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Capture and detection of extracellular vesicles derived from human breast cancer cells using a 3D self-assembled nanostructured SiO₂ microfluidic chip

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Abstract

Background Tumor-derived extracellular vesicles offer a minimally invasive approach to evaluate tumor progression and metastasis. However, detecting biomarkers, such as extracellular vesicles in body fluids during the early stages of disease, remains a significant challenge. Conventional methods like ultracentrifugation-based isolation or Western blot protein quantification are time-consuming, require large sample volumes, and offer low yield and sensitivity. Therefore, the development of new biosensors for the specific and efficient analysis of tumor extracellular vesicles is urgently needed.

Methods Microfluidic devices provide extraordinary benefits for bioanalysis, offering a large surface area for the contact between target molecules and the biosensor, significantly enhancing the specificity, efficiency, and speed. These devices also enable nanoscale and microscale work using reduced sample volumes. In this study, we developed a three-dimensional self-assembled SiO₂-based nanostructured microfluidic chip, bioconjugated with specific antibodies targeting exosomal markers for the selective capture of CD63- and CD81-positive extracellular vesicles from breast cancer-derived conditioned cell culture media.

Results The three-dimensional SiO₂-based microfluidic chip effectively captured extracellular vesicles expressing CD63 and CD81 antigens from breast cancer cell culture media. This evidence demonstrates the potential of this platform to detect extracellular vesicles as biomarkers for cancer, providing a specific and efficient, non-invasive approach for cancer diagnostics.

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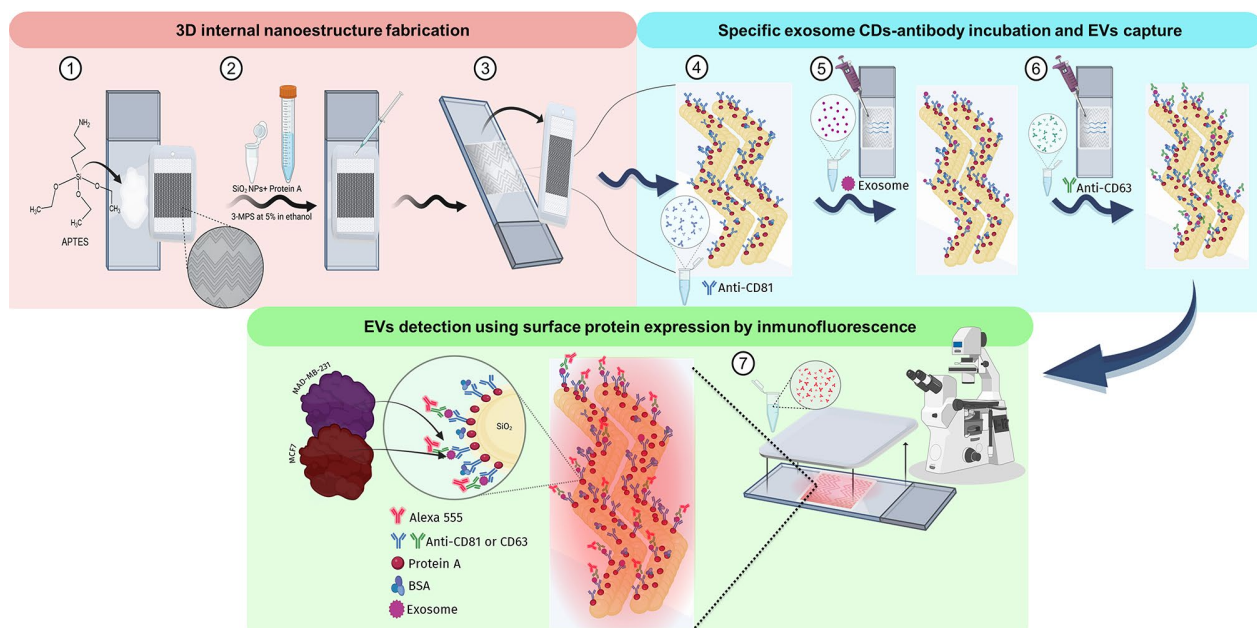


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Conclusions This study highlights the potential application of three-dimensional SiO₂-based microfluidic chips for detecting extracellular vesicles as a non-invasive liquid biopsy tool for breast cancer. The findings show a specific and efficient device as an alternative to conventional biomarker detection techniques.

Graphical Abstract

A schematic illustration of the extracellular vesicle capture process is provided, utilizing a self-assembled three-dimensional SiO₂-nanostructured microfluidic chip. (1) APTES was applied to the surface of the silanized slide, enabling the chip to adhere to the self-assembled structure; (2) a mixture of SiO₂ nanoparticles (NPs), protein A, and 5% 3-MPS in ethanol was injected to reinforce and enhance the mechanical stability of the nanostructure; (3) the chip was carefully demolded; (4) the SiO₂-NPs were bioconjugated with the CD81 primary antibody within the microchannels; (5) cell conditioned media from two different breast cancer cell lines (MCF-7 and MDA-MB-231) were subsequently incubated on the colloidal silica structure; (6) the CD63 primary antibody was then added; (7) after a 30-minute incubation with the Alexa 555 secondary antibody, the sample was observed using fluorescence inverted light microscopy.



Keywords Extracellular vesicles, Biosensors, Microfluidic chip, Liquid biopsy, Breast cancer

Introduction

Cancer remains one of the leading causes of morbidity and mortality worldwide [1]. The high mortality rate is largely attributable to late detection, as cancer is often discovered after it has advanced and metastasized, significantly limiting treatment options [2, 3]. Demographic projections suggest that the annual incidence of new cancer cases will rise to 35 million by 2050 [3]. These findings underscore the urgent need for prevention efforts, targeting key risk factors, as well as the development of innovative diagnostic and therapeutic strategies. While cancer screening and early detection are vital for improving patient outcomes, current diagnostic methods frequently lack the necessary sensitivity, specificity, or are too invasive [1, 4]. Extracellular vesicles (EVs), including exosomes and microvesicles, are a heterogeneous group

of nanoscale membranous structures (30–150 nm) of biological origin that play a crucial role in cell-to-cell communication. EV biogenesis begins with the formation of intraluminal vesicles (ILVs) through the inward budding of the endosomal membrane during the maturation of multivesicular bodies (MVBs), which are secreted following the fusion of MVBs with the plasma membrane [5]. EVs carry a diverse range of cargo, including lipids, nucleic acids and proteins, such as cell surface receptors and signaling molecules, which reflect the cell or tissue of origin, making them valuable biomarkers for various diseases, including cancer [6, 7]. In cancer, EVs are believed to play a pivotal role in intercellular communication, promoting tumor progression [6, 8–11]. Notably, EVs derived from malignant tumors induce a shift towards metastatic behavior when taken up by benign cells [12].

In addition, cancer cell lines are known to secrete significantly more EVs than noncancerous cell lines [13–16]. Several EV proteins are differentially expressed at various stages in different cancer types, becoming potential diagnostic or cancer progression biomarkers. For example, serum-derived EVs from breast carcinoma patients exhibit significantly increased levels of the oncogenic marker CD24 [17] and Lactadherin [10], while elevated levels of CD63 and Caveolin-1 are found in serum-derived EVs from melanoma patients [13]. In this line, EVs have emerged as promising biomarkers for cancer diagnosis due to their role in intercellular communication and their presence in various biological fluids, such as blood, urine, and saliva [18].

Conventional assays for EVs analysis, such as ultracentrifugation-based isolation, Western blot (WB)-based protein quantification, and enzyme-linked immunosorbent assay (ELISA) for molecular characterization, are often either time-consuming or expensive [19]. For instance, ultracentrifugation, the most widely used method for isolation, involves lengthy steps of centrifugation and requires large sample volumes, resulting in low yield and purity [20]. In contrast, microfluidic devices emerge as innovative platforms for EVs capture due to their significant advantages in bioanalysis. Microfluidic technology offers a large surface area for efficient interaction between target molecules and the sensor, which greatly enhances both analysis specificity and speed [21]. A key feature of modern microfluidic devices is their miniaturized analysis, which reduces sample consumption within a nanoscale device. Accurate fluidic control is crucial, since it not only integrates several functional components but also ensures precise and consistent measurements within the microfluidic system [21].

Immuno-affinity-based capture and analysis of EVs can be effectively performed using lab-on-a-chip devices by either modifying the microchannel surfaces or employing antibody-conjugated microbeads [21, 22]. This immuno-capture method is considered the only approach capable of selectively isolating a pure population of exosomes, whereas methods based on physical properties (such as size, density, and surface charge) often result in higher levels of nonspecific molecules or contaminants [23]. Consequently, several antibodies targeting exosomes have been used to functionalize different microfluidic devices [22]. Nevertheless, these advantages demand greater sensitivity and efficiency for detecting targets on the surface. Another advanced technique for isolating EVs from biological samples is size-based filtration. Although this method is often employed alongside other isolation techniques, such as ultracentrifugation to remove larger particles in the initial stages, it is insufficient on its own for isolating nanosized EVs. This limitation stems from

challenges such as the lack of suitable filters and the non-specific adhesion of EVs to the filters, which can lead to reduced recovery rates and concerns about EVs stability due to the pressure applied during nanofiltration [24].

