Abstract

Viruses have significant commercial application as vectors for gene and cell therapy, as well as vaccines for cancer treatment and infectious diseases. The purification of commercial viruses is a major area of bioprocess development. Current methods are mostly based on filtration and chromatography, both of which scale poorly and require clean feed streams to perform well. Viral particles are difficult to recover from culture supernatants or cell lysates because they are similar in size to cell debris. A large amount of expensive, high-quality nuclease (Benzonase) is typically needed to reduce the viscosity of the lysate sufficiently for efficient membrane and column operations. Affinity based vector purification has been developed for only one type of vector (some strains of adeno-associated virus), and is also very expensive. In summary, virus purification is challenging and significant amounts of valuable product are lost in inefficient filters.

We have developed a novel DNA-based avidity reagent, termed DeNAno, that can provide a significant advance in the development and manufacturing of commercial viruses, with applicability to a variety of viral vectors. DeNAno uses massive avidity rather than affinity to capture biologic targets such as viruses. DeNAno particles are composed of a single-stranded DNA concatemer, which contains several hundred copies of a template oligonucleotide. The goal of this project is to develop DeNAno particles that bind to a target virus, and then use the DeNAno as a capture reagent for virus purification. DeNAno libraries are produced by rolling circle replication of a circular oligonucleotide template containing a random sequence, and specific viral binders are recovered by a biopanning process. DeNAno particles can be released from their targets by disrupting their secondary structure through divalent cation removal; they can also be labeled with nano-particulate magnetite or coated onto micron-sized magnetite to allow their use as magnetic capture reagents. We have already performed a selection on adenovirus-coated beads and obtained a pool of DeNAno particles that bind the target virus. The specific aims of this project are to: 1) characterize DeNAno particles that bind to the model virus (adenovirus) and 2) demonstrate their use as a purification tool by attaching them to magnetite so that they can be used in magnetic capture. The virus will be released following capture by divalent cation removal and the magnetic DeNAno cleared from the viral preparation, again by magnet. We will assess the capture efficiency, virus yield and infectivity, residual DeNAno contamination, and the presence of host cell proteins in the final preparation. If successful, this project will demonstrate the potential of the DeNAno technology to streamline virus production and pave the way for additional DeNAno-based applications targeting other commercially important viruses.