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Microfluidic device for the analysis of MDR cancerous cell-derived exosomes' response to nanotherapy

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Abstract

Exosomes are membrane-enclosed extracellular vesicles which have been indicated as important biomarkers of cancerous cell functionality, such as multiple drug resistance (MDR). Nanoparticles based chemotherapy is a promising strategy to overcome MDR by interfering the production and composition of exosomes. Therefore, tumor-derived exosomes post-treatment by nanotherapy are implied to play critical roles of biomarkers on cancer MDR analysis. However, the efficient isolation of such exosomes from extracellular environment for their therapeutic response analysis remains challenging. In this study, we presented a microfluidic device featured exosome specific anti-CD63 immobilized ciliated micropillars, which were capable to isolate cancer-derived exosomes from cell culture medium. The captured exosomes can be recovered intact by dissolving the cilia on the micropillars using PBS soaking. Owing to the immobilized antibody in the microfluidic device, nearly 70% of exosome from the biofluid could be isolated. So the secreted exosomes of the MDR and ordinary human breast cancer cells pre-treated by free drug or nanotherapy could be isolated with high purity. The drug contents of the isolated exosomes were measured to analysis of the exosomal pathway response of MDR cells to different chemotherapeutic formulations. Such analyses and further definition of the biomarkers of these exosomes could benefit the future investigations of accurately and reliably determine design principle, functional activity, and mechanisms of nanotherapy for MDR overcoming.

Keywords Exosome · Microfluidic device · Isolation · Nanoparticles therapy · Multiple drug resistance

1 Introduction

Nowadays, chemotherapy is playing an important role to against various cancers, such as breast cancer, ovarian cancer and lung cancer etc. (Fisher et al. 1998; Le Chevalier et al. 2004; Parmar et al. 2003). However, the efficiency of chemotherapy is mainly restricted by multiple drug resistance (MDR) acquired by cancerous cells under long-time drug exposure (Foo and Michor 2014; Gottesman 2002). To overcome the MDR, a lot of treatment methods are developed: one of the effective strategies is the introducing of nanotherapy which refer to technologies of delivering chemotherapeutic agents by loading them into nanoformulations such as liposomes, polymeric micelles and other nanomateirals (Hu and Zhang 2012; Yuan et al. 2016; Zhang et al. 2017). The advantages of such strategy are obvious. Briefly, small molecule chemo drugs are transferred into cancerous cells via membrane translocators or passively diffuses, which make them easily to be refluxed back to the extracellular environment by the MDR cell membrane over-expressed P-glycoprotein (P-gp). Meanwhile, drug-loaded nanoparticles are internalized into cells through endocytosis pathways to overcome the MDR P-gp related effluxing mechanism (Bannunah et al. 2014; Oh and Park 2014). Thus, the involving of nanoparticles therapy (nanotherapy) into MDR cancer treatment did result in improved chemotherapy efficacy. However, for the further clinical translation and development of nanotherapy, it is essential to elaborate the specific process of nanoparticles on the MDR overcoming. The recent studies have shown that the cancer-derived exosome secreting pathway plays an important role during this process (Soekmadji and Nelson 2015; Wang et al. 2016).

Exosomes are extra-cellular double-layer lipid vesicles with 40–120 nm in diameter secreted by the majority of viable cells to the bodily fluids, including blood, urine, and saliva.

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(Fevrier and Raposo 2004; van der Pol et al. 2012; Yang et al. 2014). Typical exosomes sourced from epithelial cells, lymphoid cells, or tumor cells contents functional proteins, lipids and RNAs and act significant roles in intercellular communication (Kucharzewska and Belting 2013; Rak 2013). Moreover, due to the universal existence and stability of exosomes in most of body fluid and resemblance of their contents to parental owners, exosomes have great potential to perform as traceable biomarkers for various diseases (Melo et al. 2015; Tang and Wong 2015). For example, the exosome derived by cancerous cells impact in many cancer-related pathways, such as tumor growth, metastasis, and angiogenesis (Kalluri 2016; Yang and Robbins 2011). Furthermore, cancer-derived exosomes have been found to participate in the MDR generating and acted as a major role in the process (Giallombardo et al. 2016; Santos et al. 2018). Corcoran et al. discovered exosomes contributed to docetaxel excretion of prostate cancer cells, and caused phenotypic change to non-resistant cell (Corcoran et al. 2012). Roohangiz et al. documented that the exosomes secreted by cisplatin-resistant cancer cells contained 2.5 times more cisplatin than of that from normal cancer cells (Safaei et al. 2005). These studies demonstrated the exosomal pathway effects through small drug efflux during the occurrences of MDR. However, the performance of exosomes in MDR overcoming nanotherapy and the interfering of nanoparticles on exosome generation and secretion still need to be evaluated (Oves et al. 2018) .

