# Sustained release of growth factors for therapeutics

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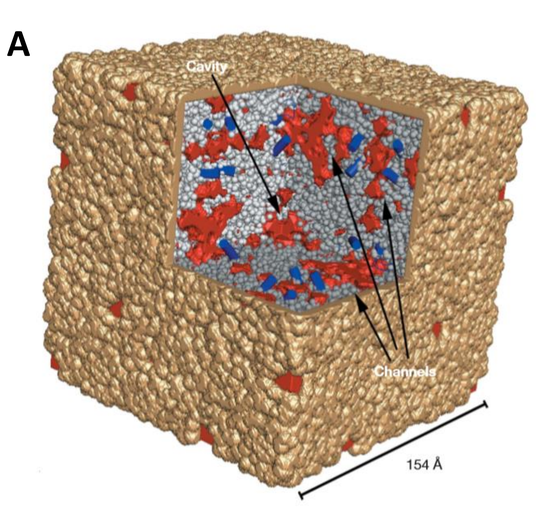
## Abstract

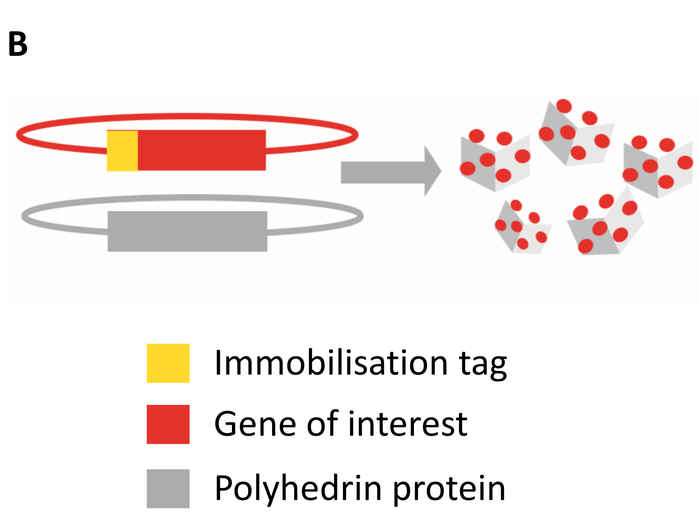
The inherent instability of recombinant growth factors, with typical half lives ranging from minutes to hours, limits their utility both in the lab and during the transition to the clinic. Furthermore, it is difficult to achieve gradients using standard recombinant growth factors without using special culturing chambers. PODS™ (**PO**lyhedrin **D**elivery **S**ystem) is a highly durable, crystalline product which encases a protein of interest within a polyhedrin protein. This technology exploits the Bombyx mori cypovirus, which encases its mature virion within a polyhedrin protein crystal in order to increase its stability. This stability means that PODS™ crystals degrade slowly, resulting in a steady release of cargo protein over several weeks. The sustained release mechanism of PODS™ growth factors can be applied in many ways, from cultivating organoid cultures to functionalising surfaces and scaffolds. We outline how PODS™ crystals were used to establish a local neurotrophic growth factor gradient. Furthermore, we also demonstrate the therapeutic potential of PODS™ crystals embedded in a collagen scaffold, using a small animal model of bone repair.

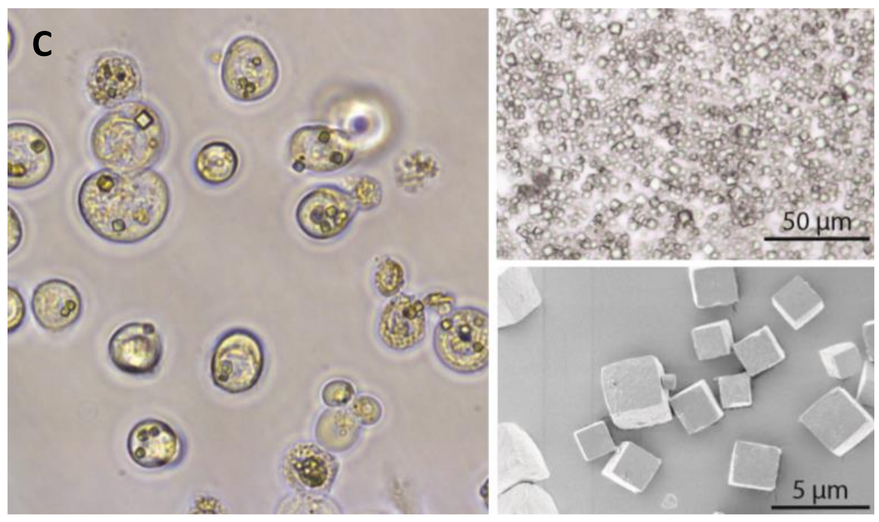
## Introduction

*Bombyx mori* larvae infected with cypovirus express a cypovirus-encoded polyhedrin protein, which crystallises within cells to encase and protect the virion. Polyhedra has a tightly-packed crystal structure[1] (A), which during infection protects the virion and enables greater durability of the virus between hosts. PODS™ are synthesised and purified from cultured insect cells by co-expression of polyhedrin protein with a cargo protein (B, C). This results in target protein being encased and protected within the polyhedrin crystal. The purified polyhedrin crystals contain intact, native, and functional protein. PODS™ are extremely stable in storage and cargo protein is slowly released in an active form in cell culture. This stability of PODS™ growth factors means they can be used to functionalise surfaces and scaffolds, create gradients, and achieve better therapeutic outcomes.