Novel technologies and devices are continually emerging due to the relevance of this field [25]. However, several challenges remain unaddressed, including the need to enhance the yield, purity, and reproducibility of methods for isolating EVs from biological samples. Additionally, there is an imperative need to automate the EV-enrichment process to enable its rapid application in the clinical setting, as well as to develop straightforward methods for retrieving bioactive EVs that are compatible with subsequent molecular analysis.

Although nanotechnology has not yet been applied clinically for cancer diagnosis, there are already several nanomedical devices on the market, such as gold nanoparticles (NPs) in home pregnancy tests [26] or SARS-CoV-2 antigen test [27]. Due to their unique optical, magnetic, mechanical, chemical and physical properties, nanomaterials have been used for more sensitive and precise biomarker detection. Those applied to sensing cancer biomarkers include gold NPs, magnetic NPs, quantum dots, carbon nanotubes and nanowires [28–30]. An advantage of applying NPs on cancer detection lies in their large surface area to volume ratio [30]. Because of this property, nanoparticle surfaces can be densely coated with specific recognition molecules, including antibodies, small molecules, and fluorescent probes, aptamers and moieties. These molecules can bind and recognize specific cancer receptors/markers on the surface of circulating tumor cells (CTCs) and exosomes or bind to cell-free circulating tumor DNA (ctDNA) and proteins [31]. By presenting various ligands for binding to receptors, multivalent effects can be achieved, which can improve the specificity and sensitivity of an assay [32, 33].

In this study, we developed a three-dimensional (3D) self-assembled nanostructured SiO_2 microfluidic chips with herringbone nanopatterns for isolating human cancer cell-derived EVs. Based on the work of Zhang et al. [34], we used a polydimethylsiloxane (PDMS) template to create the chip and then injected a mixture of SiO_2 and mercaptoethanol into the microfluidic channels to form the 3D self-assembled nanostructure. This design enhances microscale mass transfer of bioparticles, increases the surface area and improves probe density, thereby boosting binding efficiency. The nanostructure was functionalized with a CD63 monoclonal antibody. Using a small volume of conditioned medium or samples enriched with EVs from cancer cell lines, we successfully detected EVs subpopulations positive for CD63 and CD81. This method represents a potential tool for point-of-care detection of cancer-derived EVs, highlighting its applicability in liquid biopsy-based cancer diagnosis.

Methods

Reagents and antibodies

Tetraethyl orthosilicate (TEOS, purity 99%), ammonium hydroxide (NH_4OH , 28.0–30%), 3-aminopropyl triethoxysilane (APTES, 99%), ethanol absolute (EtOH , 99.5%), 3-Mercaptopropyl-trimethoxysilane (3-MPS), Rhodamine isocyanate (RITC), glutaraldehyde, were purchased from Sigma-Aldrich (St. Louis, MO, USA). SYLGARD™ 184 Silicone Elastomer Kit was from Dow Corning. SU-8 was from Gersteltec Engineering Solutions (Lausanne, Switzerland). Protein A-Agarose was from Pierce™ (Thermo Fisher Scientific, Waltham, MA, USA). Rabbit monoclonal anti-CD63 and rabbit monoclonal anti-CD81 were from Cell Signaling Technology (Cell Signaling Technology, Inc, Danvers, MA, United States). Mouse monoclonal anti-CD63 and mouse monoclonal anti-CD81 were from Santa Cruz Biotechnology (Santa Cruz, CA). Alexa Fluor 555 goat anti-mouse IgG, and Alexa Fluor 488 goat anti-mouse IgG were from Invitrogen (Thermo Fisher Scientific, Waltham, MA, USA). The Fetal bovine serum (FBS) was from Biological Industries. Cell culture media and antibiotics were from GIBCO (Invitrogen).

Design and mold fabrication of the microfluidic device using optical lithography

The microfluidic device was designed using AutoCAD®. The geometry featured a total of 110 channels arranged in zig-zag pattern (Fig. 2A), with two reservoirs for injecting and removing fluids within the microchannels (Fig. 2B).

The molds for the microdevice were fabricated at the Optic Lithography Laboratory in the Department of Physics, FCFM, University of Chile [35].

For the optical photolithographic design, a 6 cm diameter silicon wafer substrate was used. The silicon wafer was coated with SU-8 photoresist resin using a spin coater (WS-650MZ-23NPPB) under conditions of 4–5 bar pressure at 500 rpm for 120 s, resulting in a SU-8-layer height of 100 μm .

A pre-bake was then performed at 65 °C for 8 min, followed by an increase in temperature to 95 °C for 15 min to remove the solvent from the resin. The solidified resin for the PDMS molds were obtained using the well-known method of optical lithography (Heidelberg Instruments MLA 100) [36]. This was followed by a post-bake of 5 min at 65 °C, with the temperature then increased to 95 °C for 10 min to accelerate crosslinking in the UV-exposed areas. The silicon wafer was subsequently immersed in propylene glycol monomethyl ether acetate (PGMEA) for 10 min to remove the unexposed resin. To stop the reaction, the silicon wafer was rinsed with isopropanol and subjected to a final bake at 65 °C for 5 min, then at 135 °C for 2 h.

Fabrication of polydimethylsiloxane (PDMS) microfluidic chips by soft lithography

Microfluidic devices were fabricated using polydimethylsiloxane (PDMS) (Sylgard 184 silicone elastomer base) with a silane curing agent (Sylgard 184 elastomer curing agent) in a 10:1 ratio (Poly(dimethylsiloxane) as a material for fabricating microfluidic device [37, 38]. Once the two reagents were mixed, they were vigorously homogenized using a PTFE spatula. The mixture was then poured onto the mold previously fabricated by optical photolithography and placed in a desiccator connected to a vacuum pump to remove any bubbles within the resin. It was then incubated for 1 h at 70 °C. Once the curing process was completed, the PDMS chip was ready to be cut, carefully peeled from the resin mold, and punctured to create the inlet and outlet zones using a 1.5 mm biopsy punch [39].

Synthesis and characterization of colloidal silica nanoparticles

The synthesis of colloidal silica nanoparticles (SiO_2 -NPs) was performed using the Stöber method, which enables the production of monodisperse SiO_2 -NPs with a specific size depending on the concentrations of the reagents used [40, 41]. The method involves dissolving 99.9% absolute ethanol, ammonium hydroxide (NH_4OH 28–30%), Milli-Q water, and the precursor tetraethyl orthosilicate (TEOS). First, all glassware was washed with aqua regia to remove any impurities from the vials. Subsequently, 20 mL of absolute ethanol, 3.6 mL of Milli-Q water, and 776 μL of 28% NH_4OH were added. Then, 2.31 mL of TEOS (99.9%) was introduced into the mixture, which was then shaken for 24 h at 1000 rpm. The resulting solution was centrifuged twice at 6000 rpm for 15 min using a Hermle Z326K Centrifuge. The supernatant was removed, and a 1:1 ratio of Milli-Q water and absolute ethanol were added to maintain the initial volume. Subsequently, centrifugation was repeated twice at 6000 rpm for 15 min each time, followed by the removal of the supernatant and the addition of Milli-Q water to restore the initial volume. Finally, the sample was refrigerated at 4 °C. The synthesized SiO_2 NPs were characterized by scanning electron microscopy (ZEISS GeminiSEM 360 with NanoVP) (SEM) for morphological analysis. The hydrodynamic diameter and Z-potential were measured using a Nanosizer Nano ZS90 (Malvern Instruments Limited ZEN 3690) and a UV/Vis/NIR spectrophotometer (Perkin Elmer Lambda 750), respectively.

Synthesis of nanoparticles with RITC-APTES

A solution of 0.04 g of rhodamine isocyanate (RITC) in 5 mL of anhydrous ethanol was first prepared, then 200 μL of APTES was added, which acts as a linking agent between the reactive thiocyanate group of rhodamines by covalently binding to the amine group of APTES, the

solution was left in agitation for 20 h at 1000 rpm, the vial should be completely covered to avoid photodegradation of rhodamine. In a second vial, 200 μL of anhydrous ethanol was added together with 2 mL of a 28% NH_4OH solution. To this mixture 500 μL of the previously prepared RITC-APTES solution were added. This stage corresponds to the initiation of the sol-gel process, in which TEOS, in the presence of NH_4OH as a catalyst, hydrolyzes and condenses, promoting the formation of a silica network that can immobilize the RITC-APTES conjugate, finally, 50 μL of APTES was added to improve the functionalization in the formation of the silica nanoparticles, and the mixture was left stirring at 1000 rpm for 24 h. The resulting solution was subjected to two cycles of centrifugation at 10,000 rpm for 15 min. After each cycle, the supernatant was removed and resuspended in milli-Q water and anhydrous ethanol maintaining the initial volume. Subsequently, two additional centrifugation cycles were performed at 6,000 rpm for 15 min, with removal of the supernatant and resuspension only with milli-Q water maintaining the initial volume. The sample was characterized as it was previously described in Section 2.4. and then it was stored at 4 $^{\circ}\text{C}$, completely covered to avoid degradation.