To analyze the cancer-derived exosomes, it is important to develop the efficient technique to isolate the exosomes from body fluid. Different methods have been introduced. One of the conventional procedures includes the differential centrifugation which involves a series of centrifugation and filtration steps suffered of inconsistent exosome recovery rates, the breakage of exosome membrane and contamination of co-sedimentation of protein aggregates (Lamparski et al. 2002). Another procedure for exosome isolation is immuno-affinity capturing which applies the materials such as magnetic particles that are coated with a monoclonal antibody to specifically bind the exosome. This method has been implement as less time consuming and high specific exosome purity (Szatanek et al. 2015). Recently, research efforts have increased dramatically on developing immuno-affinity capturing microfluidic devices in order to isolate biological exosomes. Compare to the other procedure used as immuno-affinity capturing, microfluidic technology offers high isolation yield and functional integration for target exosomes (Kanwar et al. 2014). Chen et al. reported an effective microfluidic exosome isolation platform, whose surface is immobilized with anti-CD63 antibody for capturing of exosomes from human serum (Chen et al. 2010). Kanwar et al. developed a similar microfluidic device called ExoChip, which utilized antibody immobilized circular chamber to capture exosomes, following by fluorescent staining for on-chip isolation, quantification and characterization of circulating exosomes (Kanwar et al. 2014). Therefore, antibody immobilized exosome isolation procedures were configured to enhance the capture efficiency. However, it is quite difficult to elute the captured exosomes from the antibody immobilized magnetic particles or the microfluidic device, which can present a major limitation of the possibilities for further experimentation on the extracted exosomes (Tauro et al. 2012).

Our group has presented a microfluidic device consisting of ciliated micropillars with 30–200 nm interspaced porous silicon nanowires through simply electroless etching using electro-deposited silver nanoparticles (Wang et al. 2013). This microfluidic device was able to preferentially physical trap exosome-like lipid vesicles by the silicon nanowire cilia while allowing smaller proteins and larger nanoparticles with the size of cellular debris to pass through unhindered. Moreover, the captured liposome can be released by dissolving the porous silicon nanowires in PBS buffer, thus allowing for the high integrity and purity of isolation contents recovery. However, this platform based on physical vesicle size isolation was restricted by surface lipid saturation limit. In this paper, the alternatively improvements and application of this microfluidic device are developed. The porous silicon nanowires of the device were fabricated by depositing gold nanopattern on the sidewalls of ciliated micropillars which is proved to be more effective and less time consuming. The nanowires of the ciliated micropillars were immobilized by the exosome-specific antibody, which allows efficient and non-invasive rapid isolation of specific exosomes with high purity. We hypothesized such improvements will significantly speed up the exosome isolation and analysis; benefit to acquire more accurate biological information. Moreover, The design of this microfluidic device provide the opportunities to isolate and quality the exosomes secreted from MDR cancerous cells and evaluate the interfering of drug-loaded nanoparticles on exosomes production and composition procedure. The outcome of this study would improve our understanding the mechanisms of exosomal responding to MDR profile during traditional chemotherapy or nanotherapy, thus leading to more efficient MDR cancer treatment.

2 Materials and methods

2.1 Materials

Chloroauric acid ($HAuCl_4$), (3-Aminopropyl)triethoxysilane (APTES), doxorubicin (Dox) and paclitaxel(PTX) were purchased from Sigma-Aldrich (St. Louis, MO). P-type (100)

silicon wafers with resistivity of 0.005 Ω-cm were purchased from Silicon Quest (Santa Clara, CA). Polydimethylsiloxane (PDMS) and curing agent were obtained from Dow Corning (Midland, MI). Phosphate buffered saline (PBS, pH 7.2) was obtained from Gibco (Thermo-Fisher, Waltham, MA). Hydrofluoric acid was from Honeywell International Inc. N-maleimidobutyryl-oxysuccinimide ester (GMBS) and thiol functionalized streptavidin were purchased from Nanocs Inc. (Woburn, MA). The biotinylated anti-CD63 antibody and control IgG was purchased from Abcam Co. (Boston, MA). Human breast cancer cell line MDA-MB-231 cells were purchased from the American Type Culture Collection (Rockville, MD, USA). The multidrug resistant MDA-MB-231/MDR cell line was cultured in our lab. All the cells were cultured in DMEM (Thermo-Fisher, Waltham, MA) supplemented with 10% FBS. The exosome Labelling Kit (ExoGlow™) was purchased from System Bioscience (Palo Alto, CA). Amphiphilic copolymer methoxy-poly (ethylene glycol)-poly (ε-caprolactone) (mPEG-PCL, Mw: 5000:5000) was purchased from Polymer Source Inc. (Canada).

