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Application of heparinized cellulose affinity membranes in recombinant adeno-associated virus serotype 2 binding and delivery

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Abstract

The microporous affinity membrane based on cellulose matrices offers minimal mass-transfer effects in membrane chromatography with low nonspecific adsorption. In this research, we tested a novel application of the microporous, heparinized cellulose membranes for their affinity toward recombinant adeno-associated virus serotype 2 (rAAV2, which uses heparan sulfate proteoglycans as the primary cellular receptor) to develop a controlled, substrate-mediated viral vector delivery. We conjugated rAAV2 to an epoxy-crosslinked heparin cellulose membrane, which led to vector transduction upon cellular adhesion. When adhered, human fibroblasts exhibited proliferation kinetics similar to those on the standard polystyrene tissue-culture surface. Using fluorescent proteins as the reporter, we showed that the heparin-bound rAAV2 particles remained active and that the rAAV2-heparin binding was reversible and capable of mediating transgene delivery in cell culture. In addition, we applied the affinity membrane to adsorb unpurified rAAV2 from the crude lysate of packaging cells via the ligand–receptor binding, avoiding the use of conventional ultracentrifugation or chromatography in preparation of infectious rAAV2 for transduction. Our work explores a new application of affinity cellulose membranes in substrate-mediated viral vector delivery, which can be a useful tool in developing protocols for localized gene transfer.

Keywords: AAV2; Affinity membrane chromatography; Cellulose; Gene transfer; Heparin

1. Introduction

Adsorptive membrane chromatography offers an efficient and reliable method to purify biomolecules of interest for the biotechnology industry. Currently, adsorptive membranes have been utilized in virus capture for downstream processing of vaccines and gene therapy vectors [1–4]. The microporous structure of adsorptive membranes provides large internal surfaces for contact with viral particles with minimal mass-transfer effects. The affinity membranes, in particular, offer an additional advantage of low nonspecific adsorption in chromatography [5,6].

Despite the unique microporous structure and the specific ligand–receptor binding, the affinity membranes have thus far only been used in separation processes. In the present study, we aimed to test a novel application of the microporous, heparinized cellulose membranes (H-CM) for their affinity toward recombinant adeno-associated virus serotype 2 (rAAV2) to develop a controlled, substrate-mediated viral vector delivery. Controlled viral vector administration represents a major challenge in the clinical application of gene therapy. Diffusion of vectors away from their targets could cause reduced gene transfer efficiency, ectopic gene expression in non-target cells, and the risk of eliciting a severe immune response. One potential solution to this problem is implanting biocompatible substrates loaded with vectors for localized transgene delivery [7–9]. This strategy is particularly useful for implementing physiological cues, such as growth factor-encoding genes, in the substrate to stimulate bone regeneration and cutaneous wound healing [10,11].

Recombinant adeno-associated virus serotype 2 (rAAV2) is the vector of interest in this research. This vector has shown promising results in human clinical trials [12,13]. The primary receptor of rAAV2 is heparan sulfate proteoglycans (HSPG) [14]. In addition to HSPG, other glycosaminoglycans such as heparin are also capable of binding with rAAV2. In fact, heparin is currently used as the ligand in agarose column chromatography for rAAV2 purification [15]. These findings support the use of the heparinized cellulose membrane for rAAV2 binding and immobilization.

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In this research we applied the Sartobind heparin cellulose membrane (Sartorius AG, Göttingen, Germany) as the affinity substrate for rAAV2 binding. The microporous cellulose membrane provides large internal surfaces for rAAV2 binding and cellular adhesion/infiltration. In addition, the biocompatibility of cellulose and its derivatives has long been recognized [16–19]. Because the mechanical properties of cellulose can be engineered to match those of hard and soft tissues, studies have focused on using cellulose as the scaffold for bone and cartilage tissue engineering, connective tissue regeneration, and cultivation of functional cardiac cell constructs [17,18,20–23]. This report presents and discusses the results of using H-CM to initiate rAAV2 binding and transgene delivery upon cellular adhesion.

2. Materials and methods

2.1. Cell culture

Human embryonic kidney 293 (HEK293, ATCC, Manassas, VA, USA) cells were used for rAAV2 production. Human HT-1080 fibroblasts (ATCC) and human Saos-2 osteoblasts (ATCC) were used as targets for rAAV2 transduction. These cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cell cultures were maintained at 37 °C in a 5% CO_2/95% air humidified atmosphere.