Incubation of SiO_2 -NPs with protein A

The nanoparticles were incubated with Protein A-Agarose [1 g/mL] to ensure that the antibodies conjugated to the nanoparticles maintain their Fc regions-oriented inward, while exposing the Fab regions outward, thereby preserving the correct orientation for antigen binding. This method was based on the protocol described by Jing Tu [42].

Initially, Protein A was diluted to a concentration of 1 mg/mL in 0,01 M phosphate-buffered saline (PBS), while the SiO_2 -NPs were sonicated for 10 min. Then, SiO_2 -NPs and Protein A were mixed in a 1:1 ratio. The incubation was performed using a thermo-shaker at 400 rpm and 25 $^{\circ}\text{C}$ for 20 min. After incubation, the samples were centrifuged at 13,000 rpm for 5 min. Finally, the supernatant was discarded, and the pellet was resuspended in PBS to restore the original volume.

Generation of the internal 3D structure based on colloidal silica

The fabrication of the 3D structure within the channels of microdevice was carried out according to the methodology previously reported by Zhang P. [34]. The surface of a silanized slide was treated by applying APTES, which acts as a coupling agent to enhance bonding between the surface and other compounds through covalent interactions. This improves adhesion, in this case, between the surface of the microfluidic device and the glass slide.

Once the device was adhered to the surface of the glass slide, approximately 10 μL of SiO_2 -NPs was injected into the inlet until the colloidal solution filled all the microchannels. An inverted Olympus IX73 optical microscope was used to observe the coating and the filling of the channels by surface tension.

Subsequently, the device was dried for 1 h at 25 $^{\circ}\text{C}$. Afterward, a new injection of the sample was performed, and the device was baked again at 25 $^{\circ}\text{C}$ for 1 h. Once dry, 10 μL of 5% 3-Mercaptopropyltrimethoxysilane (3-MPS) resuspended in ethanol was injected to enhance and provide mechanical stability to the colloidal structure for subsequent disassembly. After the baking process, the device was left for an additional hour at 25 $^{\circ}\text{C}$. Finally, the nanostructure was protected by sealing it with a piece of Parafilm, which was adhered over the nanostructure, and the device edges were sealed with solid paraffin incorporated around the perimeter. This provided containment and protection to the microdevice before adding the EV samples.

Cell culture

Metastatic breast cancer cells MDA-MB-231 (purchased by ATCC, #HTB-26) and non-metastatic breast cancer cells MCF-7 (purchased by ATCC, #HTB-22) were maintained in DMEM-F12 High Glucose supplemented with 10% FBS and antibiotics (100 U/mL penicillin and 100 mg/mL streptomycin). Cells were cultured at 37 $^{\circ}\text{C}$ and 5% CO_2 .

Surface nanoparticle conjugation with anti-CD63 or anti-CD81 antibodies

The process of incubating the CD81/CD63 primary exosomal antibodies on the chip involved the injection and subsequent incubation of a CD81 or CD63 primary antibody onto a slide containing a SiO_2 NPs structure. Subsequently, the slide was incubated with 80 μL of CD81 or CD63 primary antibody in a mixture of 130 μL PBS and 90 μL 10% BSA for one hour at room temperature. Following this, the excess primary antibody was removed with PBS.

Extracellular vesicles capture and detection by Immunofluorescence assay

After preparing the microdevice with its internal 3D structure and bioconjugated with the specific exosomal CD81 or CD63 mouse monoclonal antibodies, EVs from two breast cell lines were captured in the microdevice channels. The conditioned media were obtained from the following cell lines: MCF7, a minimally aggressive breast cancer cell line and MDA-MB-231, a metastatic breast cancer cell line. Approximately 35 μL of conditioned medium were injected into the microchip and left overnight at 4 $^{\circ}\text{C}$. Positive CD81 or CD63 EVs from cells were

captured on the surface of microchannels bioconjugated with CD81 or CD63 antibodies. To detect the presence of EVs on the surface of the channels, we identified the CD63 or CD81 surface antigens using an immunofluorescence assay. First, the surface of the channels was incubated with 5% BSA diluted in 1X PBS for 30 min; then, was incubated with the specific CD63 antibody for 1 h at room temperature (for microchannels previously coated with CD81 antibody) or incubated with CD81 antibody (for microchannels previously coated with CD63 antibody). Following this, the samples were incubated with a secondary antibody conjugated with anti-mouse Alexa-555. The negative control samples were designed to assess EVs detection under three different conditions: the first negative control lacked the primary antibody on the nanostructure surface (no bioconjugation for exosome capture); the second control was not functionalized with protein A (no functionalization for bioconjugation); and the third control used an Alexa-555 antibody from a different species than the monoclonal antibody employed for EVs detection (demonstrating the specificity of the fluorescent labeling associated with the anti-CD63 monoclonal antibody) (Supplementary Figure S1). Finally, the samples were observed using an Olympus IX73P2F epifluorescence microscope.

SEM characterization

The procedure for observing exosomes (MDA-MB-231) captured on the 3D silica nanoparticle nanostructure fabricated on a silicon wafer was performed using scanning electron microscopy (SEM) (GeminiSEM 360 with NanoVP; ZEISS). The protocol was adapted from Sokolova et al. [43]. The EVs were fixed onto the nanostructure surface using 3.7% glutaraldehyde in PBS for 15 min, with approximately 40 μ L of fixative applied directly onto the surface. Following fixation, the samples were washed twice with PBS to remove excess reagents. The fixed EVs were dehydrated using a graded ethanol series (40%, 60%, 80%, and 98%). Once ethanol evaporation was complete, the samples were air-dried at room temperature for 24 h. To enhance conductivity for imaging, the dried samples were sputter-coated with an approximately 8 nm thick gold layer using a Cressington 108 AUTO sputter coater (Cressington Scientific Instruments Ltd., Watford, UK). This preparation ensured optimal imaging of exosomes captured on the nanostructure surface using SEM.

Atomic force microscopy

For the characterization by atomic force microscopy (AFM) the CoreAFM microscope (Nanosurf) was used operating in dynamic force mode, a TAP190AI-G cantilever was used, the image size used was 500 nm. The sample was prepared following the protocol described in Section 2.8 and 2.9. The characterized samples were

previously incubated with cell conditioned media from MDA-MB-231 cells, which were allowed to dry at room temperature. This step is crucial to avoid contamination of the microscope tip during measurements. The topology of nanoparticles was observed without EVs and in presence of conditioned cell cultured medium.

Flow cytometry

MDA-MB-231 and MCF-7 cells were detached using trypsin/EDTA and then incubated at 4 °C to avoid internalization of surface proteins. After blocking with BSA 2% at room temperature for 1 h, cells were immunolabeled for 30 min at 4 °C with the primary antibody rabbit monoclonal anti-CD63 (1:100) or rabbit monoclonal anti-CD81 (1:100) by Cell Signaling Technology. Cells were then washed with 1X PBS and incubated with the secondary antibody anti-rabbit Alexa 488 (1:200) for 30 min at 4 °C. Cells were analyzed using a FACSCalibur (Becton Dickinson) flow cytometry.

Statistical analysis

The statistical analysis conducted in this work primarily addressed the synthesis of nanoparticles. The variables studied were the average hydrodynamic diameter and surface charge of the nanoparticles, as well as the absorbance of the sample. Additionally, the average diameter of the nanoparticle morphology characterized by SEM was manually measured using ImageJ software, through which the images obtained from scanning electron microscopy were manually analyzed. This allowed us to measure 600 nanoparticles, as detailed in the corresponding section.

It is worth noting that approximately one nanoparticle synthesis was performed per week over the course of three months, and three replicates were conducted for each synthesis.

All the results expressed as the mean $\pm$ the standard derivation was performed at least in triplicate. The statistical analysis of the data was carried out using GraphPad Prism 10.3.1. Significance was determined by one-way ANOVA test, followed by multiple comparison Tukey post-test: **P-value < 0,01 or ***P-value < 0,001.

Results and discussion

Synthesis and characterization of SiO₂-NPs

The SiO₂-NPs were synthesized according to the procedures based on the Stöber method for silica nanoparticles [44, 45]. The synthesis consisted of a solution of ethanol, ammonium hydroxide (NH₄OH), Milli-Q water and the precursor tetraethyl orthosilicate (TEOS), under constant agitation conditions. This is a sol-gel process widely used to produce silica and involves the hydrolysis and condensation of metal alkoxides (Si(OR)₄), such as TEOS,

in the presence of an acid or a base as a catalyst, in this case ammonium hydroxide (NH_4OH) [44–46].