2.2 Device fabrication

The ciliated micropillar arrays on the microfluidic device were fabricated by modification of previously described procedure (Wang et al. 2013). We designed a large capacity microfluidic devices with 627mm^2 micropillar area (as shown in Supporting Information Fig. S1B). A close-pack arrangement of hexagonal micropillars was adapted in the devices to increase the exosome capture efficiency. As seen in Fig. 1a, an example of fabricated ciliated hexagonal micropillar array, the distance between pillars was set as constant ~1.1 μm which will block large objects like cells existing in biological fluids. The devices were fabricated in two steps. First, the fluidic channels with high aspect ratio micropillars were fabricated by the deep silicon etching on an Inductively Coupled Plasma (ICP) etcher. Second, porous silicon nanowires were etched all around the micropillars by using metal assisted nanowire etching. To do so, silicon wafer with micropillars was first dipped into HF solution to remove native oxide. Then the wafer was assembled on the Teflon electroplating cell, and sealed with a Viton O-ring. An aluminum foil was pressed on wafer backside as electrode. A platinum mesh immersed into 0.5 M $HAuCl_4$ plating solution was applied as anode. The reverse pulse electroplating is performed to deposit uniform gold nanopattern on the sidewalls of the micropillars. After that, the wafer was chemically etched in a mixture of 0.1 M H_2O_2 and 2.9 M HF solution for 2 min to generate ~0.5 μm long nanowire cilia on the micropillars. The gold nanopattern was then stripped by immersing into gold etchant (Transene Inc., Denvers, MA) for 2 min. The substrate was then rinsed in excess water, ethanol and dried by blow air.

2.3 Device construction

The ciliated micropillars on the wafer were first functionalized with the APTES by immersing into 30% H_2O_2 at 90 °C overnight for oxidation and subsequently in 1% APTES isopropanol solution at 65 °C for 24 h as reported (Gunda et al. 2014). And the polydimethylsiloxane (PDMS) caps were

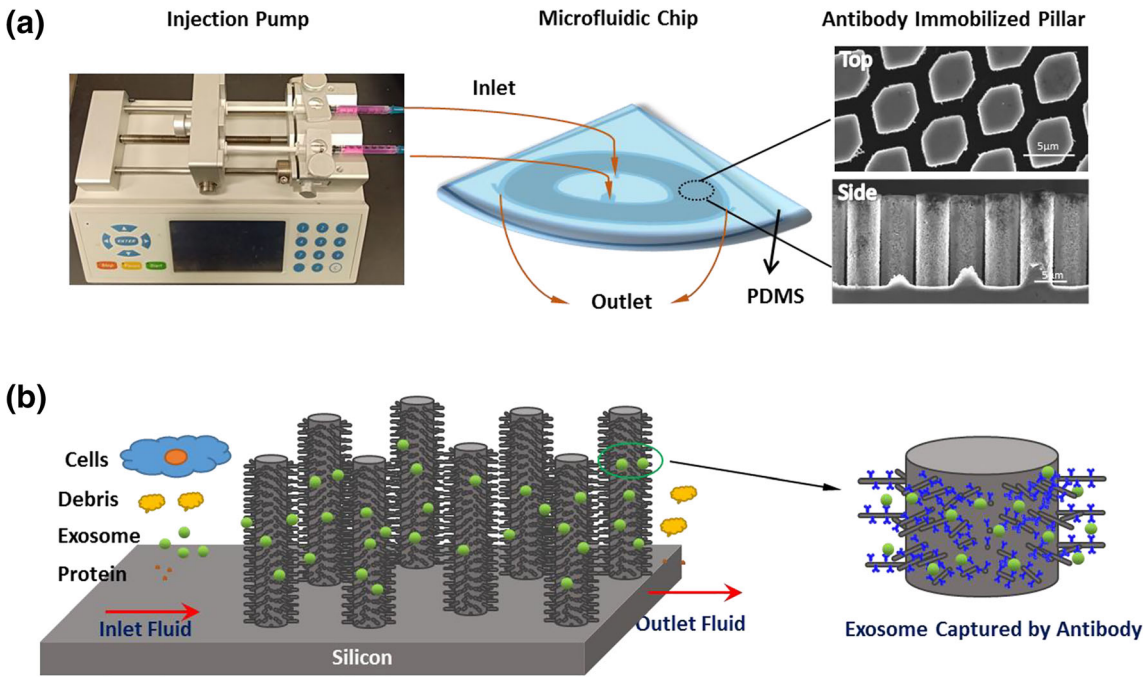

Fig. 1 Schematic of the antibody immobilized ciliated micropillar array for exosome isolation. **a** Experimental set-up of the ciliated microfluidic device system for exosomes isolation. **b** The exosomes are captured by antibody immobilized micropillars when passing the microfluidic system

made in a 10:1 elastomer base-curing agent ratio and incubated at 70 °C for 4 h under vacuum followed by cleaning with isopropanol and deionized water. The APTES functionalized wafer and PDMS cap were treated with oxygen plasma for 30 s, and then manually bonded and subsequently baked at 37 °C for 10 min to get the microfluidic device with the ciliated micropillars.

