Sensors and Actuators B 233 (2016) 289–297

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Isolation of extracellular vesicle from blood plasma using electrophoretic migration through porous membrane

Siwoo Cho[a], Wonju Jo[a], Youhee Heo[c,d], Ji Yoon Kang[c], Rhokyun Kwak[c,**], Jaesung Park[a,b,*]

[a] Department of Mechanical Engineering, Pohang University of Science and Technology, Pohang, Gyeongbuk, Republic of Korea
[b] School of Interdisciplinary Bioscience and Bioengineering, Pohang University of Science and Technology, Pohang, Gyeongbuk, Republic of Korea
[c] Center for BioMicrosystems, Korea Institute of Science and Technology, Seoul, Republic of Korea
[d] Department of Biomedical Engineering, Sogang University, Seoul, Republic of Korea

ARTICLE INFO

Article history:
Received 23 January 2016
Received in revised form 22 March 2016
Accepted 18 April 2016
Available online 19 April 2016

Keywords:
Exosome
Purification
Separation
Nanoporous membrane
Body fluid

ABSTRACT

We present a high-yield electro-migration method to isolate extracellular vesicles (EVs), which achieves ultracentrifugation (U/C)-level size exclusion from biological fluids (e.g., plasma). An electric field applied across a dialysis membrane with an appropriate pore size (30 nm), facilitates protein migration through the membrane, but captures EVs on the membrane surface. Compared to conventional procedures, this method shows up to 65% EV recovery estimated at the RNA level (~7.9 times better than U/C) and up to 83.6% protein removal (residual protein amount is approximately half of the precipitate) in ~30 min (~9 times faster than U/C). With optimized working range of voltage and use of biochemically stable buffers, isolated EV are fully compatible with biological post-processes and assays.

1. Introduction

Extracellular vesicles (EVs) are biological particles that have an lipid bilayer core-shell structure like cells, but are far smaller (50–1000 nm) than cells [1]. Most types of cells secrete EVs, and their lipid compositions, membrane proteins, enclosed proteins, and nucleic acids reflect biological states of the cells that secreted them [2]. EVs are secreted into body fluids (e.g., blood, saliva, urine, lymph and milk), and may have important functions in intercellular communication [3]. They also have functions in cell proliferation [4], immune systems [5], thrombosis [6], and tumor invasions [7]. Therefore, researchers have pursued the use of EVs as bioactive carriers in both diagnosis and therapy [8,9].

To investigate EVs, efficient and repeatable isolation from biological samples is required [2]. Isolation entails concentrating EVs in a small volume, then removing impurities such as proteins [10], lipoproteins, cellular debris, cholesterol, urine crystals, lipid aggregates, and viruses [11]. Current methods can be categorized into ultracentrifugation (U/C), precipitation, ultrafiltration, immune affinity, chromatography and electrophoretic migration [2,12,13]. Among them, an appropriate isolation method must be chosen according to the purpose of experiments and the type of original solution [14,15]. For example, if EVs are to be used to diagnose disease [16] or to treat cells or tissues [17], all isolation processes should be free of biochemical artifacts, which may alter EVs' characteristics and prohibit biological post process such as RNA isolation and immune reaction. Such artifacts may be caused by inseparable antibodies, severe pH change, and residual isolation solvent. Therefore, many researchers prefer U/C because it offers biocompatibility without biochemical reaction or solvent contamination, high selectivity for size or density, and high throughput [14,18]. However, U/C also has the disadvantages of lengthy processing time (5–6 h), low recovery rate (5–25%), and large initial volume required (4–50 mL) [19]. Especially for small samples, recovery can be low. In contrast, precipitation methods

Abbreviations: EV, extracellular vesicle; U/C, ultracentrifugation; BSA, bovine serum albumin; TAE, tris acetate ethylenediaminetetraacetic acid; PBS, phosphate buffered saline; PEG, polyethylene glycol; GFP, green fluorescence protein; DMEM, Dulbecco's Modified Eagle's Medium; IPA, isopropanol; DLS, dynamic light scattering; NTA, nanoparticle tracking analysis; PVDF, polyvinylidene fluoride; TBS, tris-buffered saline; RT-PCR, reverse transcription polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay.

* Corresponding author at: Department of Mechanical Engineering, 77 Cheongam–Ro, Engineering Building 5, Nam–gu, Pohang, Gyeong–buk, 37673, Republic of Korea.
** Corresponding author at: Korea Institute of Science and Technology (KIST) 5, Hwarang-ro 14-gil. Seongbuk-gu. Seoul, 02792. Republic of Korea.
E-mail addresses: rhokyun@kist.re.kr (R. Kwak), jpark@postech.ac.kr (J. Park).