The synthesized nanoparticles were characterized by SEM, UV-visible spectroscopy, dynamic light scattering (DLS) and zeta potential measurements to comprehensively evaluate their size distribution, surface charge, optical properties, and stability in suspension. The concentrations of TEOS used were evaluated by analyzing the hydrodynamic diameter and the obtained zeta potential, choosing the highest concentration of TEOS (Supplementary Table 1).

The particle size was first measured in scanning electron microscope (SEM) images (Fig. 1A), confirming their morphological structure corresponding to spherical shape. The average particle size was 265 ± 32 nm (histogram in Fig. 1B), which falls within the expected range for nanoparticles synthesized via the Stöber method. Similar results have been reported in the literature, where the Stöber method consistently produces silica particles in the submicron range, depending on the concentration of precursors and reaction conditions [47, 48].

The analysis of the hydrodynamic diameter (d_h) and zeta potential for SiO_2 -NPs was carried out in the Malvern Zetasizer nano-ZS. The analysis was made in triplicate, to obtain greater precision and better statistical analysis. The average of the hydrodynamic size (d_h) was 317.3 ± 70.8 nm and the polydispersity was 0.032, indicating low variability in the size of the NPs and therefore uniform distribution and similar sizes (Supplementary Table 2). This is a relevant result as it ensures that the nanoparticles produced are homogeneous, which is critical for applications that demand consistency, like drug delivery systems or catalytic processes. The data exhibited a Gaussian distribution, resulting in a characteristic pattern of a normal distribution curve, demonstrating a symmetrical and homogeneous distribution of the characterized NPs with respect to their hydrodynamic size (Fig. 1C). These findings align with previous studies that have employed the Stöber method to produce monodisperse silica particles [44]. The size characterization obtained by SEM images does not correspond to the real size of the nanoparticles, but rather to the hydrodynamic diameter, which is the effective size (apparent or average size) of a nanoparticle or molecule in a solution. However, it is important to note that the d_h obtained by DLS was slightly larger (317.3 ± 70.8 nm). This discrepancy is commonly observed due to the nature of measurement techniques. SEM images provide the physical size of dry particles, whereas DLS measures the effective size in suspension, which includes the hydration layer surrounding the particles in solution. This phenomenon has been reported in several studies comparing these two techniques, confirming that the hydrodynamic diameter is typically larger than the physical size due to solvation

effects [49]. The zeta potential, which depends on the surface charge, is important for the stability of nanoparticles in suspension [50]. Regarding the zeta potential of the SiO_2 -NPs, due to the coating of the silanol functional group ($-\text{Si}-\text{OH}$) layer with negative charge, the zeta potential was -50.6 ± 6.5 mV (Fig. 1D and Supplementary Table 2). We obtained high values of suspended nanoparticles in solutions when we evaluated synthesis concentration, which is indicative of high colloidal stability. The negative surface charge is attributed to the presence of silanol groups ($-\text{Si}-\text{OH}$) on the surface of the nanoparticles, which become deprotonated in an aqueous environment, leading to a negative charge. Particles with zeta potential values greater than ± 30 mV are generally considered stable due to the strong electrostatic repulsion between particles, preventing aggregation [51]. The high zeta potential observed here suggests that the SiO_2 -NPs synthesized in this study are highly stable in suspension, which is a desirable trait for many applications. These results agree with that reported by Zhao et al. [47] showing stable silica particles with a zeta potential of approximately -40 mV, suggesting that the functionalization of the silica surface with silanol groups contributes significantly to maintaining colloidal stability. Characterization via UV-visible spectroscopy of pure silica nanoparticles was performed to investigate their optical absorption properties, showing a λ_{max} of 195 nm for SiO_2 -NPs (Fig. 1E), indicating that SiO_2 -NPs have a low absorption capacity in the UV-Vis spectrum. This is consistent with previous studies showing by [47, 48]. The absence of significant absorption peaks beyond 195 nm suggests that the SiO_2 -NPs produced in this study may not be suitable for applications requiring strong optical interactions, such as photothermal therapy or optical imaging. However, this low absorption could be advantageous for applications where transparency and minimal light interference are required, such as in biosensors or optical coatings [52]. These results are comparable to other studies on pure silica nanoparticles. For example, Zhao et al. reported similar absorption characteristics for SiO_2 -NPs, reinforcing the conclusion that silica's inherent low optical absorption makes it ideal for applications where interference with visible light must be minimized [47].

The average concentration of particles ($4.43 \times 10^{11} \pm 5.45 \times 10^{10}$ particles/mL) occurs at a diameter of 300 nm (Fig. 1F), the same value we obtained with the hydrodynamic diameter and the scanning electron microscopy measurements. The strong correlation between the concentration and size measurements suggests that the synthesis process is reliable and produces nanoparticles that maintain their integrity in the solution. Furthermore, the fact that the measured size from SEM, DLS, and the concentration analysis are all aligned reinforces the conclusion that the synthesized nanoparticles

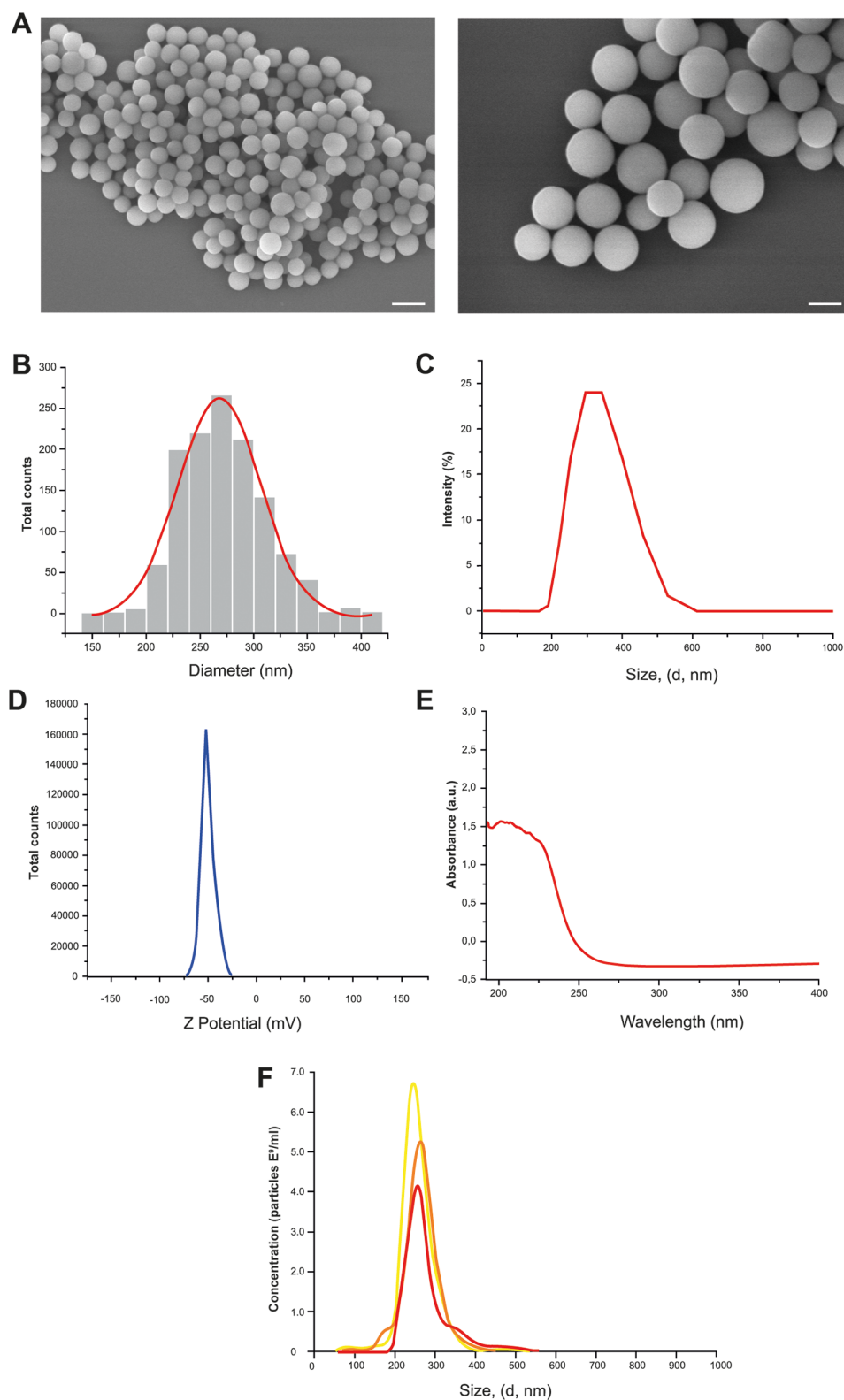


Fig. 1 Synthesis and characterization of monodisperse colloidal silica nanoparticles. **(A)** SEM images of SiO₂-NPs at 50,000x (left) and 100,000x (right). Scale Bar 500 and 250 nm, respectively. **(B)** Diameter distribution histogram of SiO₂-NPs. Measurements were made from 6500 nanoparticles of SEM images from 3 independent experiments, with a peak at 300 nm. **(C)** Hydrodynamic diameter distribution graph obtained by UV-visible spectroscopy, **(D)** Graph of the zeta potential of the nanoparticles obtained using a Malvern Zetasizer nano-ZS equipment. The value shown in the graph is the average (-50.6 ± 6.5 mV) of measurements taken in triplicate. **(E)** Size distribution graph of SiO₂-NPs. **(F)** Concentration graph of colloidal silica nanoparticles sample obtained using Nanosight equipment. The average was $4.43 \times 10^{11} \pm 5.45 \times 10^{10}$ particles/mL

exhibit a stable and uniform distribution. Therefore, we can conclude that the characterization of the nanoparticles matches the values from the different measurements in diameter and polydispersity.

