Sophistication of biological simplicity

Dr Roderick Slavcev heads up a laboratory interested in exploiting bacteriophages as a means of producing biotherapeutics, such as DNA ministrings. Here, he discusses his background, current work and the potentially vast applications of his team's research

Could you begin by providing an insight into your background and how this led to your current work on the use of bacteriophages in therapeutic applications?

My background is in microbial genetics – in particular, bacteriophage genetics. During my graduate studies I became enthralled with the malleability of bacteriophages and the ease with which they can be manipulated and exploited toward the low-cost production of new biotherapeutics. During my MBA, I learnt about the commercialisation process around this and how to focus designs on providing holistic solutions that simultaneously enhance safety, efficacy and production efficiency.

Bacteriophages were used by the former Soviet Republic of Georgia and the US during the 1920s and 1930s to treat bacterial infections. Why was their development halted?

Bacteriophages, or phages, are natural antibacterials and were used as such for many years without many controlled scientific measures until the 1940s. The advent of antibiotics replaced them as the preferred antibacterial choice. However, they have recently resurfaced as antibacterial alternatives due to the issues surrounding antibiotic resistance. Despite this, the use of phages as antibacterials poses its own hazards. The ability of phages to exchange, collect and horizontally transfer pathogenic DNA from one host to another imparts the possibility for disease transfer. A number of pathogenic genes are encoded by phages.

I have moved away from phages as antibacterials and have instead focused on the manipulation of these malleable, self-assembling entities. Phages constitute the largest reservoir of nucleic acid on the planet and, as such, offer limitless biotechnological applications to medicine, agriculture and industry.

Can you tell us more about your work on phages and what some of its applications are?

My work is best explained as the exploitation of phages and phage genetic elements in the design of new biotherapeutics, hence the establishment of our Mediphage Bioceuticals name. While phages' genetic systems may be very complex, they are the most biologically rudimentary entities and are thus, generally easily manipulated both genetically and physically. I consider our work the 'sophistication of biological simplicity' for exactly that reason. We are currently applying phages to the development of newly engineered antibacterials, vaccines and gene therapeutics.

What is gene therapy and what has resulted from it?

Gene therapy is an experimental technique that uses genes to treat or prevent disease. This can be performed by either replacing or preventing gene expression in a way best suited to maximising treatment. Bacteriophages are safe to humans as they can only infect bacteria and not mammalian cells, but it has been shown that they can be engineered to deliver genes to eukaryotic cells. Moreover, because they are encapsulated in a protein capsid, they protect the nucleic acid cargo being delivered and, in some phages, the capsids can be further engineered to target specific cells to maximise safety and efficacy.

Our laboratory has also learned to modulate the decoration of phages with other proteins to maximise utility and effectiveness. We call these systems intelligent Phage Encapsulated Gene Expression Systems (iPhAGES). There are, however, caveats to the use of iPhAGES, which we believe makes them best suited to the targeted delivery of DNA vaccines.

Which properties of bacteriophages make them ideally suited to a vast range of applications, such as DNA ministring production?

Phages comprise a virtually limitless reservoir of genetic material and systems that can be exploited for the efficient, efficacious and safe design of gene delivery vectors. Protelomerases are specialised enzymes that can act at a specialised sequence in a larger circular molecule to cut and relegate as a closed end, thus conferring a stable linear molecule, known as a linear covalently closed (LCC) molecule. With more than one site present within the parent DNA molecule, multiple smaller linear LCC molecules can be formed and this can all be done within an *E. coli* cell expressing only the protelomerase when we want it to.

Turn it on and we can generate the smallest active gene therapeutic in *E. coli*, while separating all the bacterial junk from the DNA vector that is wanted. We call the resultant minivectors 'DNA ministrings'. They are important because they improve the longevity of gene expression and prevent any unwanted immune responses in the patient. They also dramatically improve our ability to deliver the gene of interest to the nucleus of the desired cell and, perhaps most importantly, they are very safe.