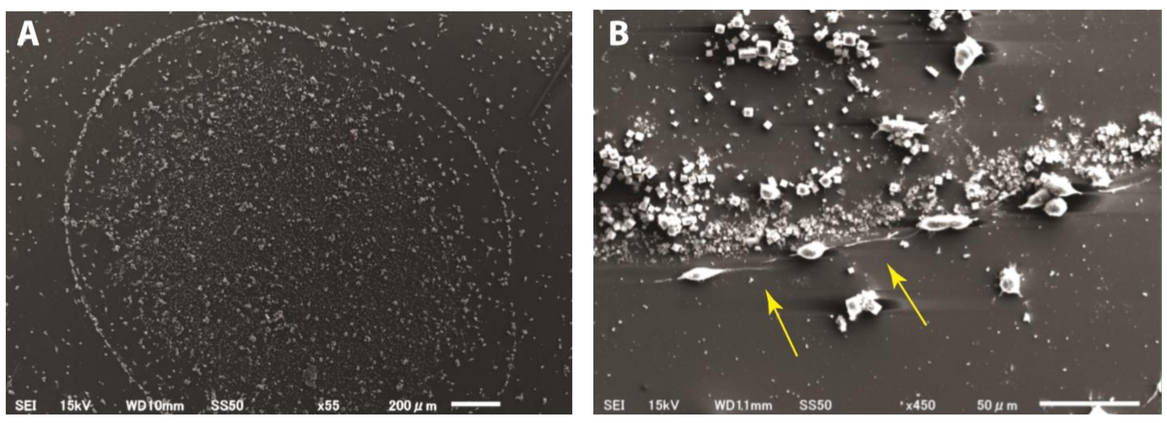
Neuronal cells migrate up a neurotrophic growth factor (NGF) gradient, which promotes survival and differentiation of neurons. However, a gradient can be difficult to achieve using standard recombinant NGF, which does not generate a long-term and stable gradient. We sought to identify whether a lower, constant and more physiologically-relevant dose and NGF gradient could be achieved through the application of PODS™ NGF. We also tested whether using PODS™ BMP-2 to provide a longer therapeutic exposure improves bone repair in a small animal model.



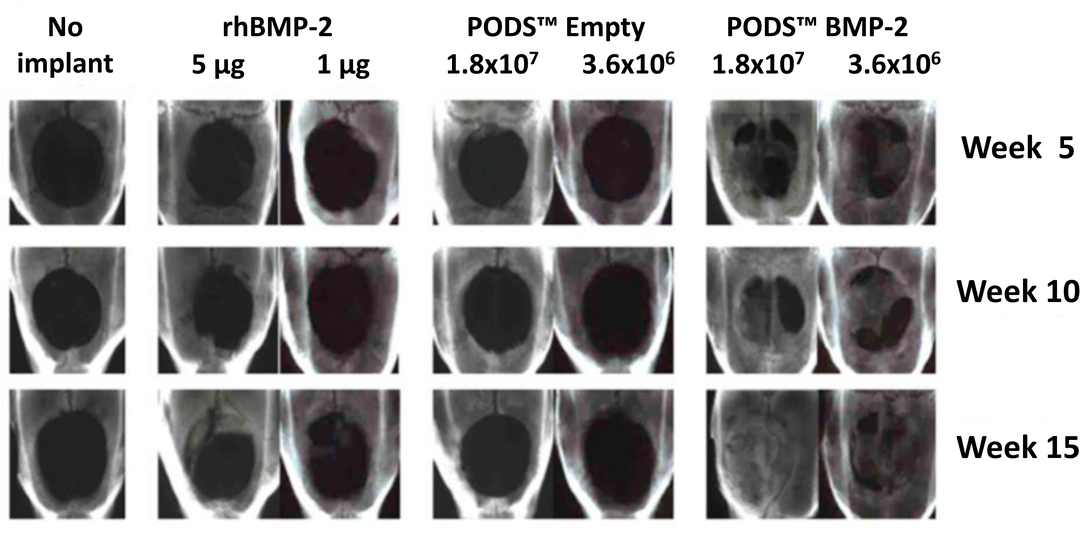




## Using PODS™ NGF to create a growth factor gradient




A) A circular field of PODS™ NGF was created by pipetting them directly onto a culture dish. PC12 cells were subsequently plated and incubated without media change for four days. This created a steady, physiologically-relevant NGF gradient at the periphery of the field. The PC12 cells migrated to form a ring encircling PODS™ NGF. (B) Higher magnification image showing PODS™ NGF (upper region) are clearly intact after four days. Neurites (arrowed) extend from the PC12 cells perpendicular to the PODS™ NGF gradient. This demonstrates that PODS™ NGF induces migration, alignment and attachment of PC12 cells via axon extension.

## Functionalising implants with PODS™ BMP-2 to promote bone formation




A 9 mm circular section of the rat skull was removed and an atelocollagen pad infused with either rhBMP-2 or PODS™ BMP-2 was implanted. Bone formation was monitored over 5, 10 and 15 weeks using x-ray radiographs. PODS™ BMP-2 at low and high doses produced significantly more bone at all time points compared with rhBMP-2 or PODS™ Empty. Histological analysis revealed that crystals were still visible after 12 weeks. No inflammatory response was observed.

## Conclusions/References

- PODS™ lock growth factors in a highly stabilised form
- PODS™ deliver on long term sustained release
- PODS™ makes it easy to functionalise and pattern surfaces and scaffolds
- PODS™ generate high levels of efficacy from low, non-toxic, targeted doses

1. Mori et al. Expression of Bombyx mori cytoplasmic polyhedrosis virus polyhedrin in insect cells by using a baculovirus expression vector, and its assembly into polyhedra. J Gen Virol 1993;74(Pt 1):9 –102
2. Coulibaly et al. The molecular organization of cypovirus polyhedra. Nature 2007;446(7131):97–101