Fabrication of PDMS microdevice via soft optical lithography and formation of the three-dimensional structure of monodisperse colloidal silica

The design of the microdevice using AutoCAD software (Fig. 2A and B) demonstrates a thoughtful approach to optimize fluid flow and nanoparticle self-assembly. The device consists of two reservoirs connected by a zigzag-shaped microchannel, which is intended to promote greater mass transfer. This design contrasts with simpler straight microchannel designs commonly found

in microfluidic devices, where laminar flow dominates and may limit efficient mixing and particle transfer [53]. The zigzag structure increases the nanoparticles residence time within the channel and enhances the interaction, improving the likelihood of uniform assembly. This approach leverages principles of fluid dynamics to optimize the device function, which has been seen in prior microfluidic devices aimed at enhancing mixing efficiency in biological and chemical assays [54].

One of the critical steps in ensuring the functionality of the microdevice was the silanization of the substrate and the subsequent treatment with APTES, to improve the adhesion of the PDMS microdevice to the underlying substrate (Fig. 2C). Silanization is commonly used to create a stable bond between PDMS and glass, as it

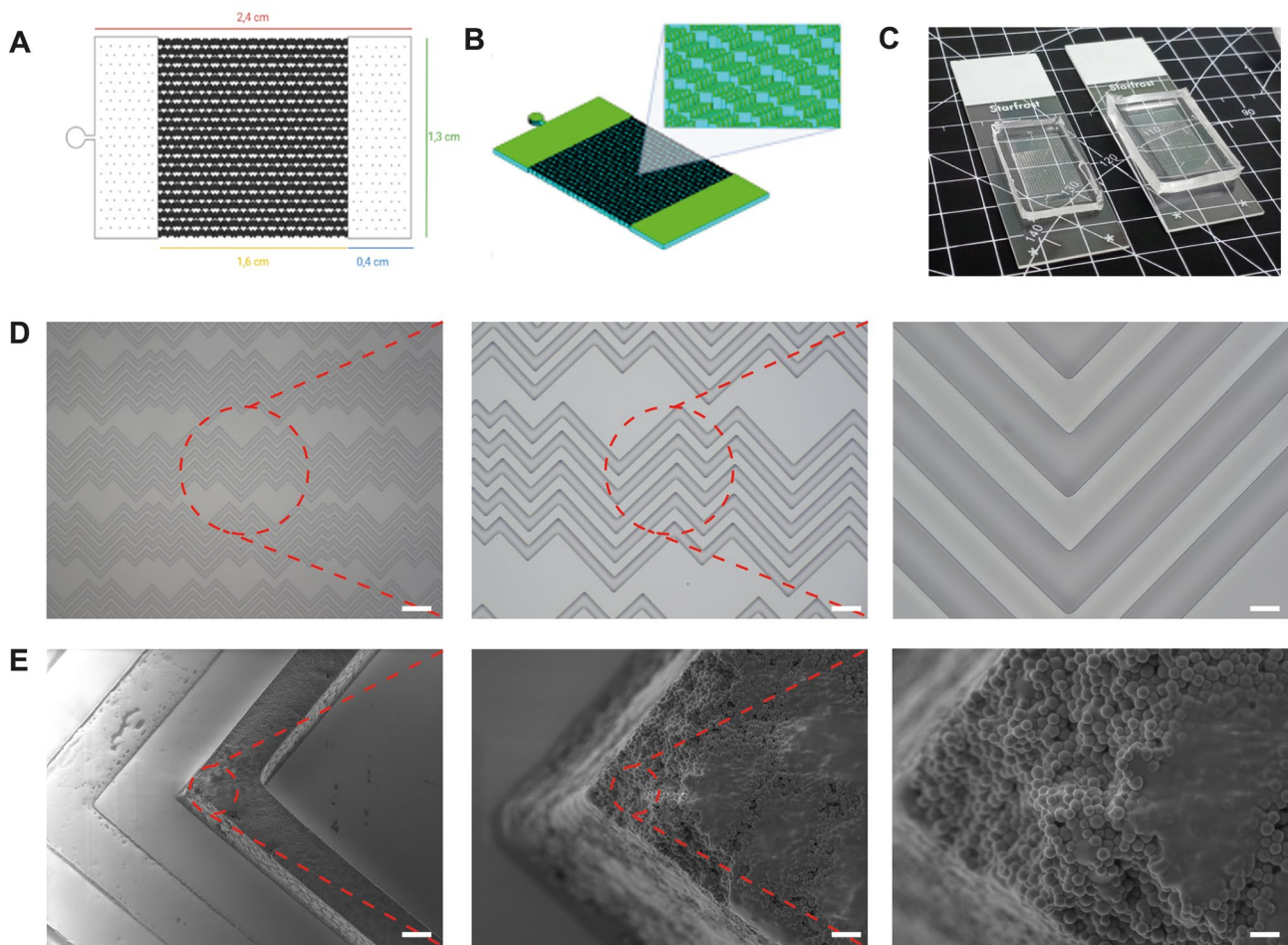


Fig. 2 Fabrication of PDMS microfluidic chips via soft optical lithography and formation of three-dimensional structure of monodisperse colloidal silica. **(A)** Design of the microfluidic chip created using AutoCAD software. This design includes two reservoirs at the ends, with a hole in one of them to incorporate the sample and 110 zigzag-shaped microchannels between two reservoirs. The dimensions of this device are 2.4 cm in total length, 1.6 cm in length for the zigzag-shaped microchannels, 1.3 cm in height, and two reservoirs of 0.4 cm in length. **(B)** Schematic of the microfluidic chip mold. **(C)** Images of PDMS microfluidic chip mounted on a silanized slide and previously treated with APTES on its surface. **(D)** Optical microscope images in bright field of the zigzag-shaped microchannels of the PDMS microfluidic chip at different magnifications, 4x (left), 10x (center) and 40x (right). The red lines correspond to the area enlarged in the following magnification. Scale bars, 100 μ m, 50 μ m and 25 μ m respectively. **(E)** SEM images of 3D structure formed by mono-assembled colloidal silica of 300 nm inside of zigzag-shaped microchannels of microchip at different magnifications. The red lines correspond to the area enlarged in the following magnification. Scale bars, scale 20 μ m (left), 2 μ m (center) and 1 μ m (right)