Going (non)viral

A multidisciplinary team based at the **University of Waterloo**, School of Pharmacy, Canada, has developed DNA ministrings – an extremely safe and effective type of DNA vector for gene delivery. The researchers' findings are establishing a new gold standard for non-viral gene therapy

DISEASE CAN BE caused by a range of influences, such as injury, infection or an unhealthy lifestyle. However, it can also be influenced by an individual's genes, which are effectively the complete set of instructions that guide the body's operations, necessary to grow and survive in a healthy manner. If a gene becomes damaged then the information it passes to the body is altered and the product or function it governs is compromised.

Gene therapy is a means of treating or preventing disease by introducing genetic material into an individual's cells. Although it is still a largely experimental endeavour, it has been shown to be an extremely powerful and effective means by which to repair compromised genes. At its simplest description, gene therapy functions by delivering a normal or wildtype copy of the gene into the cells that harbour the damaged version (allele), enabling the production of the normal gene products, thereby treating the abnormality.

During the gene therapy process, DNA is delivered to the specific cell through the use of a nucleic acid carrier known as a vector. The systems used are generally known as either viral or non-viral vectors. Viral vectors work because all viruses naturally introduce their genetic material into a cell when attacking it. This genetic material encourages the production of more copies of the virus, leading to the spread of infection. Ingeniously, scientists have found ways of manipulating this process, removing the genes in the virus that cause the disease and replacing them with 'good' genes; however, there are important safety caveats to consider. The use of viral vectors has traditionally been viewed as the most effective means of performing gene therapy, not least because of the high levels of transfection (the process of introducing nucleic acids into cells) and gene expression associated with non-viral vectors. However, recent technological developments have improved to the extent that non-viral methods are now seen as advantageous over their viral counterparts.

BACTERIA EATERS

A group of researchers at Mediphage Bioceuticals (MB) – a School of Pharmacy, University of Waterloo based team – has committed itself to developing new therapeutics that make use of gene therapy practices. Led by Dr Roderick Slavcev, the multidisciplinary team has focused its research efforts on manipulating naturally occurring bacterial viruses to develop a range of new and effective treatments with impressive findings and potential applications.

Bacteriophages (or phages) are viruses that infect bacteria and then replicate only within them – and they have been used as an effective alternative to antibiotics for many decades. First discovered to be antibacterial agents during the 1920-30s, they were used to treat soldiers in Russia's Red Army. "Phages have a rich genetic and therapeutic history and offer powerful potential for genetic and physical manipulation," Slavcev shares. It is with this in mind that he and his team have set about exploiting a specific type of bacteriophages that enable successful gene therapy for a range of ailments.

INTRODUCING DNA MINISTRINGS

The researchers have discovered a means to exploit the bacteriophage PY54 Tel/*pal* recombinase system to develop a novel platform for the efficient production of linear covalently closed (LCC) DNA minivectors. Their work centres on constructing efficient and scalable processes to generate DNA vectors *in vivo* in simplified, scalable and optimised ways. Known as DNA ministrings, these DNA delivery vectors have been proven to be both extremely safe and highly effective.

Indeed, Slavcev and his colleagues have established that the efficacy of DNA ministrings – as related to *in vitro* transfection studies – is, statistically, much higher than conventional DNA vectors and even higher than the closest substitute, DNA minicircles, in nondividing cells. "The linear and minimal nature of DNA ministrings is much safer than conventional vectors and is highly protective against any unwanted integration events that might occur in the human chromosome, which we have shown, cannot be conferred by any circular vector," explains Slavcev.

