**Dual mode sensing of binding and blocking of cancer exosomes to biomimetic human primary stem cell surfaces**

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**Abstract**

Cancer derived exosomes (cEXOs) facilitate transfer of information between tumor and human primary stromal cells, favoring cancer progression. Although the mechanisms used during this information exchange are still not completely understood, it is known that binding is the initial contact established between cEXOs and cells. Hence, studying binding and finding strategies to block it is of great therapeutic value. However, such studies are challenging for a variety of reasons, including the need for human primary cell culture, the difficulty in decoupling and isolating binding from internalization and cargo delivery, and the lack of techniques to detect these specific interactions. In this work, we created a supported biomimetic stem cell membrane incorporating membrane components from human primary adipose derived stem cells (ADSCs). We formed the supported membrane on glass and on multi-electrode arrays (MEAs) to offer the dual option of optical or electrical detection of cEXOs binding to the membrane surface. Using our platform, we show that cEXOs bind to the stem cell membrane and that binding is blocked when an antibody to integrin β1, a component of ADSCs surface, is exposed to the membrane surface prior to cEXOs. To test the biological outcome of blocking this interaction, we first confirm that adding cEXOs to cultured ADSCs leads to upregulation of VEGF, a measure of proangiogenic activity. Next, when ADSCs are first blocked with anti-integrin β1and then exposed to cEXOs, the upregulation of proangiogenic activity and cell proliferation are significantly reduced. This biomimetic membrane platform is the first cell-free, label-free, *in vitro* platform for the recapitulation and study of cEXO binding to human primary stem cells with potential in therapeutic molecule screening as it is compatible with scale-up and multiplexing.

**1.Introduction**

Cui and colleagues recently showed that cancer derived extracellular vesicles (cEVs) transfer miR-630 to fibroblasts promoting their differentiation to carcinoma-associated fibroblasts and ovarian cancer invasion and metastasis1. Similarly, several others have implicated cEVs in cancer development and progression in other types of cancers2-4. In particular, a subset of cEVs called exosomes (cEXOs), are known to mediate transfer of information between cells within the tumor and distal sites contributing to tumor growth and formation of pre-metastatic niches5, 6. For instance, interactions with breast cancer derived cEXOs induced pro-angiogenic behavior, indicated by upregulation of vascular endothelial growth factor (VEGF) in adipose-derived stem cells (ADSCs) and their subsequent differentiation to myofibroblasts7, 8. Both outcomes favor tumorigenesis by promoting tumor vasculature formation and high levels of inflammation at the tumor microenvironment (TME), respectively9-11. Therefore, hindering interactions between cEXOs and healthy cells is expected to reduce these types of negative outcomes and possibly mitigate the progression of cancer.

Blocking specific interactions as a route to inhibit the transfer of information between cEXOs and cells and subsequent outcomes is an important emerging avenue for cancer therapeutics. However, given the complexity of the plasma membrane surface and all the possible targets for cEXOs to bind to and deliver their message, screening for such interactions is a challenge that could benefit from new ways to mimic the surfaces and interactions between the cell plasma membrane and cEXOs, as well as technologies to read out these interactions. These types of screening studies are complicated because the molecular interactions between cEXOs and cells are specific to each type of cEXO-cell pair and are determined by the surface composition of both entities12-14. All possible cEXO-target cell interactions are expected to be begin by surface contact that involves binding of at least two surface components, one from the cEXO surface and one from the cell plasma membrane12, 13. Subsequently, cEXOs can take different routes for transfer of information including: binding-induced activation of signaling pathways at the plasma surface that leads to downstream cellular responses, cellular internalization via various endocytosis pathways that leads to delivery of contents to various organelles, and direct fusion with the cell plasma membrane that leads to direct release of information into the cytosol12, 13 15. Of note, however, is that a common theme among the different routes is that *binding* is the initial interaction where cEXOs begin to influence the local microenvironment to promote tumorigenesis and cancer progression. Being able to assess binding isolated from these subsequent biological processes, and with an easy readout that can be scaled up to integrate technologies for high throughput screening to identify effective blocking agents, is a current need in the field. To create a model system to study and visualize cEXO-cell binding, we report here a planar and tunable *in vitro* ADSC plasma membrane model system that allows us to study the interactions of cEXOs and ADSCs and to test potential binding blockers using both optical and electrical readout of binding interactions, isolating the membrane interaction without the complexity of the whole cell. Our platform’s central element is a hybrid supported lipid bilayer (SLB) incorporating native ADSC membrane components derived from plasma membrane blebs from human primary stem cells. We refer to this element as an Adipose derived stem cell supported bilayer (ASB).

SLBs are employed in several research fields as cell membrane models 16-21 and have been used as cell culture platforms22-24, and as tools to investigate interactions at the cell membrane interface including cell-cell interactions25 and cell-particles interactions 22-24, 26 and strategies to inhibit virus binding27, 28. In the past, our group has expanded SLB platform molecular complexity by developing methods to integrate native components from cell membranes that preserve the natural function and orientation of the proteins in mammalian cell membranes 29, 30. Here, we adapted these methods to create a stem cell supported bilayer from human primary stem cells that maintains much of the authenticity of the plasma membrane of ADSCs, preserving native constituents, molecular complexity, and lateral diffusivity of the membrane. Because this platform is a representative mimic of the plasma membrane that is free of the dynamism of live cells, it is useful for isolating and focusing on cEXO binding and blocking without competing effects of cEXO uptake and cargo delivery. Here we demonstrate using ASBs combined with two different sensing modalities to assess binding/blocking in one example of this application.

The versatility of our “native-like” lipid bilayer platform to be formed on surfaces compatible with multiple sensing modes, allows us to use both optical and label-free electrical means to readout membrane-related events. For example, glass surfaces enable optical techniques like fluorescence recovery after photobleaching (FRAP) for bilayer fluidity characterization22, 23, 29-32, and total internal reflection fluorescence microscopy (TIRFM) to detect interactions such as nanoparticle binding33-36 and blocking virus binding27, 28. Moreover, given the practicality of label-free methods for screening applications, we create these bilayers on customized bioelectronic sensors, to electrically monitor molecular events at the cell membrane37, 38.

Using these sensing modes, we validate the detection of cEXO binding to ASBs, and subsequent blocking by an antibody to integrin β1/CD29 on the surface of ADSCs. First, using fluorescence microscopy on glass surfaces. Secondly, using electrochemical impedance spectroscopy (EIS) via poly(3,4-ethylenedioxythiophene) doped with poly (styrene sulfonate), PEDOT:PSS-based devices for sensitive measuring of the ASB frequency-dependent impedance.