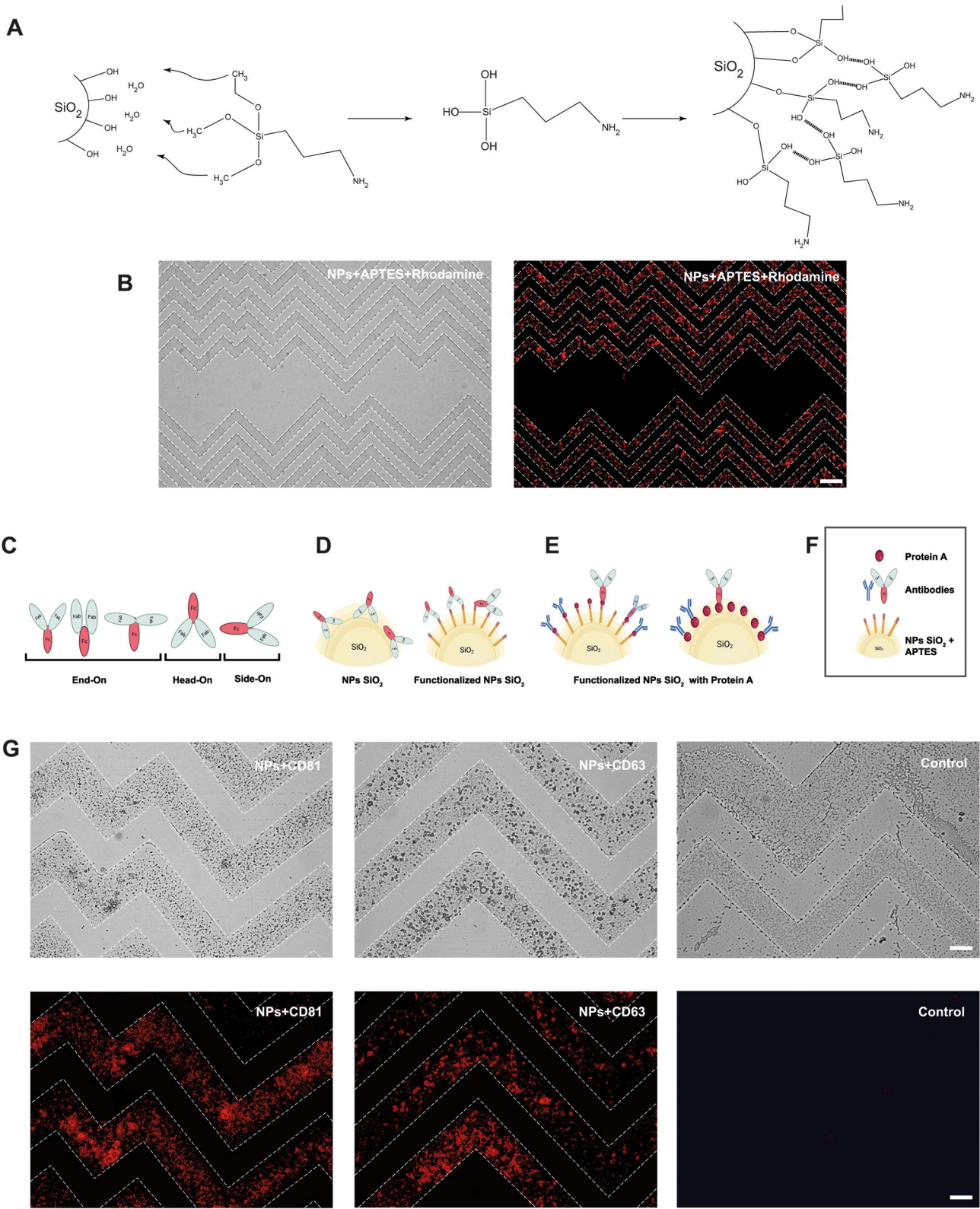


Fig. 3 (See legend on next page.)

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Fig. 3 Functionalization of the SiO₂-NPs and different approaches used to achieve the functionalization. **(A)** Schematic representation of the functionalization process of SiO₂ nanoparticles with APTES. **(B)** Representative epifluorescence images at 10X magnification from experiments of SiO₂-NPs functionalized with APTES and bioconjugates with Rhodamine B isothiocyanate in the bright field (left) and red fluorescence channel (right). Scale bar, 50 μ m. **(C)** Schematic representation of the possible orientations of immobilized antibodies on a surface. **(D)** Representative schematic of the possible orientations that an antibody can adopt when immobilized on the surface of unfunctionalized SiO₂ NPs (left) and NPs SiO₂ functionalized with APTES without protein A (right). **(E)** Schematic representation of functionalized NPs SiO₂ with protein A bioconjugated with antibodies in the correct end-on orientation, leaving their epitope-binding domain free. **(F)** Legend of the figures used in the schematic representation. **(G)** Representative epifluorescence images at 10X magnification from experiments of SiO₂-NPs functionalized with protein A and bioconjugated with monoclonal anti CD81 and CD63 antibodies, immunodetected with Alexa 555 anti-mouse. The control was SiO₂-NPs bioconjugated with anti-CD81 and immunodetected with Alexa 555 anti-rabbit. The superior panel corresponds to bright field of three experiments, SiO₂-NPs bioconjugated with anti CD81 (left), anti-CD63 (middle) and control (right). The inferior panel shows the red fluorescence channel from the same experimental conditions from superior panel respectively. The white dotted lines delineate the microchannels where the self-assembled SiO₂-NPs has formed. Scale bar, 25 μ m

introduces reactive groups that can form covalent bonds upon treatment with coupling agents like APTES [55]. This step is crucial for preventing delamination, which is often a challenge in microfluidic devices, especially when subjected to fluid flow or nanoparticle deposition. After treating the silanized slices with APTES, the microdevice demonstrated robust adhesion maintaining its integrity throughout the nanoparticle assembly process. This was further confirmed, as shown in Fig. 2D, where images of the microdevice attached to the microchannel zone are shown, ensuring stability during nanoparticle assembly.

The self-assembly of the SiO₂-NPs was achieved through the introduction of a nanoparticle's solution mixed with mercaptoethanol, followed by the structure drying within the microchannels. The mercaptoethanol contributes to self-assembly by creating favorable surface interactions between the silica particles, allowing them to form a uniform 3D structure. Similar approaches using other thiol-containing compounds, such as mercaptoethanol, have been reported in previous studies to facilitate nanoparticle organization and stabilization due to the strong interactions between thiol groups and silica surfaces [56]. The successful formation of the 3D structure within the microchannels was confirmed using SEM analysis (Fig. 2E). The SEM images showed a highly ordered, monodisperse colloidal arrangement of the SiO₂-NPs after the removal of the PDMS mold, demonstrating the efficiency of the device in directing the nanoparticle assembly. The monodispersity and uniformity of the nanoparticles are crucial for applications in which particle size and distribution significantly impact performance, such as in biosensing, catalysis or optical applications. These results are comparable to other self-assembly methods where the control over particle size and distribution is critical for achieving the desired functional properties in a variety of nanotechnological applications [57].

Characterization of the SiO₂-NPs functionalized using a fluorescent probe

A first approach to determine the correct functionalization with APTES, was to label the NPs with a fluorescent

probe. We conjugated the NPs with Rhodamine B isothiocyanate because it is an aminoxanthene that possesses carboxylic acid and isothiocyanate substituents on the pendant phenyl ring, generating links with the amine groups of NPs (Fig. 3A). In Fig. 3B, images of fluorescent silica nanoparticles obtained by rhodamine, show a homogeneous staining in all the microchannels with a dot cluster pattern characteristic of the functionalized NPs. These results indicate that functionalization with APTES confers a substrate to conjugate different molecules to the NPs surface.

For this study, initial bioconjugation of the nanoparticles (NPs) with antibodies was planned by functionalizing the NPs with APTES. However, it is challenging to guarantee the correct orientation of antibodies with their Fc domain bound to the NP surface. Antibodies may adopt various orientations on the NPs, such as end-on, head-on, or side-on, which can limit the availability of the Fab regions for binding their epitopes (Fig. 3C-F). To prevent incorrect bioconjugation orientation, Protein A-Agarose was used as a linker between the NPs and antibodies, ensuring optimal binding of IgG via its Fc domain (Fig. 3E). We performed bioconjugation with CD81 or CD63 antibodies and subsequently detected them using a red fluorescent dye (Fig. 3G). The observed fluorescence levels indicate successful bioconjugation and immunodetection of the antibodies on the nanoparticles, demonstrating the interaction of the SiO₂-NPs with the surface of the microchannels within the device.

Immunocapture and detection of EVs derived from breast cancer cell lines with a three-dimensional nanostructure of monodisperse colloidal silica

To achieve an efficient immunocapture and subsequent detection of EVs into the nanostructured SiO₂-NPs microchip, we bioconjugated the nanostructured SiO₂-NPs surface with a monoclonal anti-CD81 antibody and then, with an anti-CD63 antibody for the final detection of EVs by immunofluorescence. Both proteins are surface exosomal markers on EVs derived from breast cancer cell lines [10, 58]. EVs isolated by ultracentrifugation (UC) from the MDA-MB-231 (UC Exo

MDA) breast cancer cell line was immunocaptured with high specificity and efficiency by the nanostructured SiO₂-NPs microchip (Fig. 4A, CD63-Exosomes). Importantly, high fluorescence levels from CD63-positive EVs were detected from the captured vesicles contained in the conditioned medium (CM) from MCF7 (CM Exo MCF-7) and MDA-MB-231 (CM Exo MDA) cell lines (Fig. 4A, right panels) and the fluorescence intensity was comparable to the samples enriched with EVs via UC before mentioned, indicating that the nanostructured microchip achieves high efficiency and specificity for EVs capture. Unlike the negative control, the fluorescence intensity was almost undetectable and without a dot clusters pattern like the other EVs images (Fig. 4A, left panels; Fig. 4D, white bar). As shown in Fig. 4D, the quantification of CD63 fluorescence levels revealed a clear distinction between EV-containing samples and the negative control, where fluorescence intensity was almost undetectable in the images. This result supports the high specificity of the antibody-coated nanoparticles for EVs capture, as the fluorescence was significantly reduced in the absence of EVs (Supplementary Figure S1).