2.2. Cellular adhesion and kinetics of cell proliferation on H-CM

One day after HT-1080 fibroblasts were seeded onto H-CM (Sartorius AG, Göttingen, Germany, heparinized membrane of regenerated cellulose acetate, Cat# 94HEP-00-001) and the standard polystyrene tissue-culture surface (control, Corning Inc., Acton, MA, USA), a staining kit of actin filaments (Chemicon, Cat# FAK100, Temecula, CA, USA) consisting of tetramethylrhodamine-isothiocyanate (TRITC)-conjugated phalloidin was used to reveal the actin cytoskeleton in cells. The stained actin filaments were imaged by a Nikon TS100 microscope configured with an epifluorescence attachment and a Nikon G-2A filter set (EX 510-560, DM 575, BA 590). Cells on H-CM were also examined by the scanning electron microscopy (SEM). In this analysis, H-CM with cells was washed with phosphate-buffered saline (PBS, 0.01 M, pH 7.4, Sigma, St. Louis, MO, USA) and fixed in 4% glutaraldehyde for 20 min. After the fixation, the sample was washed twice with PBS, and then dehydrated in 50%, 70%, 90%, and 100% ethanol solutions for 15 min twice at each concentration. Afterwards, the sample was vacuum-dried and coated with gold by ion sputtering prior to examination by SEM using a JEOL (Tokyo, Japan) JSM-5600 model.

The mitochondrial activity in fibroblasts seeded onto H-CM was measured over time by the CellTiter 96 AQueous one solution cell proliferation assay system (an MTS assay, Promega, Madison, WI, USA) [24]. Cells seeded onto the blank cellulose membrane, i.e., cellulose without the crosslinked heparin molecules, and the standard polystyrene tissue-culture surface were used as controls. In this cell viability/proliferation assay, the methoxyphenyl-tetrazolium salt (MTS) compound was reduced by viable cells to form a colored formazan product, which was quantified colorimetrically at 490 nm using a spectrofluorometer to determine the optical density. Parallel cell cultures were sacrificed at time = 0.25, 1, 3, 5, and 7 days to measure the mitochondrial activity. A calibration curve of known cell number versus optical density was prepared to determine the cell number of the sample.

2.3. Viral vector preparation

Plasmid pAAV-LacZ (Supplementary Fig. 1A), carrying the expression cassette of β-galactosidase flanked by AAV2 ITRs (inverted terminal repeats), was purchased from Stratagene (La Jolla, CA, USA). Plasmid pAAV-EGFP (Supplementary Fig. 1B) and pAAV-luciferase (Supplementary Fig. 1C) was constructed by cloning the 0.8-kb EGFP cDNA from pEGFP-N2 (Clontech, Mountain View, CA, USA) and the 1.7-kb firefly luciferase cDNA from pGL3-control (Promega), respectively, downstream from the CMV promoter in pAAV-MCS (Stratagene). Production, purification, and titration of rAAV2 were performed as previously described using the AAV Helper-Free System (Stratagene) [25]. Briefly, HEK293 cells were co-transfected with the rAAV2 vector plasmid, along with pAAV-RC (Stratagene) and pHelper (Stratagene). For each 100-mm dish, HEK293 cells were transfected with 2.5 mg of each of the three plasmids and 15 ml of FUGENE 6 reagent (Roche, Indianapolis, IN, USA) according to the manufacturer's protocol. Transfected cells were harvested 3 days after transfection. Purification of rAAV2 particles followed the protocol developed by Auricchio et al. using a single-step heparin column chromatography [15]. Titration of infectious rAAV2 particles (infectious units (IU)) was performed according to the protocol of the AAV Helper-Free System (Stratagene) using HT-1080 cells as targets for transduction. The number of IU per microliter of viral stock for each batch of virus production ranged from 10^5 to 10^7. The purified rAAV2 particles were stored in the −80 °C freezer until use in the H-CM binding and transduction experiments.

Alternatively, the H-CM-mediated transduction was also carried out using unpurified crude lysate of packaging cells. To prepare the unpurified crude lysate, cells were scraped from culture surfaces and suspended in DMEM. The suspension then went through two rounds of freeze (−80 °C) and thaw (37 °C), followed by the addition of DNase I and RNase A (Roche) into the suspension and incubation at 37 °C for 30 min. Large cellular debris and the lysate were separated by centrifugation at 1000 g for 15 min. The supernatant was transferred into a 50-ml conical tube and incubated with 0.5% deoxycholic acid (Sigma) at 37 °C for 30 min. Small cellular debris was removed by filtering the lysate through a 5-mm pore size filter and then through a 0.8-mm pore size filter. Permeates were collected and stored in the −80 °C freezer until use.