PEDOT:PSS is a conducting polymer which acts as a cushioned interface between the electrode and the ASB; mimicking an *in vivo*-like environment, and enables direct electronic signal transduction for sensing biological events with excellent sensitivity39. PEDOT:PSS-coated electrodes have previously been used to detect the open/close state of transmembrane proteins in an SLB37. The addition of an insulating layer between these electrodes, here the ASB, alters the impedance characteristics of the system, and the resistance and capacitance properties of this layer can be extracted using equivalent electrical circuit (EEC) modelling40. When cEXOs bind or are blocked by antibodies, these changes can be detected in the ASB resistance and used for label-free detection of binding interactions, which we demonstrate with the same antibodies used in the fluorescence study. Finally, as a means to corroborate our platform’s results, in the context of live cells, we show that this antibody blocking strategy stops the upregulation of VEGF and cell proliferation in ADSCs that are direct outcomes of cEXO-ADSCs surface interactions. This result supports that blocking cEXO binding is a potential therapeutic strategy and validates the utility of this ASB sensing platform in finding successful targets that reduce cEXO binding and reduce proangiogenic outcomes when applied to cells.

**2. Materials and Methods**

**2.1. Cell Culture**

StemPro Human Adipose-Derived Stem Cells (ADSCs) were obtained from ThermoFisher Scientific (Rochester, NY) and cultured in ADSC growth medium kit purchased from Lonza (Walkersville, MD). Experiments were performed with cells between passages 1 and 6. MDA-MB-231 cells, highly metastatic human adenocarcinoma cells, were purchased from American Type Culture Collection, ATCC (Manassas, VA) and maintained in Dulbecco׳s Modified Eagle׳s Medium, DMEM, Corning (Corning, NY) supplemented with 10% fetal bovine serum (FBS) ThermoFisher (Waltham, MA) and 1% penicillin–streptomycin (P/S) Invitrogen, ([Carlsbad, CA](https://www.google.com/search?q=Carlsbad+California&stick=H4sIAAAAAAAAAOPgE-LUz9U3MDNLKUxS4gAxi0zK87S0spOt9POL0hPzMqsSSzLz81A4VhmpiSmFpYlFJalFxQDermitQwAAAA&sa=X&ved=0ahUKEwjCqoHHzuXaAhXsm-AKHTIACWIQmxMIzwEoATAO)). For serum free medium used for cEXO isolation, MDA-MB-231 cells were maintained in DMEM medium supplemented with 1% P/S. Experiments were performed with cells between passages 1 and 8 after received. All cell cultures were maintained at 37°C and 5% CO2 and the media were changed every two to three days as recommended by manufacturers.

**2.2. Preparation of ADSC membrane blebs**

Cell blebbing was performed through chemical induction. ADSCs were seeded in 10 cm culture dishes (Corning, NY) at a density of 1.2 x 105 cells/mL. After 72 hours of incubation at 37 ֯ C and 5% CO2 or until they reached 85% confluency, cells were washed with Giant Plasma Membrane Vesicle (GPMV) buffer (2 mM CaCl2, 10 mM HEPES, and 150 mM NaCl) at pH 7.4. Cells were then incubated in 4 mL of blebbing buffer (25 mM formaldehyde, FA, (Sigma, St. Louis, MO) and 2 mM Dichlorodiphenyltrichloroethane, DDT, (Sigma, St. Louis, MO) in GPMV buffer) on a rocker for one hour at room temperature and for one hour at 37 ֯ C. Blebs were then transferred to a falcon tube and incubated on ice for 25 minutes. Supernatant was transferred to a new tube and stored at 4 ֯ C for up to two weeks.

**2.3. cEXOs isolation and characterization**

cEXOs derived from MDA-MB-231 breast cancer cells were used for this study. Detailed isolation and characterization procedure are found in SI experimental procedures section.

**2.4. Characterization of cEXOs and ADSCs blebs**

ADSCs blebs and cEXOs size were measured by nanoparticle tracking analysis (NTA), dynamic light scattering (DLS) and further corroborated using transmission electron microscopy (TEM). Laser Doppler electrophoresis respectively (LDE) was used to measure the zeta potential of both nanoparticles. Finally, bicinchoninic acid assay (BCA) and NTA were used to find particle concentration and protein content, respectively. Experimental procedure for characterization methods is reported in SI experimental procedures section.

**2.5. Preparation of glass slides for formation of supported bilayers**

Glass coverslips 25 x 25 mm, No. 1.5, VWR (Radnor, PA) were used as a rigid surface for supported lipid bilayers. They were cleaned by piranha washing consisting of 45 mL of 50% hydrogen peroxide (Sigma, St. Louis, MO) followed by 105 mL of 70% sulfuric acid, VWR (Radnor, PA) for 10 minutes (Note: use extreme caution with this solution; highly oxidative). Then, coverslips were rinsed for 30 min with generous amounts of deionized water generated by an Ultrapure water system, Siemens pureLab (Malvern, PA). Clean coverslips were stored in deionized water at room temperature until needed. Immediately prior to the formation of bilayers, coverslips were rinsed with deionized water and dried with high purity nitrogen gas.

**2.6. Polydimethylsiloxane (PDMS) well fabrication**

A 10:1 ratio mix of PDMS, SYLGARD 184 (Ellsworth, ME) monomer and crosslinker was thoroughly stirred, degassed, and poured in a 100 mm petri dish and baked at 70֯ C for 5 hours. The cured PDMS sheet was cut into pieces to fit on top of a coverslip. A circular hole of 1 cm diameter, using a 1 cm hole puncher, was made in each PDMS piece to create a well when affixed to the coverslip. To ensure attachment of the PDMS to the clean coverslip, the well was placed in the oven at 65 ℃ for 10 minutes.

**2.7. Preparation of liposomes**

1-Oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine, POPC, Avanti Polar Lipids (Alabaster, AL) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N- [methoxy (polyethylene glycol)-2000], PEG2000-PE, Avanti Polar Lipids (Alabaster, AL) were used to create liposomes. POPC-PEG2k liposomes were made with 99.5% (mol/mol) POPC and 0.5 (%mol/mol) PEG2000. The lipid mixture was prepared by adding appropriate ratios to a previously cleaned glass vial and drying them with nitrogen gas, followed by desiccation in a vacuum chamber for 3 hours. The lipid film was resuspended in phosphate-buffered saline solution (PBS) pH 7.4 to a final concentration of 2 mg/mL and stored at -20 ֯ C overnight. After thawed, liposomes were extruded through a 100 nm membrane 12 times, Whatman (Maidstone, UK), and kept at 4 ֯ C for up to two weeks.

**2.8. Fluorescent labeling of liposomes, ADSCs blebs, and cEXOs**

To visualize the formation of supported bilayers and for diffusivity measurements using fluorescence recovery after photobleaching (FRAP), liposomes were fluorescently labeled with a membrane intercalating fluorophore, Octadecyl Rhodamine (R18). 2 µL of 0.1 mg/mL R18 (Molecular Probes, Eugene, OR) were incubated with 100 µL of liposomes, sonicated (Model # BD2500A-DTH; VWR) for 30 min, and the unincorporated fluorophore was removed using a G25 spin column (GE Healthcare, Pittsburgh, PA). The same procedure was followed to label ADSCs blebs and cEXOs, using 100 μL of each sample.