The SEM images (Fig. 4B) provide critical insights into the morphology of the silica nanoparticle nanoarchitecture and its efficiency in capturing EVs. The images show a uniform distribution of the nanoparticles, suggesting precise control over their synthesis (Fig. 4B, left panel, Control). This well-defined bioconjugated nanoarchitecture plays a crucial role as a scaffold for the selective adsorption of EVs, optimizing their capture from complex biological samples, such as the culture medium (CM) used in this work. The interaction between EVs and the nanostructure (Fig. 4B, right panels) reveals spherical vesicles adhering to that surface, confirming the successful capture of EVs. The structural integrity of the EVs appears to be well-preserved after binding, since the average diameter of these vesicles was approximately 60 nm, which is an expected size for the diameter of EVs, suggesting that the capture process does not significantly alter their morphology. A closer magnification further highlights the strong adhesion of exosomes to the nanoparticle surface, likely mediated by specific biochemical interactions between exosome surface markers and the antibody-functionalized silica nanoparticles. These results validate the efficiency of the silica-based nanostructure for EVs capture. These findings are consistent with earlier studies that demonstrate the importance of CD63 and CD81 as ubiquitous exosomal markers [59]. The ability to target these proteins, which are expressed on the surface of most EVs, ensures that the microchip is applicable to a wide range of cancer cell lines and other biological samples. The flow cytometry results presented here confirm the presence of both proteins on the surface of MDA-MB-231 and MCF-7 cells (Fig. 4D).

Quantification of fluorescence intensity shows that CD81 was expressed in 96% of the MCF-7 cell population and 83.5% of the MDA-MB-231 cell population. Regarding CD63, a similar percentage (53%) of positive cells was observed in both cell lines (Fig. 4E).

The high fluorescence intensity and SEM images observed in this study further support the conclusion that the SiO₂-NPs microchip can effectively capture and detect EVs, even in samples with low EVs concentrations, such as culture medium. These results suggest a certain specificity of the SiO₂-NPs microchip for detecting EVs directly from complex biological samples without requiring extensive sample preparation, such as ultracentrifugation or filtration. Future experiments will be conducted to determine the sensitivity of the system and to analyze the limits of detection and quantification in these micro-devices, using known concentrations of breast cancer EVs obtained by ultracentrifugation.

Previous studies have demonstrated the potential of nanostructured materials, such as gold nanoparticles or magnetic beads, for EVs isolation and capture due to their high surface area and functionalization potential [60]. However, the use of silica-based nanoparticles in this study offers distinct advantages. Silica nanoparticles are biocompatible and provide a highly tunable surface chemistry that can be easily modified for various bio-functionalizations, such as antibody conjugation, which enhances the specificity and sensitivity of EVs capture [61]. Additionally, the three-dimensional nanostructure of the SiO₂-NPs chip increases the available surface area for EVs binding, further improving detection efficiency compared to planar surfaces or non-structured chips (Supplementary Figure S2) shows the EVs capture in a planar surface. To experimentally evaluate whether the nanostructured SiO₂-NPs chip enhances the binding of EVs compared to planar surface, we conducted a binding assay. Our results indicate that there was significant reduction in EV capture on the planar surface relative to the nanostructured chip. This suggests that, in our specific experimental conditions, the presence of SiO₂-NPs did lead to a detectable increase in binding efficiency.

Our results aligned with prior studies demonstrate that 3D nanostructuring enhances EV binding efficiency. The paper by Zhang et al. [34] showed that a 3D-nanopatterned herringbone microfluidic chip increased exosome binding compared to conventional planar channels. It is due to existing an enhanced mass transfer and microscale mixing, overcoming boundary layer limitations, higher probe density for molecular recognition and reduced near-surface hydrodynamic resistance, increasing EV-surface collisions. These findings support our SiO₂-NPs based nanostructure, which similarly enhances EV interactions by providing a larger available surface area.

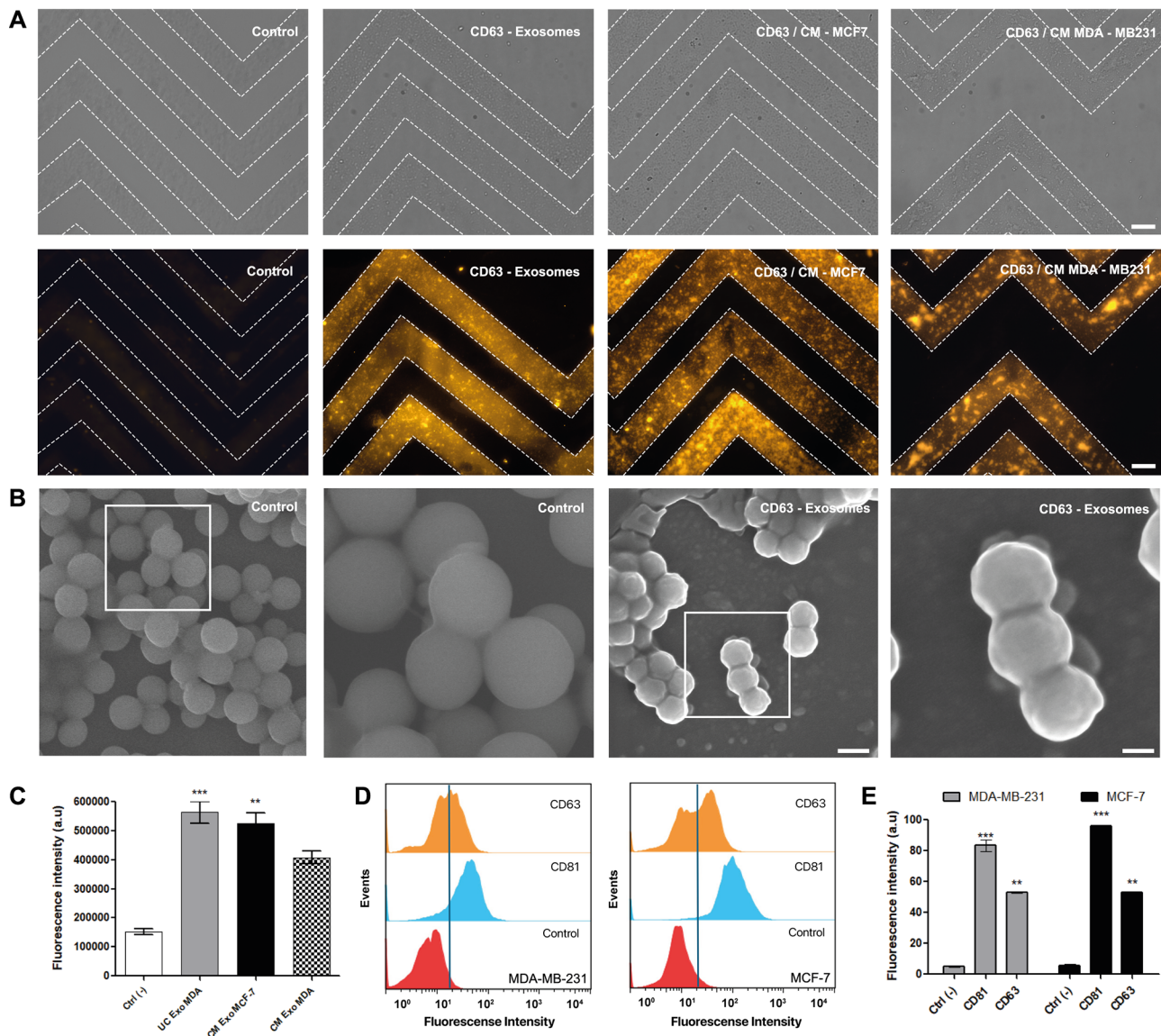


Fig. 4 Capture and detection of extracellular vesicles derived from breast cancer cell lines. **(A)** Epifluorescence images at 40X magnification of capture and immunodetection of EVs present in conditioned medium from MDA-MB-231 and MCF7 cancer cell lines. The immunocapture of EVs was performed using the 3D nanostructure bioconjugated with anti-CD81 antibody, and the immunodetection was performed through the binding of the anti-CD63 antibody present in the trapped EVs. The EVs were visualized by fluorescence using the Alexa 555 anti-mouse secondary antibody. The superior panel shows the nanostructure fixed on bright field images from different experimental conditions. The inferior panel shows the EVs immunolabeled on nanostructure. The first image (left) shows the control experiment which consists of immunodetected EVs with an Alexa 555 anti-rabbit secondary antibody. Then, the next images show the detection of EVs contained in sample enriched with EVs by ultracentrifugation (UC) from MDA-MB-231 cell line (UC Exo MDA). The last images (right) correspond to the detection of EVs contained in conditioned medium (CM) from the MCF-7 and MDA-MB-231 cancer cell line, respectively. Scale bar 25 μ m. **(B)** SEM images showing the immune capture of EVs from the MDA-MB-231 cell line. The left panel displays non-specific adsorption and immunocapture of nanoparticles in a control experiment, while the right panel shows EV capture using an anti-CD63 monoclonal antibody-coated SiO₂ NP tridimensional structure. The magnified images highlight the characteristic spherical morphology of EVs (arrowhead). **(C)** The graph shows the quantification of the fluorescence levels detected in microchips. The measurement was performed on the most intense microchannel of each image, corrected for the analyzed area. **(D)** Flow cytometry data histograms obtained by measuring fluorescent levels of CD81 and CD63 antibody, or secondary antibody (Control (-)) in MDA-MB231 (left) and MCF-7 (right) tumor cells, respectively. **(E)** The graph shows the immunofluorescence quantification of CD81 and CD63 expression in MCF-7 and MDA-MB-231 cells by flow cytometry. Data are expressed as mean $\pm$ SEM. P-values in the figure are indicated as ** < 0,01 or *** < 0,001

