Electronic Supplementary Material (ESI) for Polymer Chemistry.
This journal is © The Royal Society of Chemistry 2023

# A facile method based on superabsorbent polymer composite for concentration and separation of exosomes from cell culture medium

Wei Li,[a] Jianna Li,[b] Yuanting Xie,[a] Yan Tan,[a] Huili Wang,[a] Lei Su,*[,a] Xueji Zhang*[,a]

*a School of Biomedical Engineering, International Health Science Innovation Center, Shenzhen Key Laboratory for Nano-Biosensing Technology, Marshall Laboratory of Biomedical Engineering, Health Science Center, Shenzhen University, Shenzhen 518037, P.R. China.*

*[b] School of Basic Medical Sciences, Health Science Center, Shenzhen University, Shenzhen 518037, P.R. China.*

** Corresponding authors. E-mails: sulei@szu.edu.cn; zhangxueji@szu.edu.cn.*

**Experimental section**

*Materials and chemicals*

Anti-TSG 101 polyclonal antibody, anti-63 polyclonal antibody and goat anti-rabbit IgG (HRP-conjugated) were purchased from MultiSciences (Lianke) Biotech, China. Dulbecco's modified essential medium (DMEM), fetal bovine serum (FBS), and phosphate buffer solution (PBS), and tris-buffered saline (TBS) and tris-buffered saline with Tween-20 (TBST) were purchased from Gbico, USA. Phosphotungstic acid hydrate was purchased from Alfa Aesar, USA. 3,3-dioctadecyloxacarbocyanine perchlorate (DiO), RIPA lysis solution, BCA Protein Assay Kit, SDS-PAGE Gel Parparation Kit, PVDF membrane, protein loading buffer and bovine serum albumin (BSA) were purchased from Beyotime Biotechnology, China. Acrylamide, ammonium persulfate, N,N'-methylene bis(acrylamide) (NMBA), acrylic acid, potassium persulfate (KPS), glucose, sodium alginate (SA) were purchased from Guoyao Chemical, China. HeLa cells were obtained from Beyotime Biotechnology (China) and identified by STR (short tandem repeats). All chemical reagents obtained were at least of analytical grade and used without further purification.

*Synthesis of the sodium alginate and poly(acrylamide-co-acrylic acid) composite SAPs*

The SAPs were synthesized via a water phase polymerization method using acrylic acid and acrylamide as monomers, N, N'-methylene bisacylamide as a crosslinking agent and potassium persulfate (KPS) as an initiator. For the synthesis of the composite SAPs, 9 g of acrylic acid with 80% neutralization degree, 3 g of Acrylamide, 0.1 g of sodium alginate and 0.2 g of glucose were mixed at 25$^{o}$C. Then, 48 mg of KPS and 9.6 mg of N,N'-methylene bis(acrylamide) was added into the solution and reacted at 75 $^{o}$C for 3 h. After reaction, the obtained SAPs were dried at 50 $^{o}$C for 12 h.

*Cell culture and sample pre-treatment*

Hela cell line was cultured in DMEM containing 1% (v/v) penicillin-streptomycin and 10% (v/v) FBS, incubated at 37 $^{o}$C and with a humidified atmosphere of 5% $CO_2$. To remove exosomes derived from FBS, when the Hela cells were cultured upto about 60–70% coverage, washed with PBS, and then cultured in serum-free medium for another 12 h. And then the cells were washed with PBS, followed by culturing in the 2% (v/v) Exo-FBS (exosome-depleted FBS, purchased from Gbico, USA) medium and 1% (v/v) penicillin-streptomycin for 48 h. Next, the cell culture medium was collected and the cell pellets, debris and micro vesicles were removed by centrifuged at 300 g for 10 min, 2000 g for 20 min, and 10 000 g for 30 min at 4 $^{o}$C, respectively. Lastly, the pretreated samples were stored at -80 $^{o}$C for further use.

