. Subsequent analysis showed the release kinetics of the bacteriophage in Sorensen’s buffer at pH 7.2. The phage release was observed to be a sudden burst followed by subsequent increase over a 6 h period. Burst release would be preferred to ensure a higher multiplicity of infection (MOI) which plays a crucial role in reducing bacterial numbers.

4. CONCLUSIONS

Preliminary results are encouraging showing suitability of pH responsive polymer Eudragit® S100 in encapsulating phages for colon targeting. Controlled release in alkaline buffer illustrates the promise of using the formulation as an enteric coating for colon targeted phage delivery. Future work is planned to control the release kinetics and evaluate phage encapsulation yield.