TECHNICAL NOTE

A rapid and easy method to clean and concentrate adenoviruses for *in vitro* and *in vivo* applications

Steve Hildebrandt, Stefan Jean-Pierre Haas, Christian Andressen and Andreas Wree

Institute of Anatomy, Medical Faculty, University of Rostock, Rostock, Germany

ABSTRACT

The transduction of various cells from the nervous system *in vivo* or *in vitro* with adenoviral vectors (Ad-vectors) carrying the information for marker proteins like green fluorescent protein (GFP) is a very common method in neuroscience. By the use of membrane chromatography we describe here a rapid and easy to perform method to clean and concentrate such Ad-vectors without the use of CsCl-ultracentrifugation. Ad-vectors encoding for GFP were propagated in HEK293-cells, purified and concentrated with commercially available membrane chromatography columns. As a proof of principle these Ad-vectors were injected into the lateral ventricles of mice or cultured immortalized neural progenitor cells were transduced. Following intraventricular injections, differences in GFP-expression according to the amount of injected Ad-vectors were observed and *in vitro* a robust fluorescence of cultured cells was documented. Moreover, neurotoxic effects were not observed demonstrating the efficacy and security of the cleaning and purification process.

Keywords: *adenovirus, vector, green fluorescent protein, stereotaxy*

INTRODUCTION

The use of Adenoviral vectors (Ad-vectors) encoding GFP (Green Fluorescent Protein) or various other marker proteins, for example RFP (Red Fluorescent Protein), is very useful for many studies in the central nervous system including tract-tracing studies[1-2], intra-ventricular injections[3] or ex vivo cell labelling for cell transplantation purposes.[4] However, the purification and concentration of adenoviruses is a very difficult procedure, because according to the classical cleaning and concentration steps ultracentrifugation with CsCl-gradients is needed.[5,6,7] Centrifugation is time consuming and expensive since ultracentrifuges are needed and toxicity due to CsCl-contamination cannot be excluded.[6]

Here we describe a rapid, non toxic and easily to perform method that can be practically applied in almost every laboratory for neuroscientific research with the appropriated security level for handling adenoviruses by the use of membrane chromatography.[8]

Corresponding author: Stefan Jean-Pierre Haas, Institute of Anatomy, University of Rostock, Gertrudenstrasse 9, D-18055, Rostock, Germany, Tel: +493814948439, fax: +493814948402, E-mail: stefan.haas@uni-rostock.de

MATERIALS AND METHODS

Adenovirus propagation

All preparation dealing with adenoviruses has been performed in a biosafety level 2 facility equipped with a biological class 2 safety cabinet. Adenovirus type 5 (Adeno-X™ AcGFP1 Marker Virus) encoding the green fluorescent protein (GFP) under the immediate early promoter of human cytomegalovirus was obtained from Clontech Laboratories (Mountain View, Ca, USA). This virus lacks the E1 gene and, therefore it replicates only in E1 trans-complementing cells such as HEK293-cells. HEK293-cells were cultured and expanded in Petri dishes (9.2 cm diameter, NUNC, Hereford, UK) containing DMEM, 10% fetal calf serum (FCS), 100 U/ml penicillin and 100 µg/ml streptomycin (all media and supplements from Invitrogen, Karlsruhe, Germany) in a humidified incubator (95 % air/5% CO_2, at 37° C). 72h after adenoviral transduction HEK293-cells were scraped from the bottom of the Petri dishes and immediately, together with the supernatant frozen at -80° C, until further processing.

Adenovirus purification and concentration

Adenovirus purification and concentration by using the Vivapure® AdenoPack™ 100 kit was performed with the supplied material and according to the manufacturer's instructions (Vivascience AG, Hannover, Germany). In brief, for purification, cells and supernatant from three Petri dishes containing about 20 ml solution were frozen at -80° C and subsequently thawed three times at 25°C. Then the cell debris was centrifuged (Megafuge 1.0R, Heraeus, Düsseldorf, Germany) at 3500g for 15 min and 20 ml of the supernatant, containing the viruses, was incubated for 30 min at 37° C with 20 µl Benzonase®. The solution was then transferred to a Vivaclear Maxi tube, centrifuged at 500 g for 15 min and the flow through was gently mixed with the loading buffer according to the manufacturer's protocol. After equilibration and wash through of the AdenoPACK Maxi spin column with washing buffer the column was then filled up with the loading buffer containing the viruses and centrifuged at 500 g for 5 min. The AdenoPACK Maxi spin column was then again rinsed two times with washing buffer followed by centrifugation at 500 g for 5 min. The membrane bounded adenoviruses were then, after incubation for 10 min in elution buffer, centrifuged at 500 g for 5 min and the adenovirus containing eluate was collected. To exchange the buffer and further concentrate the viral solution the eluate, about 1 ml, was transferred to a Vivaspin 20 concentrator tube, filled up with about 9 ml of sterile phosphate buffered saline (PBS, 0.1M, pH 7.4) and centrifuged at 800 g for 30 min. The amount of concentrated viruses in several