2.4 Surface modification

For antibody immobilizing, the device was rinsed in the 0.1 mg/ml GMBS solution for 30 min followed by washing with purified water. Then the microfluidic device was sequential dipped into thiol functionalized streptavidin and biotinylated anti-CD63 antibody PBS solution at 4 °C. The antibody immobilized microfluidic device was obtained after washing by 1% BSA solution and PBS. The control IgG immobilized microfluidic device was achieved as the similar method.

2.5 Exosome isolation and recovery

The cells were cultured DMEM supplemented with 10% exosome depleted FBS (System Bioscience Palo Alto, CA) for 12 h. Then the culture medium were collected and centrifuged at 2000 rpm for 10 min to remove cell debris. The exosome in the medium was labelled by Exo-Green Exosome Labelling Kit according to protocol. The labelling exosome solution was continuously injected into the anti-CD63 or control IGG immobilized microfluidic device through the inlet at a flow rate of 10 μL/min by the Fusion 200 high precision syringe pump (Chemyx, Inc. Stafford, TX). The waste was then collected at the outlet. The fluoresce intensity of inlet and outlet exosome was measured by BD plate reader (BD Biosciences, Billerica, MA). The isolation efficiency of exosome was calculated by the ratio fluoresce intensity value of inlet and outlet. For exosome imaging, PBS solution was injected into the device to wash untapped impurity. The exosomes in the device were fixed by 0.5 × Karnovsky's fixative and then sequencing dipped with water, 25% ethanol, 50% ethanol, 70% ethanol and 100% ethanol for 10 min each. The exosome on the micropillars of microfluidic device were examined using FEI Nova Nano Scanning Electron Microscope with 2 nm gold-palladium layer and Nikon A1 Confocal Laser Microscopy Imaging System with 488 nm excitation (Melville, NY, USA).

To recovery the isolated exosome from micropillars, the microfluidic device channels were immersing by PBS for 8 h and then flushed by PBS. The obtained the exosomes in PBS was placed on a mica slide and observed by Multimode Atomic Force Microscope with tapping mode (Bruker, Billerica, MA).

2.6 The preparation of drug loading nanoparticles

The doxorubicin encapsulated liposome (Doxoves®, 83 nm) was purchased from FormuMax Scientific, Inc. (Sunnyvale, CA). The paclitaxel containing micellar nanoparticles was prepared by co-solvent evaporation method. Briefly, mPEG-PCL (10 mg) and 1 mg of PTX were dissolved in 0.2 mL acetone. This solution was added to 1 mL of water. The mixture was then sonicated in ice bath for 2 min. The excess organic solvent was removed under vacuum. The micellar nanoparticles solution was filtered through a 0.22 μm Minisart syringe filter (Sartorius, Germany) to remove free PTX and subsequently lyophilized. The size and Zeta potential of PTX loaded nanoparticles were characterized by Zetasizer Nano (Malvern, UK) and the morphology of the nanoparticles was observed by Atom Force Microscopy in tapping mode. The PTX content in the nanoparticles was measured by Agilent 1200 High Performance Liquid Chromatography System (HPLC) (Agilent, Santa Clara, CA).

2.7 Multiple drug resistance characterization

MDA-MB-231/MDR and MDA-MB-231 were seeded in a 96 well plate at 5×10^3 cells/well for 24 h. Then the cell culture medium was replaced with new medium containing free Dox, Doxoves®, free PTX (dissolved in DMSO) or PTX-micellar nanoparticles with different concentration for culturing another 72 h. The chemo-sensitivity of the MDA-MB-231/MDR and MDA-MB-231 cells were measured by MTT assay as literature reported (Napierska et al. 2009)

2.8 Cell treatment and exosome analysis

MDA-MB-231/MDR and MDA-MB-231 were pretreated with free Dox, Doxoves®, free PTX or PTX-micellar nanoparticles by a fixed drug concentration of 40 μg/mL for 4 h. Then the medium were discarded and the cells washed twice with cold PBS and incubated with DMEM supplemented with 10% exosome depleted FBS for another 12 h. The culture mediums were collected and centrifuged at 2000 rpm for 10 min to remove the cell debris. For exosome isolation, the collected mediums were injected into the anti-CD63 immobilized microfluidic device through the inlet at a flow rate of 10 μL/min and washed by PBS twice. Then the isolation exosomes were collected by immersed the micropillars into PBS containing 0.1% Triton X-100 for 8 h and the channels were flushed by PBS containing 0.1% Triton X-100 to increase the drug solubility. The drug content in the obtained exosomes was tested by Agilent 1200 High Performance Liquid Chromatography System.