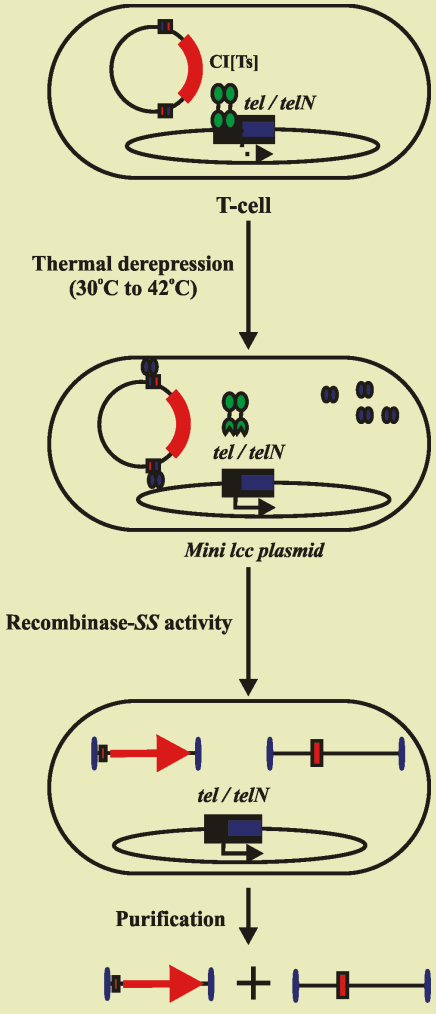
Ultimately, the researchers' findings have resulted in the most efficient *in vivo* LCC DNA vector production system to date, and their modified and optimised DNA ministrings are currently the most effective vectors for transgene delivery. They have demonstrated superior cellular trafficking and an efficiency of transfection to rival adenoviral vector transduction and gene expression.

BOUNDLESS POTENTIAL

The team's research has led to its current work which focuses on purifying and optimising concepts to improve the efficiency of DNA ministring production. Although its use as a one-step *in vivo* system is both scalable and inexpensive, it is not yet 100 per cent efficient and so additional genetic tricks are required to move the researchers' development forward. It is essentially a case of honing in on what they want and removing precisely what they do not want. "We hope to eliminate all other intracellular by-products produced as a result of the DNA ministring conversion production and perfect the purification process," Slavcev discloses. "This will be done through the use of a quick, simple and high-volume membrane chromatography technique that provides uncontaminated human grade DNA ministrings. The goal is maximal production efficiency, perfect safety and viral-level efficacy."

Excitingly, bacteriophages have potentially limitless therapeutic applications and, now the use of them has been shown to be both safe and effective, research in this area is set to expand and thrive. Indeed, the researchers' ambitions stretch to meeting the global need for treatments in a wide range of areas including an HIV vaccine, novel antibacterials, an ovarian cancer therapeutic and even an anti-acne cosmeceutical.

Additionally, bacteriophages can be freeze-dried without reducing their efficacy, meaning that they can be packaged and shipped across the world to warmer climates without the requirement for refrigeration. Their applications are bound only by imagination, especially given the vast amount of genetic information they carry.



Ministring production system. At 30 °C, *E. coli* process genetic material into DNA ministrings. At 42 °C, bacteria produce linear covalently closed DNA ministrings via Tel protelomerase activity.

SOPHISTICATION OF BIOLOGICAL SIMPLICTY

OBJECTIVES

- To manipulate of bacteriophages and develop novel therapeutics and cosmeceuticals from them
- To investigate phage-based treatments to global issues, especially in less developed countries

KEY COLLABORATORS

DNA ministring team: **Dr Nafiseh Nafissi**, **Ms Shirley Wong** (PhD candidate), **Ms Jessica Nicastro** (PhD candidate)

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RODERICK SLAVCEV gained a PhD in Molecular Genetics from the University of Saskatchewan before taking up a position as a medical liaison for the pharmaceutical industry and later completing a postdoctoral fellowship at the University of Toronto. Currently, he is an Associate Professor at the University of Waterloo, School of Pharmacy. He is also a member of the Waterloo Institute for Nanotechnology and the Waterloo Biomedical Engineering Institute.