*Sample concentration by the UF method and the SAP method*

The collected culture medium was concentrated by the UF method with an ultrafiltration tube (Merck Millipore, 100 kDa) or the SAP method. For the former, the pre-treated cell culture medium was centrifuged at 2000 g at 4 °C for 10 min. After centrifugation, the concentrated solution was collected for further use. For the latter, a certain amount of the SAPs was mixed with the cell culture medium and let the mixture sit at 4 °C for 2 h. Then, the swelling SAPs were removed and the concentrated exosome samples were obtained.

*Exosome isolation and detection with the commercial kit*

The concentrated cell culture medium was used to isolate the exosomes with the commercial Exosome Extraction kit (Liaoning Rengen Biosciences Co., Ltd.). All the operation followed the instructions of the commercial kit. Firstly, the concentrated samples were mixed with the precipitation agent (provided by the kit) for 30 min at 4 °C. Then, the mixed solution was centrifuged at 12000 g at 4 °C for 30 min to collect the precipitate. Next, the precipitate was dissolved by PBS and centrifuged at 12000 g at 4 °C for 5 min with purification column (provide by the kit). Finally, the solution was collected and stored at -80 °C for further use. The commercial Exosome detection kit (Liaoning Rengen Biosciences Co., Ltd.) was employed for exosomes detection, all the operations were carried out under Manufacturer's instructions.

*Exosome characterizations: transmission electron microscopy (TEM) characterization and dynamic light scattering (DLS) analysis*

For TEM characterization, 10 ml of the exosome samples was dropped onto a copper grid (Cat. No. 01340, Ted Pella, Inc. USA) and stood for 10 min. After that, the excess sample solution was absorbed with a piece of filter paper, then the exosomes were stained with 2% (w/v) phosphotungstic acid for 3 min. Next, the grid was dried at infra-red lamp for 10 min and observed under a TEM at 120 KV (HITACH HT7700, Japan). The size distribution and polydispersity index (PDI) of the exosome samples were measured with DLS equipment (ZetasizerNS, Malvern, UK) at 25 °C.

*Exosome characterizations: western blotting analysis*

The exosomes or cells were treated with RIPA lysis solution, and then the protein concentration of the concentrated exosome samples was measured with the commercial BCA Protein Assay Kit according to the manufacturer’s protocol. Next, the protein samples were mixed with the loading buffer after adjusting protein concentration, and heated at 100 °C for 5 min to denature proteins. Then, the proteins were separated by the SDS-PAGE electrophoresis and transferred onto a piece of PVDF microporous membrane through the wet membrane transfer device. The PVDF membrane was rinsed with TBST for three times (each time for 10 min) firstly and then blocked with 5% BSA

solution (dissolved in TBST) for 2 h at 25 °C. Next, the PVDF membrane was incubated with different antibody (anti-TSG 101 or anti-CD63) at 4 °C overnight. After rinsing with TBST, the PVDF membrane was incubated with the HRP-labeled secondary antibody for 2 h and washed with TBST. Densitometry of each protein was obtained by the chemiluminescence (ECL) kit.

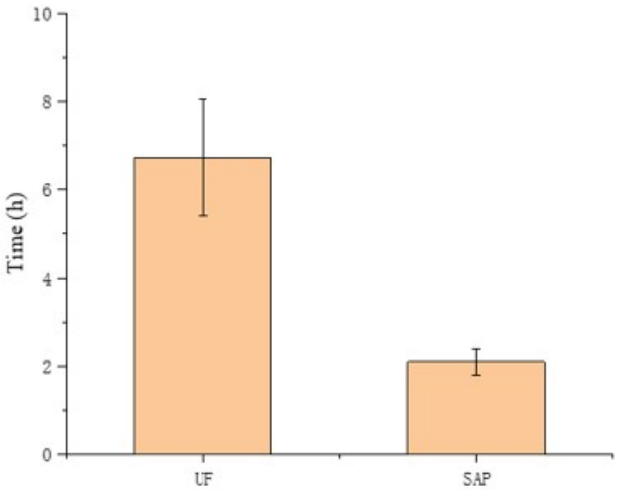


**Figure S1**. The time for exosome enrichment with UF method using an ultrafiltration tube and our SAP method.