3 Results and discussion

3.1 Design of the anti-CD63 antibody immobilized microfluidic device

A microfluidic device capable of simultaneously multi-dimensional filtration of the biofluid is highly desirable to fulfill the mission to isolate the exosomes. We designed and fabricated the microfluidic devices with hierarchical ciliated micropillar structure (Fig. S1) as we reported previously (Wang et al. 2013). In order to increase the capture efficacy, we laid out a large capacity microfluidic devices with 627mm^2 micropillar area (Fig. S1B, C). As illustrated in Fig. 1a, the assembled microfluidic device consists of a PDMS caps and a concentric ellipses-flow fluidic channel containing antibody immobilized ciliated hexagonal micropillars with 5 μm across and 15 μm in height. The gaps among the micropillars are set as constant ~1.1 μm for a steady fluid-micropillar interaction. Circular inlet and outlet ports were connected to the micropillar area with a syringe pump for sample introduction and extraction. We hypothesized that the interstitial sites of the micropillars capable of physically (1) bypassing the unwanted components of sample, such as small molecular protein and submicron-size cellular debris; (2) filtering the larger sample components, such as cells. Moreover, the antibody immobilized nanowires on the sidewalls of ciliated micropillars are dedicated to selectively capture the exosomes flowing through the device (Fig. 1b), and the porous silicon nanowires could be dissolved in buffers to integrity isolate the captured exosomes for their further analysis. Thus, such microfluidic device is capable to apply for the MDR cancerous cell-derived exosomes isolation and the analysis of their response to nanotherapy.

The nanowire cilia were fabricated on micropillars to increase the contacting area between micropillars and bypassing fluid, in order to enhance exosome capturing efficiency. Briefly, the nanowire was fabricated by the combination of electroplating, electroless metal-assisted etching techniques. First, by the governing of the electrochemical reaction and reactant diffusion, as well as the properties of the reactants and silicon substrates, a uniform gold nanopattern is deposited onto the sidewalls of hexagonal micropillars (Fig. 2a). Second, the nanowire cilia were obtained by subsequently metal-assisted chemical etching of the micropillar surface and stripping the excess gold nanopartterns (Fig. 2b). Finally, the nanostructured silicon surface was functionalized with amine groups followed by decorating of streptavidin, thus the biotinylation anti-CD63 antibody could be stable immobilized on the nanowire cilia of micropillars for exosome capturing (Fig. 2c).

3.2 Isolation of exosomes by the microfluidic device

Fluorescein-labelled cancer cell-derived exosomes were labelled and used as the traceable samples to verify the functionality and isolation efficacy of our antibody immobilized microfluidic device. The samples was injected to the inlet through the PDMS cap and extracted at the outlet. After that, the captured exosomes by the ciliated micropillars on the microfluidic device were observed by SEM. As shown in Fig. 3a, b, the captured exosomes are visualized on the sidewalls of micropillars and trapped in the porous of the nanowire, which demonstrated the capacity of microfluidic device with ciliated micropillars to capture the exosomes from body fluid. To quantity of the efficacy on exosomes isolation, the eluting outlet samples were collected and the fluorescence intensity was divided versus that of the totals injected samples. The unspecific IgG immobilized microfluidic device was used as control. As shown in Fig. 3c, 75% of the total exosome retention was observed by the anti-CD63 immobilized microfluidic device, while only less than 20% of exosome retention rate was shown for no-selective IgG immobilized one. The functionalization of antibodies against CD63, an antigen commonly overexpressed in exosomes greatly contributes to this significant efficacy of exosome capturing (Szatanek et al. 2015). The captured exosome by the antibody immobilized microfluidic device was further visualized by confocal laser microscopy. As shown in Fig. 4a, b, the continuously green fluoresce dots which represent the captured exosomes clusters were observed to absorb on the sidewalls of micropillars. Notably, the three-dimension imaging showed the majority of the exosomes was trapped around the micropillars rather than the bottom of the microfluid device, which demonstrated the forming of turbulence among the micropillars enhanced the absorbing chances of exosome and anti-CD63 leading the high isolation efficacy. These revealed results showed that our microfluidic device with anti-CD63 immobilized ciliated micropillars offers obvious advantages on exosome isolating.

3.3 The recovery of captured exosomes

It has been reported that the porous silicon nanowires degraded in the PBS buffer (Chiappini et al. 2010). Thus, the microfluidic device prototype we developed is not only applied for exosome capturing but also recovering the captured exosomes through the degradation of the porous nanowire of the ciliated micropillars. The degradation of nanowire was accomplished by soaking the channels of microfluidic device with PBS for 8 h after capturing exosomes. As shown in Fig. 5a, the nanowires of ciliated micropillars in the

