DEVELOPING A NEW CLASS OF PATHOGEN SURROGATES FOR WATER APPLICATIONS

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ABSTRACT

In the last a few years, we have conducted multidisciplinary research in developing a new class of surrogates for studying pathogen removal and transport in subsurface media and engineered water systems. This innovation has progressively advanced from developing protozoan surrogates (polystyrene microspheres coated with protein or vitamin), to virus surrogates (DNA-labelled silica nanoparticles with/without protein-coating), and currently bacterial surrogates (food-grade biopolymer microparticles). The new surrogates are harmless, user-friendly and inexpensive.

So far, surrogates for *Cryptosporidium parvum*, rotavirus, adenovirus and norovirus have been developed by mimicking the physiochemical properties and surface characteristics of the target pathogens. Experiments demonstrated that these new surrogates significantly outperformed the most used existing surrogates, which are unmodified microspheres for *Cryptosporidium* oocysts and MS2 phage for viruses. The *Cryptosporidium* surrogates have also been satisfactorily validated by overseas collaborators in filtration processes and water treatment (rapid sand filtration and coagulation).

The "micro mimics" approach has opened up a new avenue for understanding and assessing pathogen removal and transport in water systems, without the risk and expense involved in working with pathogens. Working with the water industry, we have been demonstrating the new surrogate technology in real-world settings, including pilot-scale sand filters in a drinking-water treatment plant and point-of-use domestic filters. For the first time, NZ's drinking-water filtration systems (also commonly used overseas) are being assessed for their efficacies in protozoan and virus removal. Our research findings will help implement preventive measures to reduce drinking-waterborne infection risks. Research outcomes will promote uptake of new surrogate technology for better protection of vital freshwater resources and drinking water supplies.

KEYWORDS

Water treatment, Microbial pathogens; Surrogates; Water Microbial Safety

PRESENTER PROFILE

Dr Liping Pang is a Science Leader at ESR. Her expertise is on the experimental investigations and modelling of contaminant transport in porous media, in particular in subsurface microbial transport. Her research has ranged from identifying groundwater contamination pathways using synthetic DNA tracers, to modelling subsurface transport of microbes, nanoparticles, colloid-associated contaminants, heavy metals, phosphorus and pesticides, and to setback distances and the impact of on-site disposal systems on groundwater quality.

In recent years she has pioneered multidisciplinary research in developing micro mimics using biomolecule-modified particles and lately food-grade biopolymer particles for studying pathogen transport and removal in water systems.

1 INTRODUCTION

Each year, tens of thousands of New Zealanders suffer from gastro illness due to microbial contamination of drinking-water supplies. Testing for pathogens is expensive, labor intensive, and often impractical. Current tools available for assessing microbial removal from water supplies are limited to *E. coli* (an indicator of faecal contamination) and turbidity for protozoa. However, protozoa can break through treatment filters even when the turbidity meets the guideline values and the absence of *E. coli* is no guarantee that there are no viruses present.

In our research we have focused on developing surrogates for *Cryptosporidium parvum* (Pang et al., 2012) rotavirus, adenovirus (Pang et al., 2014; Farkas et al., 2014) and norovirus (unpublished). These pathogens pose major health concerns and are frequently detected in surface waters in New Zealand and overseas.

Cryptosporidium and norovirus cause acute gastroenteritis in all age groups, while rotavirus and adenovirus are the leading causes of childhood gastroenteritis. *Cryptosporidium*, rotavirus and norovirus and are extremely infectious. The ingestion of a single norovirus (Teunis et al., 2008) or rotavirus (Graham et al., 1987) particle could lead to infection. Haas and Rose (1995) determined the action level of *Cryptosporidium* and proposed that 1-3 oocysts/10L water is sufficient for illness. Thus, these pathogens are often used in quantitative microbial risk assessments (WHO, 2011).

These pathogens, especially *Cryptosporidium*, tend to be more resistant to chlorination than bacteria (WHO, 2004). Adenovirus is also exceptionally resistant to ultraviolet irradiation (WHO, 2011), and it is the best marker for human viral contamination because of its prevalence in environmental waters (WHO, 2011).

2 SURROGATE DEVELOPMENT AND VALIDATIONS

The physiochemical properties (e.g. size, surface charge, hydrophobicity, surface macromolecules, density, shape etc.) of a pathogen play a very important role on its attention and transport in porous media. We have initiated a new approach in using

biomolecule-modified particles to mimic the physiochemical properties of a target pathogen.

The *C. parvum* surrogates were synthesised by covalently coating glycoprotein or biotin onto carboxylated fluorescent polystyrene microspheres that have size, density, and shape similar to *C. parvum*. These biomolecules have isoelectric points similar to that of *C. parvum* (pH ≈ 2) and glycoprotein is a major type of surface protein that oocysts possess. The surrogates can be counted by using an epifluorescence microscope and a spectrofluorometer. Filtration experiments with different sand media demonstrated that, compared to the unmodified microspheres, the surrogates achieved a superior match to the retention of the *C. parvum* oocysts, showing the same log-reduction. In contrast, results from the unmodified microspheres differed one-order of magnitude. The surrogates remained stable in size and charge for at least 22 months.

The *Cryptosporidium* surrogates have also been satisfactorily validated by overseas collaborators in filtration processes (Stevenson et al., 2015) and water treatment such as rapid sand filtration and coagulation (Monis et al., 2018).