**2.9 Formation of phosphatidylcholine** **supported lipid bilayers**

Supported lipid bilayers (SLBs) self-assemble on a clean coverslip using the well-established vesicle fusion method as shown previously 29, 30. 100 µL of a 2.0 mg/mL liposome solution (POPC-PEG 2k) were added into a well and incubated for 20 minutes at room temperature. Wells were thoroughly washed with PBS pH 7.4 to remove excess lipid vesicles remaining in the bulk phase after bilayer formation. This surface was used as a negative control surface.

**2.10. Formation of ASB**

For ASB formation, 100 µl of ADSC membrane blebs (2.48 × 108 blebs/mL) in GPMV buffer were added to a PDMS well and incubated at room temperature for ~10 min. Then, the well was thoroughly rinsed with PBS pH 7.4 to remove unabsorbed blebs. Formation of the bilayer was induced by adding 100 µL of 2 mg/mL POPC-PEG2k into the well and incubation for 20 min at room temperature followed by rinsing with PBS.

**2.11. Observing ASB formation and measuring diffusivity with fluorescence microscopy**

The rupture of fluorescently labeled ADSC blebs was observed using an inverted Zeiss Axio Observer.Z1 microscope with α Plan-Apochromat objectives, a Hamamatsu EM-CCD camera (Image EM, model C9100- 13, Bridgewater, NJ), and a X-Cite 120 microscope light source (Lumen Dynamics Group Inc., Canada). To accumulate the fluorescence emitted by the fluorophores (R18), an ET MCH/TR filter cube (49008, c106274, Chromatech Inc.) was used. Labeled ADSCs blebs were first incubated on the coverslips or PEDOT:PSS-coated coverslips as described above, followed by addition and incubation of POPC-PEG2k vesicles (unlabeled) to rupture them. The process was recorded with the camera and complete rupture observed as shown in the supplemental movie, further corroborated by FRAP and additional techniques.

Diffusivity and mobile fraction measurements were carried out using photobleaching experiments. Using a 4.7 mW 488 nm krypton/argon laser, a 25 μm spot was photobleached for 300 ms followed by 40 min (30 s intervals) of recovery of the photobleached spot. The fluorescence intensity of the photobleached spot was determined by subtracting the background from a reference spot that was unbleached and normalizing to each image captured to the maximum difference between the initial photobleached spot and the initial intensity of that area. Using MATLAB, the intensity recovery data was fit into a Bessel function model using the Soumpasis30 method to extract the half time to recovery, t1/2. The following equation was used, Eq. 1: *D = w2 /4* t1/2, to determine the diffusion coefficient (D) for each bilayer type, where *w* is the full width at half-maximum of the Gaussian profile of the focused beam. The mobile fraction was determined by the final intensity over the initial intensity of the bleached area.

**2.12. Testing integrin β1/CD29, a native component of ADSCs membrane, presence in ASB**

ASB was formed as stated above. After washing the residual POPC-PEG2k vesicles away with PBS, 70 uL of 20% normal goat serum (GS, ThermoFisher Scientific (Waltham, MA)) was added to the well and incubated for 30 min at room temperature. The well was thoroughly washed with PBS one more time to remove GS excess. 100 µL of 1:100 anti-integrin β1/CD29 antibody solution (MAB1778, R&D Systems, Minneapolis, MN) was added to the well and incubated for 2 hours at room temperature. The well was thoroughly washed with PBS to remove excess primary antibody and incubated with 100 µL of 1:2000 Donkey Anti-Mouse IgG NorthernLights (NL009, R&D Systems, Minneapolis, MN) conjugate antibody for one hour at room temperature in the dark. After, the bilayer was washed one last time with PBS to remove excess secondary antibody, then was imaged using total internal reflection fluorescence microscopy (TIRFM) to detect specific binding of integrinβ1/CD29 antibody to ASB. As a negative control, an ASB was treated just with the secondary conjugated antibody to show nonspecific binding of integrinβ1/CD29 to the ASB with the absence of the primary antibody. Additionally, treatment with both antibodies as done with the first condition was repeated with a POPC-PEG2k SLB (no ADSC material) as a negative control to show nonspecific binding of integrinβ1/CD29.

**2.13. Binding of cEXOs to ASB**

ASB was formed as stated above and blocked using 70 uL of 20% normal GS for 30 min at room temperature to prevent nonspecific binding. After thoroughly washing the well with PBS to remove excess GS, 100 uL of R18 labeled cEXOs were added to the ASB and incubated for 30 min in the dark to allow binding. Finally, the well was thoroughly washed with PBS to rinse unbound vesicles. Binding of cEXOs to ASB was imaged using TIRFM and quantified by ImageJ software.

**2.14. Blocking binding of cEXOs to ASB using Integrin β1/CD29**

An ASB was incubated with 100 uL of 20% normal GS for 30 min at room temperature, followed by a thorough wash with PBS to remove excess GS. 100 µL of 1:100 anti-integrin β1/CD29 were added to the well and incubated for 2 hours at room temperature. The well was thoroughly washed with PBS to remove the primary antibody, followed by incubation with 100 uL of R18 labeled cEXOs at room temperature for 30 minutes to allow binding. The ASB was washed with PBS to rinse unbound cEXOs and images were captured using TIRFM.

**2.15. TIRFM Setting and Operation**

Total Internal Reflection Fluorescence Microscopy (TIRFM) was conducted on an inverted Zeiss Axio Observer.Z1 microscope with an α Plan-Apochromat 100× objective; 488 nm and 561 nm wavelength from solid-state lasers were used to excite the samples. A Laser TIRF 3 slider (Carl Zeiss, Inc., [Oberkochen, Germany](https://www.google.com/search?sxsrf=ALeKk03QdfiqAh8bXcySXXYvxJ5NEgI1MA:1582220567218&q=Oberkochen&stick=H4sIAAAAAAAAAOPgE-LUz9U3MEwutChWAjNNTTPMy7S0spOt9POL0hPzMqsSSzLz81A4VhmpiSmFpYlFJalFxYtYufyTUouy85MzUvN2sDICAP8BmH9UAAAA&sa=X&ved=2ahUKEwiK3_X21uDnAhXGlXIEHcyoDowQmxMoATAiegQIDBAD&sxsrf=ALeKk03QdfiqAh8bXcySXXYvxJ5NEgI1MA:1582220567218)) was used to adjust the angle of incidence at approximately 68.2° generating an evanescent wave at 100 nm and total internal reflection. The excitation light was filtered by a Semrock LF488-B-ZHE filter cube and sent to the electron multiplying CCD camera (ImageEM C9100-13, Hamamatsu).

