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Evaluation of viral clearance in the production of HPV-16 L1 virus-like particles purified from insect cell cultures

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Abstract

Biopharmaceutical products produced from cell cultures have a potential for viral contamination from cell sources or from adventitious introduction during production. The objective of this study was to assess viral clearance in the production of insect cell-derived recombinant human papillomavirus (HPV)-16 type L1 virus-like particles (VLPs). We selected Japanese encephalitis virus (JEV), bovine viral diarrhea virus (BVDV), and minute virus of mice (MVM) as relevant viruses to achieve the aim of this study. A downstream process for the production of purified HPV-16 L1 VLPs consisted of detergent lysis of harvested cells, sonication, sucrose cushion centrifugation, and cesium chloride (CsCl) equilibrium density centrifugation. The capacity of each purification/treatment step to clear viruses was expressed as reduction factor by measuring the difference in log virus infectivity of sample pools before and after each process. As a result, detergent treatment (0.5% v/v, Nonidet P-40/phosphate-buffered saline) was effective for inactivating enveloped viruses such as JEV and BVDV, but no significant reduction ($<1.0\log_{10}$) was observed in the non-enveloped MVM. The CsCl equilibrium density centrifugation was fairly effective for separating all three relevant adventitious viruses with different CsCl buoyant density from that of HPV-16 L1 VLPs (JEV, BVDV, and MVM = 4.30, 3.10, $\geq 4.40\log_{10}$ reductions). Given the study conditions we used, overall cumulative reduction factors for clearance of JEV, BVDV, and MVM were ≥ 10.50, ≥ 9.20, and $\geq 6.40\log_{10}$ in 150 ml of starting cell cultures, respectively.

Keywords: HPV-16 L1 VLPs; Viral clearance; JEV; BVDV; MVM

1. Introduction

The main potential risks associated with the use of biopharmaceuticals produced from animal cell cultures are directly related to contaminants from cells including viruses and other transmissible agents, cellular DNA and proteins [1]. While viruses of concern in cells originated from humans or vertebrate animals are relatively well documented, viruses in cells originated from invertebrates are not extensively characterized in terms of their reality in production cells and consequences in human health risks.

Insect cells originated from invertebrates have some advantages over mammalian cells in that many mammalian viruses may not replicate effectively in them and that synthetic media free of bovine serum are well established to support the cell growth and maintenance. To date, there are no licensed products derived from insect cell cultures. Studies on the expression of a particular gene using insect cells and recombinant baculovirus vectors have been actively conducted, and have also been used in the manufacturing process [2–4]. Currently at least one promising candidate human papillomavirus (HPV) vaccine under the phase III clinical study is produced from insect cell cultures [5]. The HPV candidate vaccine is composed

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of virus-like particles (VLPs) when L1 protein of HPV is expressed in insect cells by the baculovirus expression system. HPV-16 L1 VLPs were reported to reduce the incidence of HPV-16 infection. Of known many genotypes of HPV, HPV-16 virus is the most prevalent HPV type associated with cervical cancer (more than 60% of cervical cancer) [5,6].

Various strategies for virus testing and assessment of virus removal and inactivation achieved by the manufacturing process have been developed and applied to assure the safety of biopharmaceuticals. These two different approaches are complementary and have been evolved to control the potential viral contamination of biopharmaceuticals produced from a variety of cell substrates [7]. In principle, these approaches shall be applied for safety evaluation of biopharmaceuticals produced from insect cell cultures as well. For evaluation of insect cell-based products intended for use in humans, yet there is no guidance for regulators of which viruses should be of specific safety concerns in terms of virus testing and/or clearance validation. Developing a virus testing program must be a straightforward task, whereas studies of viral clearance validation should carefully consider the choice of viruses and the validation conditions employed.

The objective of this study was to assess viral clearance in the production of insect cell-derived human papillomavirus (HPV)-16 type L1 virus-like particles (VLPs). To achieve the overall study objective, proper justification for choosing viruses and purification/treatment steps was necessary.