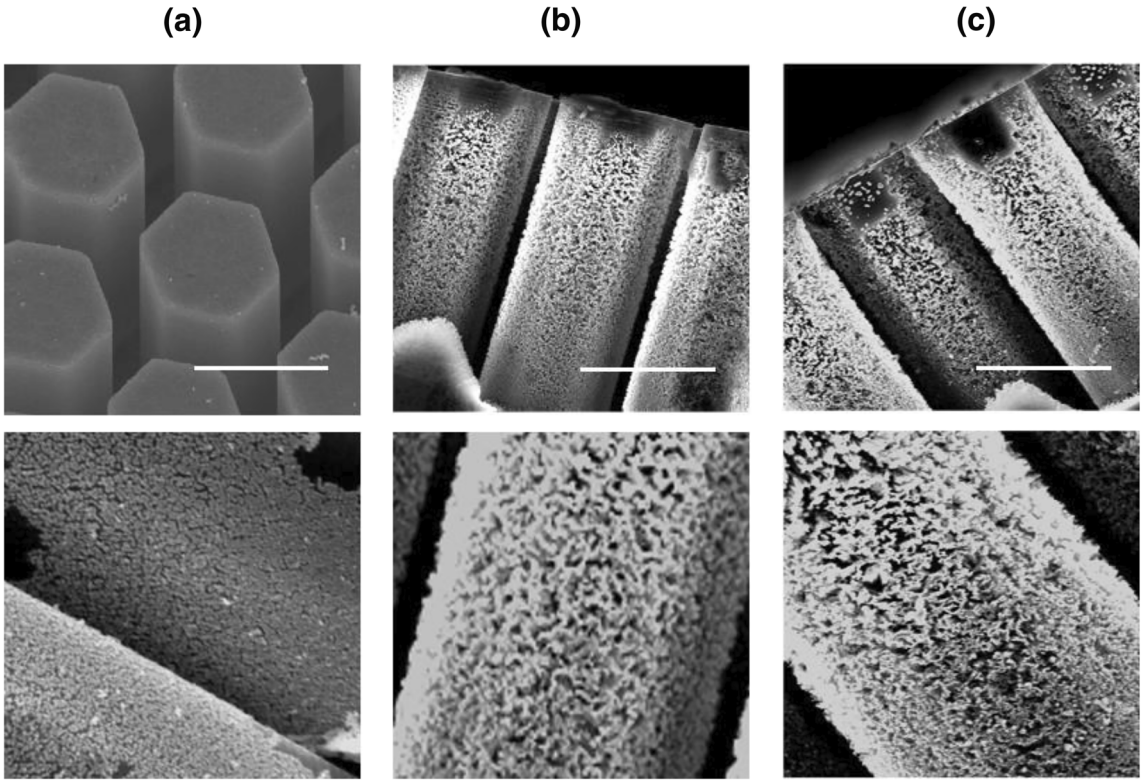

Fig. 2 SEM images of the antibody immobilized ciliated micropillars preparation stages. **a** Hexagonal silicon micropilliars before(top) and after (bottom) gold deposition(bottom). **b** Porous silicon nanowire cilia are formed on the sidewalls of the micropillars after etching (top) and its close-up view (bottom). **c** Anti-CD63 antibody were immobilized on the ciliated micropillars(top) and its close-up view (bottom). All scale bars are 5 μm

microfluidic device fully disappeared, which lead the stripping of the anti-CD63 immobilizing layer and trapping sites to release the capturing exosomes. Commonly, the traditional methods of isolating exosomes always interfere with the structure of the exosomes and hardly to show the morphology of native exosomes (Szatanek et al. 2015). However, the recovered exosome from our microfluidic device maintained its 100 nm sphere morphology and the integrity of surface structure as characterized by Atom Force Microscopy (Fig. 5b). Overall, the microfluidic device we designed presents the significantly capacity on high purity exosomes selectively isolating and recovering from biofluid.

3.4 Measuring of drug content of isolated exosomes

Intrinsic or acquired MDR which considerably limits the efficacy of chemotherapeutics, is mediated by different mechanisms, such as P-gP effluxing (Hayeshi et al. 2006; Kim et al. 2016). Recently, nanotechnology holds great promising development for drug delivery systems with controlled-release and drug-resistance overcoming profiles for MDR cancer treatment (Hayeshi et al. 2006). It is reported that the altering biophysical properties of anticancer drugs by nanoparticles conjugation or encapsulation was able to bypass the recognition of

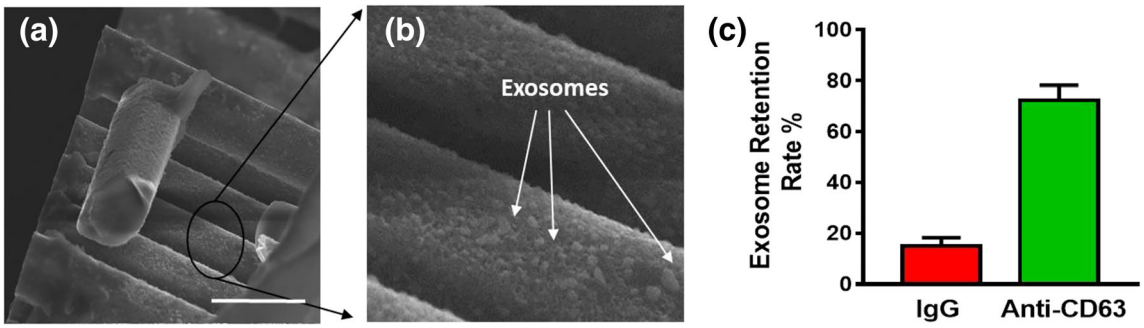

Fig. 3 Exosome retention by the antibody immobilized micropillar array. **a** SEM images of exosome captured by ciliated micropillars (Scale bar 5 μm). **b** Close-up view of captured exosome on the micropillar (Scale bar 500 nm). **c** Comparison of exosome retention rate of anti-CD63 antibody immobilized micropillars and IgG immobilized micropillars

