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Editorial Open Access

Empty Capsids in Production of Viral Vector: Separation and Reassembly

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Editorial

Viral vectors have become promising weapons against many illness, such as vaccine and gene therapy to cancer and genetic disorder disease. Production and purification of different viral vectors have been widely studied. In the production process of the virial vector, empty viral capsids, which do not contain nucleic acid material, were reported as an undesired by product in many gene therapy studies. Several groups have demonstrated the function of specific immunogenicity of empty capsids because of their integrated foreign antigens. Research studies have raised the point that empty capsids may act like a natural decoy rather than 'contamination' of the product [1]. Even though the potential function of the empty capsids, the ratio of empty capsids to full viral capsids is very difficult to control in the production process. The empty capsid can be produced more efficiently as virus-like particles, which are another type of on-purpose empty capsid, instead of being the side product. A rational process will be separation of the empty capsids first and mix them later at a certain ratio, if necessary. From a process development point of view, we discuss the efforts for separation, disassembly, and assembly of the empty capsids.

Production of different viral vectors have been reported from different host cell cultures. It is reported that the ratio of the full capsid to empty capsid depends on the virus and subtypes [2]. It is reasonable to speculate that the cell culture condition, including pH, ion type and ion concentration, and some other molecules will affect the assembly of the viral vector. Many factors of the viral vectors, such as the nucleic acid material, size and units of the structural protein will also affect the assembly efficiency in the host cell culture.

Compared to the full viral capsids with genome material, empty capsids have different densities due to the absence of nucleic acid material. Traditionally, the empty capsids can be separated by gradient centrifugation. However, more efficient and scalable procedures need to be developed for commercial production. The surface electrical property of the empty capsids are also different, because of the lack of interaction between nucleic acid materials with the capsid proteins. For certain virus such as adeno-associated virus (AAV), size of the empty capsid is close to the full capsids, which have been confirmed using electron microscope by different groups. For some other virus, the size of the virus vector also depends on the nucleic acid material.

Separation of Empty Capsids by Chromatography

Chromatography has been widely applied for purification of viral vectors. The most common chromatography methods include ion-exchange, size exclusion, and affinity chromatography. They have been widely studied for purification and polish steps in downstream process because of their scalability and robustness. The empty capsids have different surface electrical properties from the full capsids, which can be separated using ion-exchange chromatography. Qu et al. applied anion-exchange column chromatography to separate empty capsids of adeno-associated virus (AAV) type 2 [3]. Results indicated that pH plays an important role in separating the empty particles. pH has a significant impact on the surface electrical property of the capsid, which can be used to distinguish the empty capsids from the full capsids. Lock et al. developed a method with anion-exchange monolith to separate empty capsids of AAV8 using differential elution [4]. Leuchs et al. succeed in using DEAE monolith column to separate the empty particle of Parvovirus H-1 virus [5]. The quantification of empty capsids has also been reported using optical density measurement by Sommer et al. [6]. The ratio of vector genome and capsid particle can be measured using absorbance at 260 nm and ratio of absorbance of 260 nm to 280 nm. The optical quantification method offers online detection of the empty capsids for the anion-exchange chromatography.

Disassembly and Assembly of Viral Vectors

In vitro disassembly and assembly of viral vectors is an option to increase the productivity of the process. The assembly mechanism of virus or virus-like particle has been studied both theoretically and experimentally. The kinetic and driving force of self-assembly of virus surface protein has also been reviewed [7]. Couple of studies have revealed different triggers for disassembly of virus/virus-like particle such as light, pH, salt concentration, and temperature [8-10].

Changes in environmental condition can trigger both disassembly and self-assembly of viral vectors. Depending on the molecular attraction among capsid proteins, the self-assembly of viral vectors can be triggered by change of pH and ions. Chuan et al. reported the assembly of Murine polyomavirus capsid protein by changing pH or the calcium concentration. The authors observed that dramatic increase in strength of protein molecular attraction is coupled with assembly of capsid protein. Other salt such as ammonium sulphate which is commonly used to precipitate many different proteins of solution, can also trigger the assembly of virus capsid protein. The effect of nucleic acid material was not studied in the paper [11]. In the presence of nucleic acid material, such as RNA, the assembly between RNA and capsid protein is dominated by the electrostatic interactions. There are other parameters will affect the interactions such as the length of the RNA. Some models for assembly mechanism were summarized by Perlmutter and Hagan [7]. Kler et al. showed the control of the geometry of the assembled virus particles with nucleic acid material. It was demonstrated that both dsDNA and ssRNA change the geometry of the particle to form different sizes [12]. The

difference between dsDNA and ssRNA play a significant role in the assembly of the viral vectors [13,14]. However, under certain circumstances, assembly of capsid protein can form an 'alternative' structure, such as mutation of the capsid protein, presence of ions, and different nucleic acid materials.

One of the most successful applications is post-purification disassembly and reassembly of human papillomavirus (HPV) virus-like particles, which have been reported to increase the immunoreactivity and stability [8,15]. However, most of the reassembly studies of HPV vaccine were performed without nucleic acid material. Zhao et al. demonstrated improved stability of the HPV virus like particle after disassembly and reassembly treatment. The change of immunoreactivity was observed, which can be attributed to the orientation of antibody epitopes [15]. It is important to examine the change of immunoreactivity after the disassembly and assembly of the virus like particles since they can be changed during the interaction between different capsid proteins, especially for multivalent vaccine. Similarly, Mach et al. showed improved stability and immunogenicity after disassembly and assembly of HPV virus like particle from yeast-derived monovalent subtypes: 6, 11, 16, and 18. The results indicated change of particle size and thermal stability after disassembly/assembly. The environment conditions for triggering disassemble/assemble are pH, ionic strength, and reducing agent [8]. The current challenge is to control and improve the disassembly and assembly process with increased immunoreactivity and stability. If the ratio of the empty capsids is significant in the production, it is worth studying the reassembly process.

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