**2.16. Multi-electrode array Fabrication**

Multi-electrode arrays (MEAs) were fabricated using an established photolithography process on 4‐inch glass wafers. The wafers were cleaned using a piranha (H2O2:H2SO4, ratio 1:3–4) bath, washed with water and cleaned with O2 plasma (Nanoplas DSB 6000). The electrode areas were defined using standard photolithography steps. To perform the lift‐off step, the wafers were coated with a photoresist bilayer consisting of LOR 5B (Microchem) and S1813 (Shipley) and exposed to UV light using the EVG 6200 mask alignment system and developed using MF319 developer. A 10 nm layer of Cr and a 100 nm layer of Au were deposited using magnetron sputtering (Equipment Support Company Ltd. ESCRD4) and lifted using appropriate solvents. After the lift‐off step, the first Parylene C layer was vaporized to a thickness of 1.7 µm using a SCS Labcoater 2 with Silane as an adhesion promoter. A second Parylene C layer was vaporized to act as the sacrificial layer for polymer film patterning. A layer of AZ9260 was spun cast and developed using AZ developer as a mask for reactive ion etching (Oxford Instruments Plasmalab 100–ICP 380) which was used to expose the device channels and pads for polymer deposition. Each chip consists of four circular electrodes of 500 μm in diameter (0.00196 cm2).

**2.17. Electrochemical Impedance Spectroscopy (EIS)**

EIS was utilized as a label-free approach to assess cEXO binding capabilities. ASBs were formed on PEDOT:PSS-coated MEAs and cEXOs binding and blocking experiments were carried out in the same manner as described for glass substrates in 2.18 and 2.19. ASBs were prepared fresh on the devices and were stable for 48 hours, meaning device performance did not decrease during this time nor did the membrane resistance change. A potentiostat (Autolab PGSTAT128N) equipped with a frequency response analysis module was used to record impedance spectra at the frequency range between 100 KHz – 0.1 Hz. Commercially available Ag/AgCl and platinum mesh were used as reference and counter electrodes, respectively. The PEDOT:PSS-coated Au MEAs were used as the working electrodes. The PEDOT:PSS solution contained 95% v/v Clevios PH 1000 (Heraeus), 5% v/v ethylene glycol (Sigma-Aldrich), 0.002% v/v 4-dodecylbenzenesulfonic acid (Sigma-Aldrich), and 1% v/v (3-glycidyloxypropyl) trimethyoxysilane (Sigma-Aldrich). PEDOT:PSS was spin coated onto MEAs (dried under nitrogen and treated with oxygen plasma for 2 min just prior to use) at 3,000 rpm for 35 s and baked at 140 °C for 1 h. A glass cloning cylinder was glued onto the MEA using PDMS to act as a well. The electrodes were circular, 500 μm in diameter, and thus, the active electrochemical area was 0.00196 cm2. The applied AC voltage was 0.01 V and a DC voltage of 0 V versus open circuit potential. All measurements were taken in 1X PBS buffer contained in a glass well attached to the MEAs. To monitor cEXOs binding, we took EIS measurements on individual electrodes after each step as follows: (1) bare PEDOT:PSS electrodes, (2) ASB formed on top, (3) after incubation with anti-CD29 antibody, in the case of blocking, and (4) after addition of cEXOs. Nova software was then used for data analysis and electrical equivalent circuit (EEC) modeling to extract the membrane resistance values.

**2.18. Assessment of VEGF secretion by ADSCs**

ADSCs were seeded on a Millicell EZ-slide (Millipore, Burlington, MA) at a density of 8 x 104 cells/well. After 48 hours, ADSCs were changed to 2% FBS media to slow down cell proliferation and incubated overnight at 37 °C and 5% CO2. Each well had a different treatment, as follows: negative control with no treatment (NT), ADSCs treated with 40 μg of cEXOs, ADSCs incubated with 20 µg/mL of anti-integrin β1/CD29 antibody (MAB 2253Z, Millipore, Burlington, MA) for two hours at 37°C followed by addition of 40 μg of cEXOs, and ADSCs treated with 10 ng/mL of TGFβ1 (ThemoFisher Scientific, Waltham, MA). Cell media was changed every other day along each of the treatments. In addition, 100 µL of media from each well were removed on days 0, 2, and 5 and stored at -20°C for VEGF-ELISA assay. As a measure of proangiogenic activity, VEGF secretion by ADSCs in all mentioned conditions was assessed using a Human VEGF DuoSet ELISA kit (R&D Systems, Minneapolis, MN) following the manufacturer's protocol. VEGF concentration was normalized by the number of alive cells in each condition. The number of alive cells was assessed for each well/treatment on days 2 and 5 by staining cells nuclei using Hoechst (ThermoFisher Scientific, Waltham, MA) and getting the average number of cells in 30 frames per well in images acquired by an inverted Zeiss Axio Observer. Z1 microscope with α Plan-Apochromat objectives, a Hamamatsu EM-CCD camera, Image EM, model C9100- 13 (Bridgewater, NJ), and an X-Cite 120 microscope light source, Lumen Dynamics Group Inc. (Ontario, Canada). Four independent replicates of the experiment were performed.

**2.19. Statistical Analysis**

For all experiments performed in this study, variance analysis was performed using a *t*- test with unequal variances to find significant differences between substrates and conditions. All data were plotted and analyzed using Microsoft Excel, and it is presented as mean ± SD. Statistical significance levels were determined as follows: \*p ≤ 0.05, \*\*p ≤ 0.01, \*\*\*p≤0.001, \*\*\*\*p≤0.0001.

**3. Results and discussion**

**Overview.** Here, we present the use of ADSCs-derived SLB (ASB) to facilitate the studies of cancer cEXOs-ADSCs binding and blocking strategies. First, we use optical means to characterize the formation of an ASB and binding of cEXOs, followed by screening the potential of blocking β1/CD29 with a specific antibody to reduce cEXOs binding. Next, these results are repeated using a label-free electrical approach. Lastly, to corroborate the results obtained using our cell-free ASB platform, we used a cell culture assay to investigate the effect of blocking integrin β1 in the surface of ADSCs for the prevention of cEXOs binding and show that this stops the upregulation of VEGF secretion and cell proliferation, which are direct outcomes of cEXOs and ADSCs binding7, 23.

**3.1.** **ASB as an *in vitro* model of ADSC membrane**

We chose to study ADSCs because they are an important cell group in the tumor microenvironment (TME) with a critical role in cancer progression and aggressiveness11, 41. In particular, ADSCs secrete inflammatory biomarkers, such as VEGF, that promote angiogenesis and a **TME w**ith high levels of inflammation, fostering tumorigenesis9, 42. Notably, surface interactions between cEXOs and ADSCs, are known to stimulate VEGF secretion in ADSCs contributing to their angiogenic potential23. Because of these malignant implications, it is imperative to study cEXOs-ADSCs binding and to find treatments to block it as a mean to decrease ADSCs angiogenic properties. Towards this end, we developed a tool to study cEXO-ADSC binding by replicating it in an *in vitro* setting using an ADSCs-membrane model, ASB.