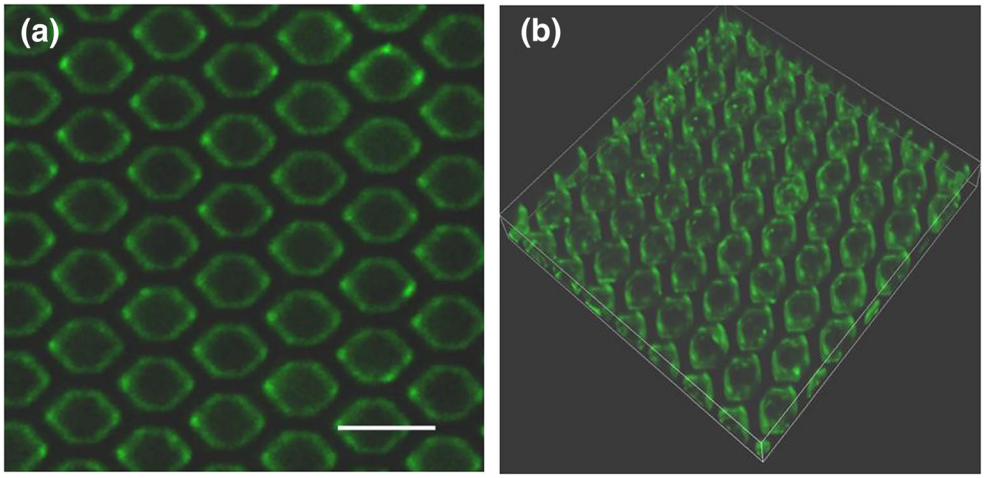

Fig. 4 Confocal microscopy images of fluorescein-labelled exosomes captured by **a** ciliated micropillar and **b** its 3D imaging. Scale bars represent 10 μm

P-gP (Brigger et al. 2012). Moreover, the paramount overlap of exosome expelling and the uptake of nanoparticles are still unclear. Especially considering that the secreting of cancerous cell exosomes plays critical roles in drug resistance generating and transferring (Soekmadji and Nelson 2015; Wang et al. 2016), the effects of exosomal pathway related to the drug-loaded nanoparticles MDR cellular internalization, further to the MDR overcoming need to be well evaluated.

As the results above, our microfluidic device with antibody immobilized ciliated micropillars is a well-suited platform for effective and non-destructive exosome isolation. By isolating exosomes from MDR cells treated with nanotherapy with this device, the drug efflux profile in the exosomal pathway can be determined, which lead a better understanding of drug expulsion mechanism through the exosomal pathway and circumventing MDR effect. For this purpose, two different of drug-loaded nanoformulations were prepared: the commercialized doxorubicin-loaded liposome (Doxoves, 83 nm) and synthetized paclitaxel-loaded mPEG-PCL micellar nanoparticle with sphere morphology of 74 nm diameter and − 4.12 mV surface potential (Fig. S2). The normal human breast cancer cell MDA-MB-231 and its MDR type MDA-MB-231/MDR were both treated by free small drugs and nanotherapeutic formulations. After that, the expelled exosomes in the cultured mediums were isolated through anti-CD63 immobilized microfluidic device and the total drug contents in the exosomes was measured to study exosomal pathway corresponding to different forms of drug treatment. As shown in Fig. 6a, b, both the exosomes from nanoparticles treated non-resistant and MDR cell showed much lower drug content than free drug treated counterpart. Moreover, it is notably that free drug treated MDR cells showed more drug efflux than its counterpart sensitive cell due to the MDR profiles (approximately 2 times and 1.5 times higher of DOX and PTX, respectively), while nanoformulations treated MDR cells showed comparable drug efflux to its sensitive counterpart.