http://dx.doi.org/10.1016/j.snb.2016.04.091
0925-4005/

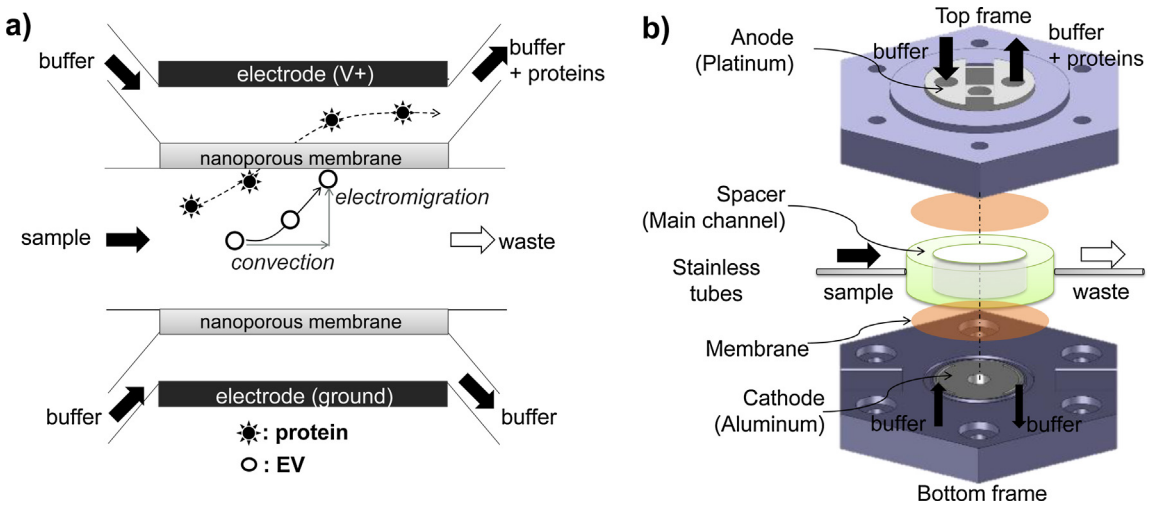

Fig. 1. a) Schematic of electrophoretic migration isolation processes. Proteins smaller than the pore diameter can pass through the membrane into the buffer flow (stars on the dotted arrow), but EVs cannot pass through the membrane and are captured on it (circles on the solid curved arrow). Between the membranes, the sample flow is flow-rate driven, and yields a parabolic velocity profile ($\vec{u}$). Buffer flows on the electrodes rinse the transported proteins and products by Faradaic reactions. b) Implementation diagram of the device. Membranes used for dialysis also separate EVs physically from Faradaic reactions (possible pH changes and bubbles on electrodes); tubes are embedded in the spacer as inlets and outlets.

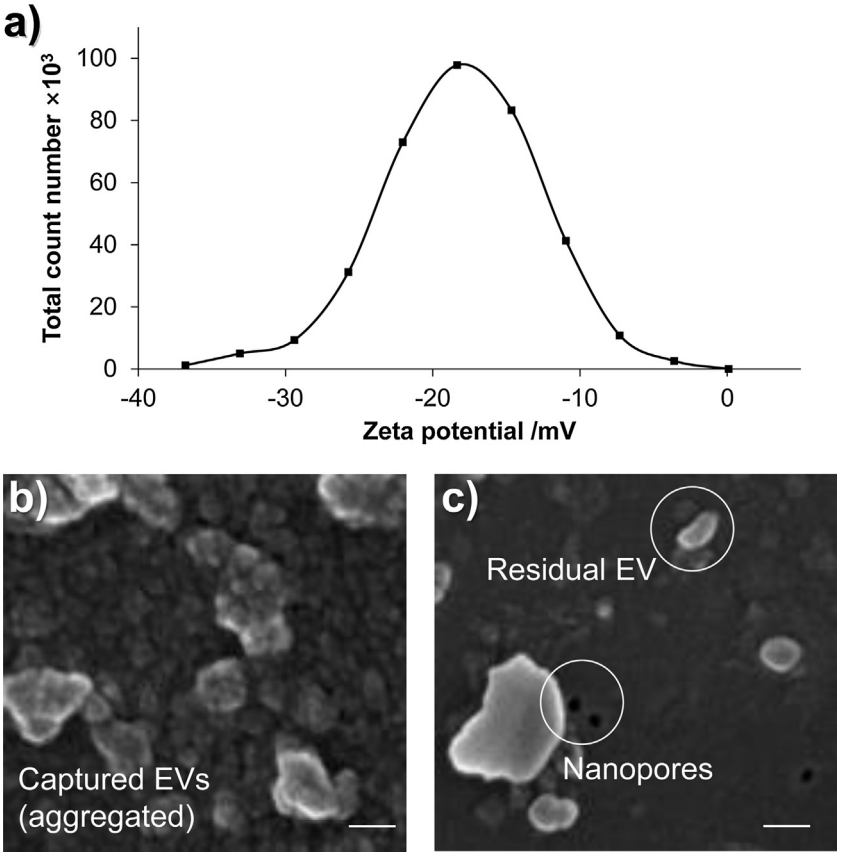

Fig. 2. a) Zeta potential distribution was measured using a Zetasizer ZS (Malvern Instrument). Relative particle number corresponding to each zeta potential value is measured. b) SEM image of EV-deposited membrane; scale bar: 100 nm. EVs collected on the membrane show numerous grains as forming a layer. c) SEM image of the membrane after elution. Small black dots in the lower circle are pores; the white grain in the upper circle is a single EV that could not be eluted out from the membrane. c) SEM image of the membrane after elution. Small black dots in the lower circle are pores; the white grain in the upper circle is a single EV that could not be eluted from the membrane.

can concentrate EV from sub-milliliter samples effectively, but can cause significant coagulation, and do not eliminate impurities from serum and plasma [20].

Separation criteria of U/C are mass and density, which are volumetric parameters. Due to the scaling laws of nanoparticles such as EVs, U/C is ineffective to induce sufficient migration, and its ability to reduce processing time is limited [21]. Among many other types of physical interaction, electrophoretic migration and size exclusive filtration can separate nanoparticles, because the relation between zeta potential and electrophoretic migration is proportional to par-