To recapitulate the membrane of ADSCs in the ASB, we incorporated native components of the cell membrane into a SLB using ADSCs membrane blebs29, 30.Cell blebs are proteoliposomes that protrude and bud from the cell surface and retain the lipid and protein composition of the mother cell membrane30. Here, ADSCs membrane blebs were obtained by chemical treatment and characterized by several techniques. Size was obtained by complementary methods such as nanoparticle tracking analysis (NTA), dynamic light scattering (DLS), and transmission electron microscopy (TEM). Concentration was determined also by NTA. Total protein concentration was determined by bicinchoninic acid assay (BCA). Finally, laser Doppler electrophoresis was used to assess their zeta potential. The size of the blebs found by the three methods were highly comparable ranging between 214 ± 22.8 nm (NTA) and 232 ± 39.3 nm (TEM), and zeta potential of membrane blebs in GPMV buffer was 14.3±1.22 –mV; these are both in the range of values reported in the literature30, 43, 44.

cEXOs were isolated from breast cancer cells (MDA-MB-231) and characterized in the same manner as cell blebs. cEXOs isolation and characterization is described in detail in the supporting information section. Our average cEXOs size, 109 ± 12.3 nm, agreed with reported values in literature in which their diameters range between 30-120 nm45-48. A complete characterization of cell blebs and cEXOs is reported in Table 1 and Figure S1.

**Table 1. Characterization of cEXOs and ADSC cell blebs**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Particle** | **Diameter Size by NTA (nm)** | **Diameter Size by DLS (nm)** | **Diameter Size by TEM (nm)** | **Zeta potential (-mV)** | **Concentration (particles/mL)** | **Protein content (μg/mL)** |
| **cEXOs** | 93.9 ± 22.2 | 116 ± 11.9 | 118 ± 28.3 | 10.3±0.41 | 2.26 ×108 ± 9.21 ×107 | 638 ± 11.9 |
| **ADSCs blebs** | 214 ± 22.8 | 229 ± 33.6 | 232 ± 39.3 | 14.3±1.22 | 6.25×108± 5.70 ×107 | 1.23x103 ± 89.2 |

**3.2. Formation and characterization of ASB**

ASBs were produced from membrane blebs from ADSCs using a protocol previously established by our group to form proteinaceous bilayers from mammalian cells (Figure 1a)29. In brief, cell membrane blebs labeled with octadecyl rhodamine (R18) a membrane-intercalating fluorophore, were incubated, and adsorbed on a cleaned glass slide. The fluorophore, R18, was initially confined to cell blebs and visualized as bright dots, as shown in the left image of the fluorescence images (Figure 1a). Next, fusogenic POPC-PEG2k liposomes were added to the well to induce rupture of blebs and spreading of R18 signal through the bilayer, as seen in the middle image of the fluorescence images30, 49. After 30 min of incubation followed by thorough PBS rinsing, a contiguous supported bilayer was formed and R18 signal from blebs uniformly spread out, diffusing freely within the planar bilayer surface as seen in the right fluorescence image. The dark scratch in the lower right (Figure 1a and 1b) of microscopy images was made to assess focus at the z-plane of the bilayer during image acquisition. The formation of an ASB can be found in Video S1.

Lateral diffusion of components within the cell membrane is a fundamental process involved in many biological functions including binding and fusion of external particles50 51-53. For proper mimicking of ADSCs plasma membrane, ASB should retain the fluidity of the cell membrane since it allows diffusion within its 2D plane53, influences binding-avidity54, and facilitates lateral rearrangement of ligands to optimize binding55. We assessed the diffusivity of octadecyl rhodamine (R18) in the 2D plane of ASB using Fluorescence Recovery After Photobleaching (FRAP). Using a laser beam, a 22 µm diameter circle was photobleached in the bilayer at time zero as indicated by an arrow in the fluorescence recovery graph in Figure 1c and as shown in the top fluorescence image in Figure 1b. Partial recovery of the photobleached circle can be visualized in Figure 1b and 1c at around 350 sec for ASB. Lastly, final recovery of the photobleached circle was achieved at about 1600 sec for ASB as shown in the fluorescence recovery graph in Figure 1c and in the bottom fluorescence image in Figure 1b. The ability of the ASB to undergo fluorescence recovery and its resultant high mobile fraction value of 90% and above validates the formation of a diffusive and mobile supported bilayer, minimally free of defects. Fluorescence recovery curves (Figure 1c) formed as described in the experimental section and used to determine the diffusion coefficient (D) of ASB and a control POPC/PEG-2k SLB are reported in Figure 1d. ASB was found to be less diffusive (D: 0.297 ± 0.035 µm2/s) than POPC/PEG-2k SLB (D: 0.486 ± 0.096 µm2/s). These results were expected since POPC/PEG-2k SLB contains only lipids and ASB consists of not just lipids, but also ADSCs membrane proteins from ADSCs blebs, which have been established to reduce diffusion within the bilayer56. Therefore, ASB conserves the characteristic fluidity properties of cell membranes.

Diagram, engineering drawing

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**Figure 1. ASB formation and characterization**. a) Diagram and fluorescence images of ASB formation. *Created with BioRender.com.* b) Pictures depicting pre-bleached, photobleached, partially recovered, and completely recovered ASB. c) ASB fluorescence recovery after photobleaching curve. d) Coefficient of diffusivity of ASB and POPC/PEG2k SLB. e) TIRF microscopy images of ASB treated with CD29/integrin β1 antibody shows specific binding of CD29/integrin β1 to ASB and its presence in it. f) Absence of CD29/integrin β1 in ASB treated with secondary antibody in the absence of primary antibody. g) Absence of CD29/integrin β1 in POPC/PEG2k-SLB. N = 4, mean ± SE, \*\*\*p ≤ 0.001.

**3.3. Detection of Integrin β1/CD29, a native component of ADSC membrane, in ASB**

To recapitulate the cell membrane of ADSCs, ASBs should retain its native components. Integrin β1 is a protein highly expressed in the plasma membrane of ADSCs and is implicated in several biological functions including cell adhesion, wound repair, tumor directed angiogenesis, and tumor cell growth57, 58. ASB, as an *in vitro* model of ADSC membranes, should preserve this component. Here, we confirmed the presence of integrin β1 in the ASB using immunofluorescence with an-anti integrin β1/CD29 antibody, directed to recognize the extracellular portion of this transmembrane protein. We then labeled with a fluorescent secondary antibody for integrin β1 detected using TIRFM. Figure 1e depicts the bright spots associated with binding and presence of integrin β1 in an ASB. Furthermore, given that the antibodies bound to the appropriate epitope that faces away from the membrane, it suggests that the orientation of the integrins is also intact. This result agrees with our previous observations for protein orientation where the extracellular portions face the bulk phase after bleb rupture29, 30. To reassure that our results were specific and not reflective of nonspecific adsorption, we used two negative controls shown in Figure 1f and 1g. Figure 1f depicts an ASB treated with conjugated secondary antibody in absence of primary antibody. Since the secondary antibody label is specific for the primary antibody59, absence of the latter led to lack of detection of integrin β1/CD29 in the ASB. Moreover, in Figure 1g, a POPC/PEG2k-SLB is treated with primary and secondary antibodies, and no presence of integrin β1 is detected since it is a synthetic and inert bilayer that does not contain adhesion molecules like integrins. Collectively, these results suggest that our ASB preserves native membrane components, making it a good platform to study cEXOs interactions with ADSCs.