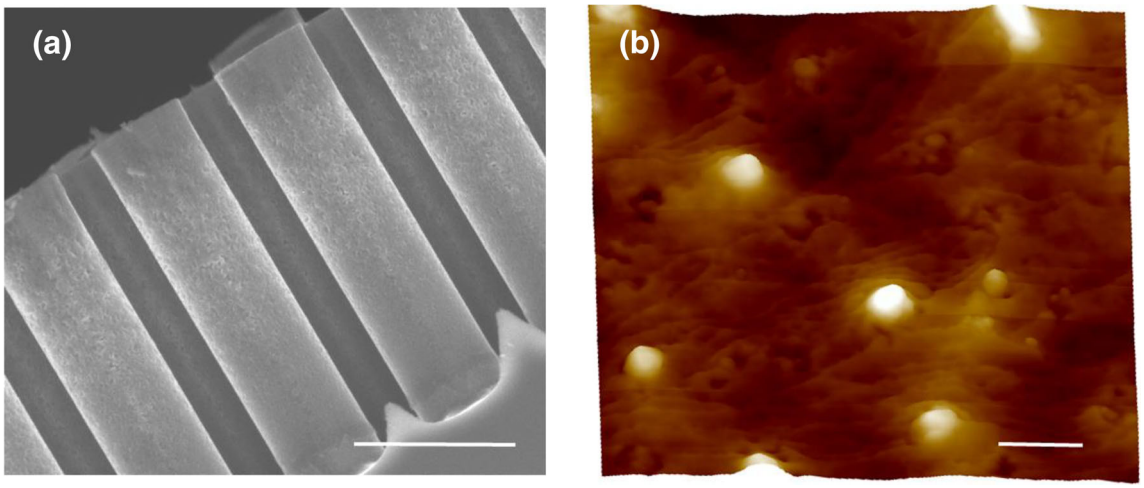

Fig. 5 **a** SEM image of the micropillars after soaking in PBS buffer to recover the captured exosome. (Scale bar represents 5 μm) **b** Atom forced microscopy image of the recovered exosomes from micropillars. (Scale bar represents 200 nm)

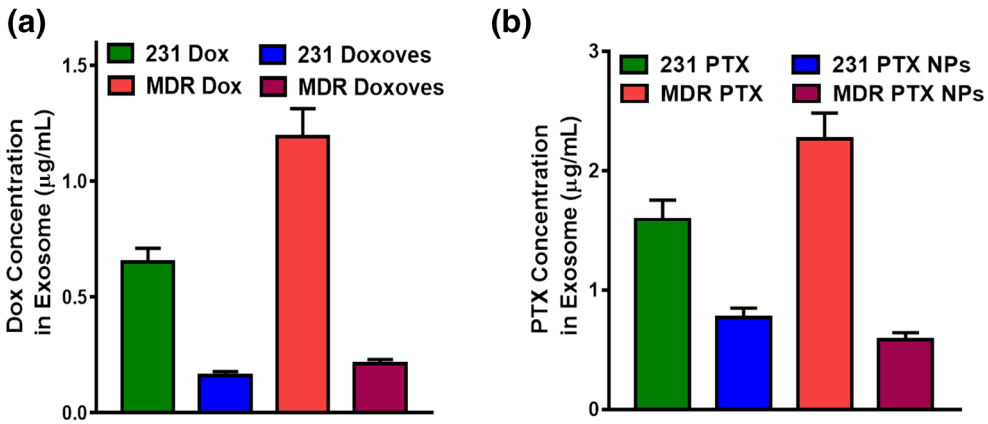

Fig. 6 **a** Doxorubicin content in the exosomes isolated from MDA-MB-231 human breast cancer cells (231) and MDR MDA-MB-231 human breast cancer cells after treating by different drug formulations. **b** Paclitaxel content in the exosomes isolated from MDA-MB-231 human breast cancer cells (231) and MDR MDA-MB-231 human breast cancer cells after treating by different drug formulations

Considering the same treated drug concentration on both sensitive/MDR cancer cells, these results demonstrated the existing overlap of the cellular exosomal expelling pathway and bypassing P-gP effluxing effect of nanoparticles. However, the enhanced cellular internalization of different nanoformulations were not inducing the actual MDR overcoming (Fig. S3, Table S1). The slow drug releasing from the nanoparticles or other mechanisms related MDR such as anti-apoptosis may be responsible to the nanotherapy insensitivity of cancerous cells (Hu and Zhang 2009; Notarbartolo et al. 2002; Song et al. 2012, 2013, 2014). Thus, developing a novel drug-loading nanoplatform not only bypassing exosomal effluxing pathways when internalized by the cancerous cells but also showing intracellular burst release profiles and personalizing capacity against the multi-mechanisms of MDR is essential for the future clinical applications of nanotherapy.

4 Conclusion

We developed a microfluidic device consisting of nanowire enhanced ciliated micropillar arrays. By the immobilization of anti-CD63 on the nanowires, this device was capable of the specifically capturing the exosomes in the biofluid, while simultaneously bypassing smaller proteins/cell debris and filtering larger particles such as cells. The captured exosomes were able to be recovered without structure destructive by dissolving the porous nanowires in buffers. Therefore, this microfluidic device could be used as a rapidly and efficiently tool to selectively isolate exosomes from complex biological samples while maintaining their structural integrity, offering potential benefit to a range of both diagnostic and therapeutic applications. This device was further applied for the analysis of exosomes derived by MDR cancerous cells treated by drug-loading nanoparticles. Due to the superior performance of the microfluidic device, the relationships between the exosomal pathway and the MDR-related drug expulsion under the nanotherapy were identified. These results insure the future studies of MDR biomarkers on secreted exosomes under varies treatment procedure, as which will contribute to our further mechanism understandings on MDR acquiring and overcoming.

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