The virus surrogates were synthesised by covalently labelling a double-stranded 302 bp synthetic DNA onto 70 nm or 30 nm carboxylated silica beads with/without coating with specific proteins. The DNA marker and selected proteins had similar surface charges to the viruses. The surrogates are analysed by using the quantitative polymerase chain reaction (qPCR). Studies have demonstrated that the new surrogates significantly outperform the existing virus model MS2 phage in predicting filtration removal of the target viruses. The surrogates demonstrated the same log-removals as the target pathogens. Conversely, MS2 deviated from the target pathogens in terms of their removal by 1–2 logs.

Working with the water industry, we have been demonstrating the new surrogate technology in real-world settings, including pilot-scale sand filters in a drinking-water treatment plant and point-of-use domestic filters. For the first time, NZ's drinking-water filtration systems (also commonly used overseas) are being assessed for their efficacies in protozoan and virus removal.

Under a Marsden Fund project, we are currently developing micro mimics for investigating *Legionella* mobility and persistence in plumbing systems using food-grade biopolymer microparticles.

3 CONCLUSIONS

Our studies suggest that biomolecule-modified particles are promising new class surrogates that can be used to study the abiotic aspects of microbial pathogens in their interactions with water systems - such as adsorption/desorption, filtration and transport. This approach has opened up an exciting new direction for studying pathogens in water systems without the health risk and extreme cost that would normally be involved with pathogens. With further validations, the new surrogates developed in our studies could be a new tool to assess the performance of water and wastewater treatment and to study pathogen retention and transport in subsurface media after land disposal of effluent and

biosolids. With a new tool that enables the quantitative prediction of contamination risk, we can better protect public health. It will help implement preventive measures to reduce drinking-waterborne infection risks.

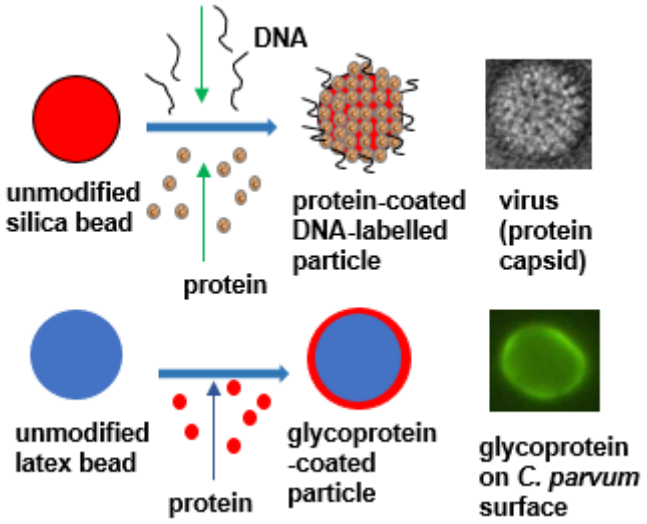

Figure 1: *Illustration of micro mimics – surface modification. Surrogates were synthesised by covalent coupling of biomolecules (protein, DNA) onto particle surfaces.*

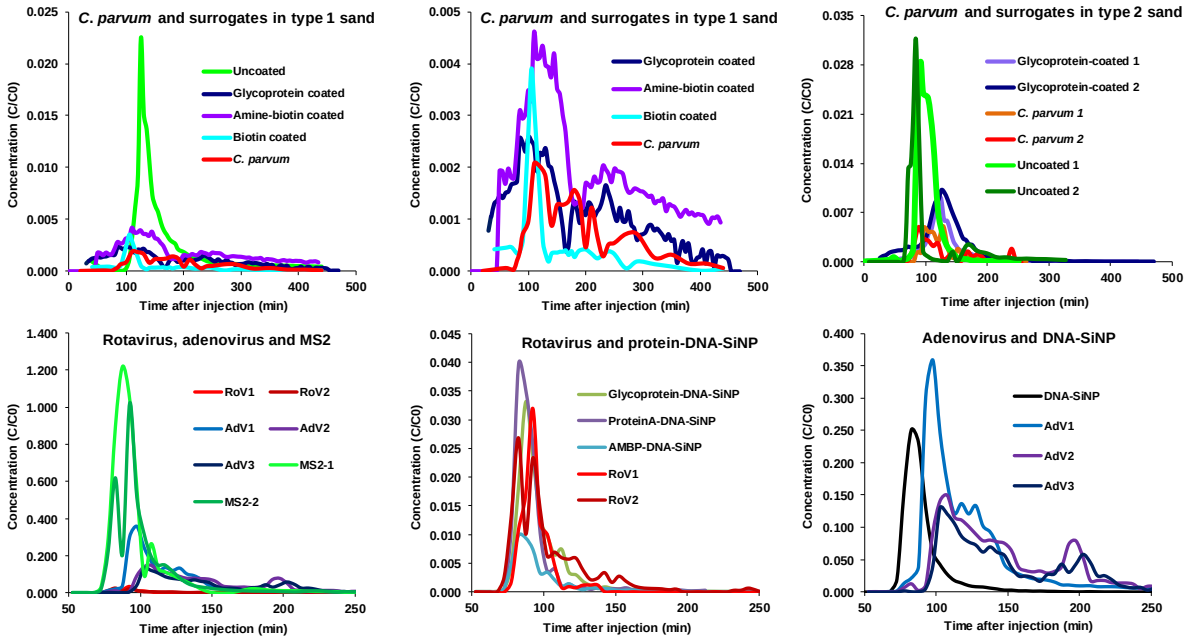

Figure 2: *Micro mimics – mimicking pathogen filtration removal in aquifer media. In the filtration experiments with natural sand media, the new surrogates predicted the concentrations and attachment/detachment of rotavirus, adenovirus, and Cryptosporidium better than existing models (unmodified microspheres and phage MS2).*

ACKNOWLEDGEMENTS

This work was supported by the Royal Society of New Zealand and the Institute of Environmental Science & Research Ltd.

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