**3.3. Optical detection of cEXOs binding to ASB**

Independent of the route taken by cEXOs after initial contact with the cell membrane, binding is a common initial step for all mechanisms. Therefore, targeting it as a way to universally inhibit these malignant outcomes of cEXO-cell interactions is a strategy with high therapeutic potential. To detect binding to the ASB platform, R18-fluorescently labeled cEXOs were incubated with an ASB, and their initial interactions were captured using TIRFM and reported in Video S2. We observed cEXOs binding to the ASB within 3 min of contact, with some of them being stably bound and some others binding and detaching from the ASB. Images captured by TIRFM after 30 additional minutes of incubation, show that cEXOs remained attached to ASBs (Figure 2a) suggesting a stable binding, however further experiments must be performed to confirm that. In contrast, Figure 2c shows a negligible amount of cEXOs binding to POPC/PEG2k-SLB as expected since POPC/PEG2k-SLBs are inert, non-fouling surfaces, and do not contain adhesion proteins to mediate binding. It also suggests that in the ASB, the cEXOs binding specificity comes from the ADSCs membrane components that come from the blebs.

**3.4. Blocking of Integrin β1/ CD29 reduces cEXOs binding to ASB**

Binding between cEXO and ADSCs is known to be mediated by at least two proteins, one on the surface of each entity. Since it is established that integrins, including β1, α3, and α5 have been involved in binding of different types of EXOs to recipient cells60, 61, we suspect that integrin β1, present on our ASB, could be one of the proteins involved in cEXO-ADSC binding. Figure S2a, an schematic of the EXOs-cell surface interactions previously established13, shows that

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**Figure 2.** **Integrin β1/CD29 plays a role in cEXOs binding to ASBs.** TIRFM images of cEXOs binding toa) ASB with no treatment. b) ASBs treated with CD29/integrin β1 antibody. c) POPC/PEG2k SLB. d) Quantification of cEXOs bound to ASBs, non-treated and treated with integrin β1/CD29 antibody, and POPC/PEG2k SLB after 30 min incubation. *Created with BioRender.com.* N = 3, mean ± SE, \*\*\*\*p ≤ 0.0001.

integrins on the surface of the target cell potentially bind to ICAM and/or ECM proteins (fibronectin, laminin and collagen) on the EXOs surface13. To confirm the presence of those ligands on the surface of breast cancer cEXOs that will facilitate binding to integrin β1 on the surface of ADSCs, we performed a proteomics analysis available on File S1. Figure S2b shows several integrin β1 ligands on the surface of cEXOs including ICAM1, fibronectin, laminin, and collagen subunits, among others. Therefore, we decided to focus on investigating the role of integrin β1 in ADSCs membrane, on cEXOs-ADSCs binding, specifically the ability to block that binding by antibodies directed against integrin β1.

To examine the potential of blocking integrin β1 as a strategy to decrease cEXO binding, the surface of the ASB was treated with an anti-integrin β1/CD29 primary antibody (referred as CD29 Ab here) prior to incubation with cEXOs. Following the same procedure previously stated, treatment of the ASB with CD29 Ab resulted in significantly reduced binding compared to cEXOs binding to the untreated ASB, Figure 2 b and 2a respectively. Additionally, as seen in figure 2d, the number of cEXOs binding to the CD29 Ab-treated ASB is significantly less than cEXOs bound to untreated ASB, and similar to the number of cEXOs binding to the POPC/PEG2k-SLB. These results indicate that integrin β1 plays a specific role in cEXO-ADSCs binding, recapitulated in ASB, and its blockage decreases attachment of cEXOs to ASB.

A convenient aspect of our platform is that ASBs are free of maintenance required for live cells, simplifying that aspect of assay and drug screening development. Furthermore, we can focus on binding, isolated from cEXOs internalization by cells, to target that conserved aspect of cEXO-cell interactions. We demonstrated that ASB is a functional *in vitro* ADSC cell membrane model able to detect cEXOs binding using optical approaches and can be leveraged to develop strategies to block it. A great advantage of SLBs is their high amenability to scale up, through rapid functionalization and patterning of surfaces and integration of microfluidic and electrical components. Toward this end, we coupled the ASB with an electrical device forming a bioelectronic platform to allow label-free and sensitive detection of cEXOs binding.

**3.5 Electrical monitoring of cEXOs binding to ASB**

By coupling the ASBs with MEAs we can probe changes in the electrical signals resulting from modifications of the membrane properties associated with the binding of cEXOs. We have previously demonstrated the capability of electrical methods for monitoring biological events at the cell plasma membrane37, 62, 63. This led us to integrate ASBs with poly(3,4-ethylenedioxythiophene) doped with poly(styrene sulfonate) (PEDOT:PSS) -based MEAs (Figure 3a) for electrical sensing of cEXOs binding to ASB using EIS. PEDOT:PSS has overall low impedance and can hence be ideal for biological sensing applications. Additionally, PEDOT:PSS films modified with silane based crosslinkers swell in aqueous biological environments and act as a hydrogel-like cushioned support well suited for cell - cell membrane integration while preserving mechanical and electrical properties64-66. This more native-like environment ensures transmembrane protein functionality by facilitating their free lateral movement in the membrane62.

ASB lipid membranes were successfully formed on PEDOT:PSS transducers and initially characterized using FRAP to reassure the formation of a proper bilayer to the electrical transducer. Results in Figure S3 show FRAP images and fluorescence recovery of an ASB on PEDOT:PSS after 400 sec suggesting successful formation of a diffusive lipid bilayer on PEDOT:PSS. The reported coefficient of diffusivity of ASB on PEDOT:PSS (0.500 ± 0.022 μm2/s) is slightly higher than on glass (D: 0.297 ± 0.035 µm2/s), and we hypothesize that this increase in diffusivity is due to the ability of PEDOT:PSS to act as a cushion for the ASB, as it has been previously observed for other hybrid-SLBs formed on polyelectrolyte cushions67.