Japanese encephalitis virus (JEV) is a member of arthropod-borne flavivirus genus in *Flaviviridae* family and causes central nervous system diseases such as meningitis and severe encephalitis. The virion size of JEV is 40–50 nm and single-stranded positive-sense RNA of approximately 11 kb in length [8,9]. Insect cells, susceptible to JEV, have the potential for contamination since JEV can infect invertebrates as well as vertebrate hosts [10]. Bovine viral diarrhea virus (BVDV) is a typical virus that has a potential for contamination in the biopharmaceutical manufacturing process because of fetal bovine serums (FBS), used in cell cultures [11]. BVDV is a member of pestivirus genus in *Flaviviridae* family with its single-stranded positive-sense RNA of about 12 kb. Although a direct harm of BVDV to human is not known yet, BVDV causes diarrhea, malformation during pregnancy, respiratory diseases, bleeding symptom, and miscarriage in infected cows [12]. Cases of contamination by pestivirus including BVDV were reportedly found in the biopharmaceutical products of measles–mumps–rubella (MMR) combination vaccines used for preventive injections in infants [13–16].

The minute virus of mice (MVM) belongs to the family of *Parvoviridae*, small and non-enveloped DNA virus, whose members can infect a wide variety of hosts ranging from vertebrate to insects. Parvoviruses are known to be highly resistant to physico-chemical treatment, such as heating, drying, low pH [17–19]. In biopharmaceutical manufacturing process, many products are also produced by rodent-derived cell substrates, such as Chinese hamster ovary (CHO) and baby hamster kidney (BHK) cells which are highly susceptible to MVM infection [20–22].

In this study, a series of downstream process in the production of HPV L1 VLPs consisted of detergent lysis of pelleted cells with Nonidet P-40 in phosphate-buffered saline (NP-40/PBS), sonication, sucrose cushion centrifugation, and cesium chloride (CsCl) equilibrium density centrifugation. Each step was chosen for its virus inactivation and/or removal capacity because it has well been documented in a variety of literature as a standard procedure for the production of purified HPV-16 L1 VLP [23,24]. At the end of the study overall cumulative reduction factors were calculated for a summarized result. Here, we report the assessment of JEV, BVDV, and MVM clearance in the production of purified HPV-16 L1 VLPs from insect cell cultures.

2. Materials and methods

2.1. Viruses and cell cultures

The Nakayama-NIH strain of JEV (mouse brain source) was obtained from the Department of Virology at Korea Center for Disease Control and Prevention (Seoul, Korea, Rep.) and baby hamster kidney (BHK)-21 was used for cell culture. The NADL strain of BVDV and Madin–Darby bovine kidney (MDBK) cell were obtained from the National Veterinary Research and Quarantine Service (Anyang, Korea, Rep.). MVM (ATCC VR-1346 prototype, MVM_p) and A9 cells were obtained from Division of Biotechnological products in KFDA. Characterizations of these viruses are compared in Table 1. Infectivity titration of JEV, BVDV, and MVM was measured using median tissue culture infectious dose ($TCID_{50}$/ml) according to the method of Spearman-Kärber [25]. *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV) HPV-16 L1 recombinant baculovirus expression vector was produced as described previously [26]. Sf9 insect cell (Invitrogen, USA), used as a cell substrate for the production of HPV-16 L1 VLPs, was cultured in Grace's insect medium (Gibco–BRL, USA) complemented with 10% (v/v) FBS and 1% (v/v) antibiotic–antimycotics.

2.2. Production and purification of HPV-16 L1 VLPs

HPV-16 L1 VLPs was produced from Sf9 insect cell line using a 250 ml spinner culture flask with working volume of 150 ml. Recombinant baculovirus was infected in Sf9 cells at a multiplicity of infection (moi) of about 20 PFU/cell [23,24], and cultured at 27 °C for about 5 days. The

Table 1
Viruses used in this study and their characterization

Attribute of virus	JEV	BVDV	MVM
Family	*Flaviviridae*	*Flaviviridae*	*Parvoviridae*
Virus size (nm)	40–50	50–70	18–24
Nucleic acid	RNA	RNA	DNA
Envelope	Yes	Yes	No
Resistance to physico-chemical agents	Low	Medium	Very high
Virus density (g/ml)	1.20	1.20	1.39–1.42
Cells for infectivity assay	BHK-21	MDBK	A9