Designing Recombinant Baculovirus/Nanomaterial based Hybrid Hydrogels for Gene Delivery Applications

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Statement of Purpose: Baculoviruses are a diverse group of enveloped DNA viruses belonging to the class Baculoviridae. They are pathogenic to arthropods and are known to mainly infect insect cells. Although they can enter mammalian cells but cannot replicate unlike mammalian viruses. Due to this unique non-pathogenic feature baculoviruses, containing mammalian expression cassettes to direct gene expression into mammalian cells, have immense potential for gene therapy applications. However, in most studies the baculovirus vector cannot match the transfection efficiencies of mammalian viral vectors, nor do they allow long-term transfection. To overcome many of these drawbacks, we propose a system whereby baculoviruses, conjugated to single walled carbon nanotubes (CNT), will be entrapped in a three-dimensional collagen hydrogel matrix. It is hypothesized that the collagen matrix will serve as a reservoir for the nanohybrids complexes and will result in a slower rate of baculovirus release leading to delayed cell transfection, and longer transgene expression time.

Methods: The hydrogel system consists of denatured collagen matrices. The matrix is loaded with MGFP reporter gene carrying baculoviruses (Bac) attached by ionic interaction to polyacrylic acid (PAA) wrapped CNT. Here, the hydrogel works as a reservoir to carry, protect, and simultaneously deliver the recombinant baculoviruses to the cells in a sustained manner. Rat mesenchymal stem cells were overlaid on the collagen hydrogel formulated with CNT (25µg/ml), Bac_{MGFP}, CNT/ Bac_{MGFP} or (f) functionalized CNT (f-CNT)/Bac_{MGFP} and observed under fluorescence microscope for transgene expression over time.

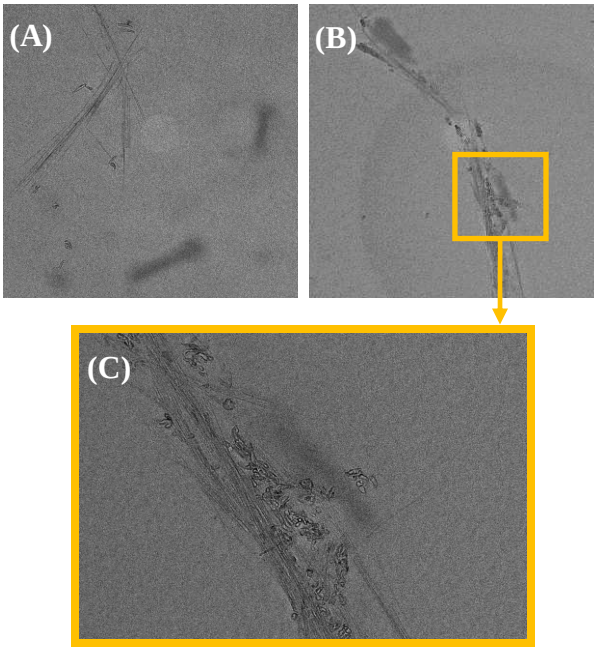
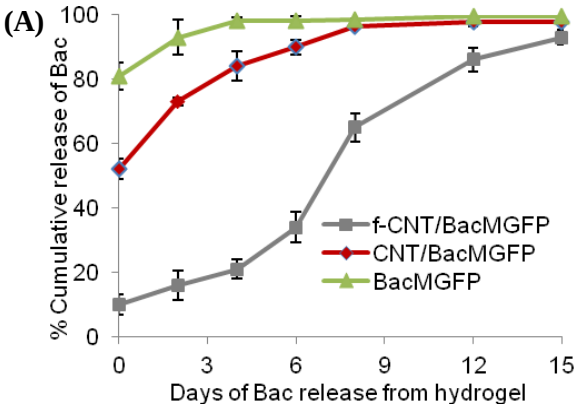


Figure 1: TEM images of CNT/baculovirus complexes.



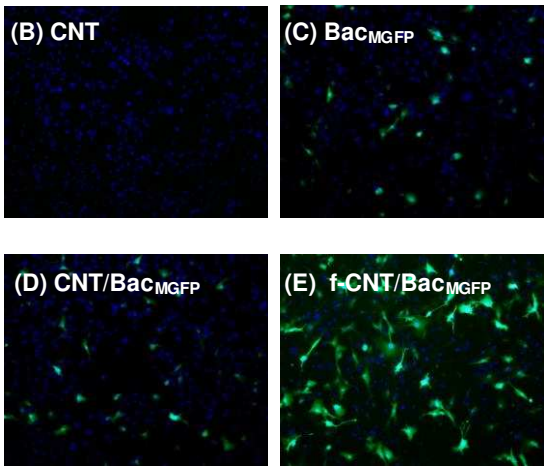


Figure 2: Sustained gene delivery features of CNT/baculovirus reinforced hydrogel

Results: Controlled release of baculovirus using CNT reinforced hydrogel was noticed. Figure 1 shows the TEM images of **(A)** non-functionalized CNT with Bac and **(B&C)** CNT functionalized Bac**.** Scale bar indicates 100nm length. Figure 2(A**)** demonstrates that CNT helps in sustained release of Bac incorporated in denatured collagen hydrogel (2.5mg/ml) over time. Fluorescent microscope data obtained after 14 days in CNT/ Bac_{MGFP} group show higher number of MGFP expressing stem cells compared to other groups (Figure 2B-E). This data confirms the extended release behavior of Bac from f-CNT reinforced hydrogel which resulted in sustained transgene expression in transduced stem cells.

Conclusion: The study demonstrate that the CNTs incorporated in the hydrogel can play a major role in extending the gene expression time for baculovirus mediated gene therapy applications.

References: 1. Holladay C et al. J Contr Release 136:220-225 (2009); 2. Paul A et al. Adv Drug Deliv Rev 71:115-30. (2014).; 3. Paul A et al Sci Rep 2013; 3:2366. (2013)