A picture containing diagram

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**Figure 3. Electrical readouts support the role of integrin β1/CD29 in cEXOs binding to ASBs.** a) Experimental setup showing ASB formed on top of PEDOT:PSS-coated Au electrode. RE: Reference electrode, CE: Counter electrode. The EEC is shown with being the solvent/electrolyte PBS 1X, and being the membrane resistance and capacitance, respectively, and the PEDOT:PSS modeled as a constant phase element (CPE), *Created with BioRender.com.* b) Bode plot of cEXOs binding to the ASB. c) Bode plot of cEXOs binding to the CD29 Ab-treated ASB. (d). Rm normalized to the ASB prior to cEXO binding showing ASB + cEXOs in the absence and presence of CD29 Ab treatment. e) Comparison of the normalized percentage increase in between the ASB + cEXOs addition and between the ASB+CD29+cEXOs addition as defined by equation 2. N= 5, mean ± SE, \*\*p ≤ 0.01.

EIS measurements of ASBs formed on PEDOT: PSS-coated electrodes were performed for the electrical monitoring of the bilayer formation too. Here, the impedance magnitude, , as a function of frequency, is captured in the Bode plot shown in Figure 3b and 3c and Figure S5 and S6, and the fitted resistance values were used as figures of merit. The magnitude of the calculated membrane resistance relates to the membrane ion permeability and has thus been successfully used in similar setups as a figure of the membrane “leakiness” or the membrane “integrity”. For example, we have shown in the past that membrane binding events result in an increase in the membrane resistance whereas membrane events that could potentially result in membrane “leakiness” (i.e., disruption, pore formation, ion channel opening) result in a decrease in the calculated resistance values. EEC model was fitted to the raw impedance as illustrated in the Nyquist plot of Figure S4b, allowing extraction of the membrane resistance ( ) using the EEC. was calculated for ASB and for ASB with integrin β1 blocking (*ASB+CD29*), and again after the addition of cEXOs (*+cEXOs*) for each condition.

The experimental protocol was carried out using multiple single MEAs for each condition to control for MEA batch-to-batch variability. Measurements were taken from multiple electrodes within the same MEA (i.e., of the same ASB) for each condition to control for electrode-to-electrode variability and to ensure that measurements were only collected from electrodes where there was good coverage of the ASB. ASB sealing quality was assessed based on the increase in impedance compared to the bare PEDOT:PSS impedance, as observed in the Nyquist and Bode plots (see Figure S4-5) and as determined from the increase in the derived value. It was typical to achieve a 25-50% success rate of electrodes whose reflected a tightly-sealed lipid bilayer. The calculated values were normalized for each measurement due to the variability in absolute between MEAs, which ranged between 0.01 and 1.02 . This range in values was likely caused by small differences in the ASBs, as the MEAs are very sensitive to defects found in the bilayer. These values are comparable to values obtained from similar setups that used mammalian cell-derived SLBs. To address this variation, normalization was performed to assess the relative change in magnitude of before and after cEXOs binding for each condition (Figure 3d). This allows cEXOs binding to be inferred from changes in , essentially rendering a function of the abundance cEXOs binding to the membrane and allows comparison across multiple experiments performed on different MEAs regardless of the baseline obtained.In the case of the CD29 Ab condition, the of the ASB after treatment with CD29 Ab was used as a reference for cEXOs binding. The addition of anti-CD29 Ab to the ASB resulted in a small increase in ~14% (from 0.010 to 0.011 ) (Figure S6). The relative change in normalized , , is defined as:

Eq. 2

where is either ASB or ASB+CD29, we can calculate the relative change in before and after cEXO binding. The results presented here are an average of these. The addition of cEXOs caused an average increase in normalized of ~54% and ~9% for the non-treated and anti-CD29 Ab-treated conditions, respectively (Figure 3e). These results align with the ones from the optical experiments and provide further proof that blocking integrin β1 receptors with anti-CD29 Ab reduces cEXO binding to the ASB.

In summary, using our ASB platform with optical and electrical analytical approaches, we were able to detect cEXOs binding and to verify the mediating role of integrin β1 on it. Fluorescence/label-based methods are more established in this field and in this study, and we confirmed the fluorescence data with the novel electrical data and demonstrated the capability of detecting cEXOs binding in a label-free manner for the first time. Further analysis to compare the two techniques is part of our future work as it will require us to establish a more thorough understanding of the EIS data, such that we can tune the sensitivity of the devices to quantitatively assess cEXOs binding events.

To validate our results obtained by both sensing methods (optical and electrical), we investigated the biological outcome of blocking integrin β1 in ADSCs in an *in vitro* cell setting. However, the utility of this platform is only promising if the results obtained here translate to changes in biological outcomes. To assess this, we next carry out the same studies in cell culture and measure changes in pro-angiogenic behavior to determine if the binding we block here leads to a reduction in negative outcomes.

**3.6. Blocking of integrin β1 decreases cell proliferation and VEGF secretion by ADSCs in culture**

We previously used an *in vitro* cEXO-membrane platform (EVSB) to isolate interactions between cEXOs surface and ADSCs and found proangiogenic markers including upregulation of VEGF and cell proliferation to be a direct outcome23. Therefore, to validate the mediating role of integrin β1 in cEXOs-ASDCs binding, found in the previous sections, we investigated its influence on VEGF and cell proliferation upregulation, as binding outcomes. Towards this end, we conducted an *in vitro* assay to assess the number of alive cells and concentration of VEGF secreted by ADSCs treated with cEXOs in the presence and absence of CD29 Ab, as a blocking treatment for integrin β1, in ADSCs.

ADSCs grown to 80% confluency were treated during 5 days with four conditions: 40 μg of cEXOs (cEXOs), CD29 Ab followed by cEXOs (CD29), transforming growth factor beta (TGFβ) as a positive control, and no treatment (NT) as a negative control (Figure 4d). ADSCs with no treatment (NT) were expected to secrete less VEGF than the treated counterparts7, 23. Conversely, ADSCs treated with TGFβ were expected to show increased VEGF secretion since TGFβ plays a key role in VEGF regulation and induction of proangiogenic factors68-70. Secreted VEGF concentration per cell for all conditions was assessed by ELISA assay at days 2 and 5 of treatment. For easier data interpretation, results displayed in Figure 4 represent VEGF secretion per cell for all conditions normalized by the no treatment (NT2) values on day 2. Therefore, NT2 values were used as a baseline to analyze VEGF concentration by ADSCs.

As expected, VEGF secretion is significantly upregulated in ADSCs treated with cEXOs and with TGFβ compared to the NT counterparts for both days. Results displayed in Figure 4a show that cells treated with cEXOs and TGFβ for two days secreted approximately 3 and 4 times more VEGF than NT2 cells. Interestingly, ADSCs treated with CD29 Ab, previous to cEXOs addition, secreted similar VEGF concentration as NT2 cells. The same behavior was observed on day 5 of treatment where cells treated with cEXOs and TGFβ secreted around 6 times more VEGF than NT2 and NT5 cells, and cells treated with CD29 Ab secreted just 2 times more VEGF than N2 and N5 cells. As expected, VEGF quantity was slightly higher on day 5 than on day 2, for all conditions as shown in Figure 4b. These results indicate that blocking integrin β1 in ADSCs prevent the pro-angiogenic activity, indicated by upregulation of VEGF observed in ADSCs treated with cEXOs (Figure 4d).