One limitation of the current system is the extended assay time required. In our study, samples were incubated overnight at 4 °C to maximize EV binding efficiency to the functionalized SiO₂-NP nanostructured surface, thereby enhancing the interaction between EVs and capture antibodies. Attempts to reduce the incubation period to 3 h at room temperature did not yield efficient EV capture, indicating that prolonged incubation is essential for adequate binding. Although this extended assay duration represents a limitation for rapid analysis, future work will focus on optimizing this step—through modifications in surface chemistry, the application of microfluidic flow conditions that promote more efficient capture, or preconcentration techniques—to improve process kinetics.

To observe the EVs capture, AFM imaging was performed (Supplementary Figure S3). This figure presents AFM images of silica nanoparticles in two distinct conditions: SiO₂-NPs without EVs (Supplementary Figure S3A-C) and SiO₂-NPs with captured EVs (Supplementary Figure S3D-F). In Supplementary Figure S3A, the SiO₂-NPs appear with a relatively uniform morphology, showing characteristic size and shape with minimal surface features. Since no EVs are present, the particles should exhibit smooth surfaces and well-defined boundaries (Supplementary Figure S3B and C). This condition serves as a baseline, indicating the unmodified physical properties of SiO₂-NPs. In Supplementary Figure S3B, the presence of EVs alters the surface characteristic of nanoparticles. The AFM image reveals additional structures on the silica surface, indicating successful EVs capture. This is evidenced by increased surface roughness (Supplementary Figure S3D) and the increase of apparent size of the nanoparticles or modified shape, as indicated by additional structural layers or aggregates on their surfaces (Supplementary Figure S3E). These differences suggest effective EVs capture and adherence.

The ultracentrifugation method has been the gold standard for isolating EVs from cell culture media or bodily fluids, as it separates EVs based on size and density [62]. While ultracentrifugation is highly effective, it is also time-consuming and requires expensive equipment, making it less accessible for routine EV isolation in many laboratories. It is important to clarify that the SiO₂-NPs microchip is specifically designed for the capture and detection of EVs and not for their physical isolation as free particles from complex biological fluids. Unlike ultracentrifugation, which separates EVs into a recoverable state based on size and density, the EVs captured by our platform cannot, for now, be physically separated from the chip. However, the SiO₂-NPs microchip developed in this study offers a faster and more cost-effective alternative for EVs capture. The ability to achieve comparable levels of EVs detection, as seen

with ultracentrifugation, highlights the practical advantages of using nanostructured materials for this purpose. This is particularly advantageous for applications where real-time EV analysis is more critical than their physical recovery, such as in non-invasive cancer diagnostics or other biomedical investigations.

The efficient immobilization of EVs on the functionalized nanostructured surface of the chip was supported by fluorescence imaging (Fig. 4A), SEM (Fig. 4B) and AFM results (Supplementary Figure S3), which demonstrate the adhesion of EVs to the three-dimensional SiO₂-NPs structure. This distinction highlights the main purpose of the SiO₂-NPs microchip: to provide a highly efficient and specific on-chip detection method for EVs in complex samples, such as conditioned culture media, without requiring extensive preparation steps like filtration or ultracentrifugation. While ultracentrifugation remains the gold standard for physical EV isolation, our platform serves as a complementary tool, prioritizing rapid and cost-effective EV detection.

Additionally, the SiO₂-NPs microchip provides several other benefits over traditional methods. First, it allows for on-chip EV detection, reducing the need for multiple handling steps that could lead to sample loss. Second, the use of fluorescence-based detection on the same platform enables real-time monitoring of EV capture, which is not feasible with ultracentrifugation. Lastly, the simplicity of the microchip setup allows for easy integration into lab-on-a-chip systems, potentially enabling high-throughput EV detection and analysis.

The results of this study demonstrate that the SiO₂-NPs microchip is a highly efficient and specific platform for EV capture and detection, with the potential for widespread use in cancer diagnostics and other biomedical applications. The ability to efficiently isolate and detect EVs from complex media, such as conditioned cell culture medium, suggests that this technology could be applied to other biological fluids, including blood or urine, for non-invasive cancer screening or monitoring. Furthermore, the use of nanostructured materials opens opportunities for the development of multiplexed detection systems, where multiple EV markers could be detected simultaneously on the same platform, enhancing diagnostic accuracy.

Additionally, the robust biofunctionalization of the SiO₂-NPs with antibodies targeting exosomal markers could be expanded to include other surface proteins of interest, potentially enabling the detection of specific cancer subtypes or the identification of disease progression markers in a clinical setting.

Conclusions

This study represents significant progress in the synthesis, characterization, and application of nanostructured SiO₂-NPs, from nanoparticle assembly to immunocapture technologies. Using the Stöber method, we synthesized highly uniform and stable SiO₂-NPs, with excellent control over particle size and surface charge, as evidenced by the SEM, DLS, and zeta potential measurements. The three-dimensional arrangement of these nanoparticles within microchannels demonstrated the feasibility of fabricating structured nanomaterials for potential applications in catalysis, biosensing, and drug delivery. The combination of optical and soft lithography techniques enabled the creation of a stable PDMS microdevice, facilitating effective nanoparticle assembly.

The SiO₂-NPs microchip developed for the immunocapture and detection of EVs from breast cancer cell lines represents a significant leap forward in biomedical applications. The bioconjugation of SiO₂-NPs with anti-CD81 and anti-CD63 antibodies enabled highly efficient and specific detection of EVs, demonstrating comparable or superior performance to traditional isolation methods like ultracentrifugation. The use of fluorescence-based detection provided real-time insights into EV capture efficiency, making this platform a promising candidate for diagnostic applications in cancer research. This technology also has the potential for high-throughput analysis and non-invasive biomarker detection, presenting a valuable tool for future clinical and research settings.

Overall, the combination of nanostructured materials, microfabrication techniques, and biofunctionalization strategies has opened new avenues for developing innovative solutions in areas such as cancer diagnostics, nanoparticle-based therapies, and environmental sensing. The ability to achieve uniform self-assembly, stable EV capture, and high detection sensitivity highlights the versatility of the approaches discussed. Future work may focus on the further functionalization of SiO₂-NPs for multiplexed detection systems, improved surface modifications, or broader applications in various nanotechnological and biomedical fields.

Abbreviations

APTES	(3-Aminopropyl) triethoxysilane
MCF7	Michigan Cancer Foundation-7. Human Caucasian breast adenocarcinoma with epithelial phenotype
MDA-MB-231	Human Caucasian breast adenocarcinoma mesenchymal phenotype
MPS	(3-Mercaptopropyl) trimethoxysilane
TEOS	Tetraethyl orthosilicate
SiO ₂	Silicon dioxide or silica

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13036-025-00512-0>.

Supplementary Material 1

Supplementary Material 2

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Author contributions

Carolina Cabeza: Investigation, Methodology, Formal Analysis, Validation, Data curation, Visualization, Writing - Original Draft. Felipe Rojas: Investigation, Formal analysis, Methodology and Validation. Lorena Lobos-González: Formal Analysis and Methodology. Juan Villena: Formal Analysis and Methodology. Dominique Lemaître: Investigation, Writing- Reviewing and Editing. María Luisa Cordero: Validation, Data curation, Formal Analysis and Methodology. Natalia Hassan: Conceptualization, Methodology, Validation, Funding acquisition, Writing- Reviewing and Editing, Visualization, Supervision, Resources. Rina Ortiz: Investigation, Conceptualization, Methodology, Formal analysis, Validation, Funding acquisition, Project administration, Writing- Reviewing, Editing and Submission, Visualization, Supervision, Resources.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics and consent to participate

Not applicable for this investigation.

Consent to publish

We hereby authorize the publication of this manuscript.

Competing interests

There are no Competing Interests in this investigation.

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