Since cell proliferation is also influenced by cEXOs binding to ADSCs, we counted the number of alive cells under the same conditions. As seen in Figure 4c, treating ADSCs with cEXOs and TGFβ leads to a higher number of alive cells than NT, as we previously reported. Conversely, a decrease on cell number was observed with CD29 Ab treatment before cEXOs addition. Given these results, it suggests that integrin β1 plays an important role in cEXO-ADSC binding and blocking it is a strategy with therapeutic potential to decrease pro-angiogenic activity that occurs from VEGF upregulation and proliferation of ADSCs in the tumor microenvironment.

**Graphical user interface

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**4. Conclusions**

We have successfully generated an ASB platform that conserves lateral fluidity and presence of integrin β1, important characteristics of ADSCs surface, making an adequate *in vitro* model of human primary ADSC plasma membrane. Using this system, we were able to replicate cEXO binding, isolating it from subsequent cEXO internalization and cargo delivery, in a cell-free manner. The planar nature of the stem cell membrane model renders it amenable to surface sensitive techniques, optical and electrical, which hold potential for extracting valuable information regarding cEXOs binding and function that would otherwise remain elusive to cell-based systems and its complementary analytical techniques. This geometry facilitated the integration of the ASB to a PEDOT:PSS-based MEA and detection of cEXO binding using optical (TIRFM) and electrical (EIS) techniques, allowing visualization and label-free monitoring of such interaction. Moreover, we showed a significant decrease of cEXO binding to the ASB with anti-CD29 Ab treatment (as a mean to block integrin β1) with both optical and electrical readouts. These results suggest that integrin β1 receptors were bound to CD29 Ab and not available to interact with cEXOs, supporting our initial hypothesis that integrin β1 facilitates cEXO binding to ADSCs. Likewise, treatment of cultured ADSCs with CD29 Ab prior to cEXO addition led to a small change in VEGF secretion and cell proliferation, both processes highly upregulated in ADSCs treated with cEXOs in the absence of CD29Ab. This validates our results obtained with the ASB showing the ability of CD29 Ab to reduce cEXOs binding to ADSCs and its malignant outcomes, and it further corroborates an important role of integrin β1/ CD29 on cEXOs-ADSCs binding.

Moreover, the detection of low levels of remaining cEXOs-ASB binding and some increase in VEGF secretion and cell proliferation in ADSCs despite CD29 blocking, suggests the involvement of other molecules in this binding. This possibility opens a new avenue to use our ASB system and study the role of other ADSCs and cEXOs surface components in cEXOs-ADSC binding and find strategies to inhibit it through drug screening. The proteomics analysis available in the SI offers several cEXOs surface molecules, including possible ligands for integrin β1, that can be further analyzed. Furthermore, our ASB will facilitate the study of cEXOs binding kinetics and the mechanisms behind it to further attain its complete inhibition along its malignant outcomes.

Here we showed how our ASB platform allows the recapitulation of human primary ADSCs plasma membrane and the monitoring of cEXOs binding in a simpler manner than the available conventional methods such as optical tweezers71 and immunofluorescence72. Although previous groups have used native cell membrane models to screen virus binding inhibitors27, 28, to the best of our knowledge, we are the first study to propose the use of biomimetic membrane models incorporating native components of human mammalian cells to screen strategies to block EXOs binding to cells. Our system offers several advantages including: (i) it is free of the dynamic complexity of cells; (ii) it does not need the aseptic conditions required in cell culture; (iii) reduces the need for extensive human primary ADSCs culture which are highly sensitive, not widely available, and have a limited number of cell culture passages allowed before they differentiate; and (iv) the ability to use complementary analytic techniques like TIRFM and EIS, for visual detection in real time and for label-free and sensitive electrical monitoring of cEXOs binding/blocking, respectively. This versatility makes the ASB an excellent option for screening potential therapeutic molecules as it is compatible with scale-up and multiplexing, with the option to be label-free. Therefore, multiple testing conditions can be screened using ASBs originated from a single batch of human primary cells. Lastly, the ASB can be tuned to generate model systems of the plasma membrane of different types of cells to investigate their binding to diverse types of EXOs. This model will facilitate the study of different types of cancer and other diseases for which the prognosis is worsened as communication between diseased and healthy cells progresses via EXOs 3, 7, 73-75. Our platform will make then a great tool for drug discovery and the development of treatments and therapeutics to mitigate the strong effect that cEXOs have on tumorigenesis and metastasis.

**5. Supporting information available**

The following files are available free of charge at [SUPPLEMENTAL INFORMATION LINK]

* A detailed experimental section for cEXOs and ADSCs blebs isolation and characterization techniques used and cEXOs proteomics analysis, including all the materials utilized and thorough procedures for easy reproducibility; cEXOs and ADSCs blebs size, particle concentration, and protein content characterization results; schematic of possible surface interactions between EVs and recipient cells and table of ligands on the surface of cEXOs that can possibly bind integrin β1 on the surface of ADSCs; complete proteomics analysis of breast cancer cells, MDA-MB-231, derived cEXOs; characterization of ASB on PEDOT:PSS by FRAP; Nyquist plots of cEXOs binding to ASB; Nyquist plots of cEXOs binding to ASB treated with CD29Ab; and bode plot showing effect of CD29 treatment in ASB resistance.
* A video capturing the formation of an ASB using FRAP.
* A video showing surface interactions and binding of cEXOs to ASB upon contact, using TIRFM.

The authors declare no competing financial interest.

**6. Authors contributions: JU:** conceived the presented idea; cEXOs and ADSCs blebs isolation and characterization; ASB formation and characterization by FRAP, cEXOs binding/blocking by TIRFM; cell culture and VEGF assessment experiments; data analysis and interpretation; and drafted the manuscript and designed the figures. **WT:** ASB-MEA formation and characterization by EIS, cEXOs binding/blocking by electrical means, data analysis, and manuscript writing and edition. **AH:** cEXOs proteomics analysis. **VD:** MEA fabrication. **ZM:** TEM imaging acquisition. **AO:** cEXOs proteomics analysis. **AMP:** Electrical data analysis, modelling, and interpretation; optimization of EEC models to fit data for Rm extraction. **MH:** ADSCs blebs size characterization, manuscript writing and editing. **SI:** Oversaw the project, coordinated cEXOs proteomic analysis and device fabrication. **RO:** Oversaw the project, coordinated electrical experiments performance, data analysis, and interpretation. **SD:** conceived the presented idea, oversaw the project, results interpretation, manuscript writing and editing. All authors discussed the results and commented on the manuscript.

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**Dual mode sensing of binding and blocking of cancer exosomes to biomimetic human primary stem cell surfaces**

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