Biosensor-based exosome immobilization: achieving enhanced specificity with SophoMer F10 polymer

Exosomes are nanoscale extracellular vesicles (typically 30-150 nm in diameter) that are naturally released by nearly all cell types into bodily fluids like blood, urine, saliva, and cerebrospinal fluid (1). They serve as intercellular communication messengers, carrying a complex cargo of biomolecules such as proteins, lipids, and various types of RNA—reflecting the state and type of their originating cells. This makes exosomes invaluable in biomedicine, as they can act as biomarkers for a range of diseases, including cancer, neurodegenerative disorders, and infectious diseases (2). Detecting and characterizing exosomes allows for the non-invasive diagnosis and monitoring of these conditions, as changes in exosome quantity or content may reflect pathological processes in real time. Exosomes' accessibility in biofluids, stability, and disease-specific molecular content position them as promising tools for precision medicine applications, such as early diagnosis, monitoring treatment efficacy, and even drug delivery.

Biosensors for exosomes characterization

Biosensors capable of detecting and capturing exosomes have transformative potential in diagnostics and research, as they allow for the profiling of specific exosome markers linked to various diseases. Specific interactions between exosomes and the biosensor surface, typically facilitated by marker-specific antibodies, are essential to ensure accurate and reliable detection. These targeted interactions enable the selective binding of exosomes with certain surface markers, allowing researchers to perform precise analyses either by direct capture on the sensor surface or through secondary staining techniques (3).

However, it is equally crucial to control and prevent nonspecific interactions, as these unintended bindings can interfere with the biosensor's performance, leading to false-positive signals and decreased sensitivity. Nonspecific interactions, often caused by unwanted protein adhesion or charge-based attraction, can be minimized by employing effective surface-blocking agents. Among available options, polymer-based blockers such as **SophoMer F10 (Sophomer s.r.o.)** have shown excellent results in preventing nonspecific binding by passivating the sensor surface, which ensures that only specifically bound exosomes are detected. **SophoMer F10** is a fully synthetic material designed to replace bovine serum albumin in immunology assays. Here, we present typical results from exosome on chip immobilization, using **SophoMer F10** during fabrication of biochemically modified surface.

Refined exosome detection: SophoMer F10 integration for maximized immobilization efficiency

- Glass slides are treated overnight with a piranha solution (1:3 H_2O_2/H_2SO_4) for surface activation.
- Activated slides are silanized by gentle shaking in 2% (3-aminopropyl)triethoxysilane (APTES) in acetone for 2 minutes, rinsed twice with acetone and distilled water, and cured on a hot plate at 110°C for 45 minutes to ensure complete solvent removal.
- To introduce biotin to the surface of the slide, the amino-functionalized surface is modified with biotin-$(PEG)_{11}$-COOH, which is mixed with a reaction solution of EDC and NHS in MES buffer (0.3 M EDC and 0.36 M NHS in 0.1 M MES buffer pH 6.0) and incubated for 30 minutes. The resulting solution is applied to the slides and incubated at 5°C in a humid environment for 1.5 hours, followed by rinsing with distilled water and drying with N_2 gas.
- Immediately before use of the slide, a streptavidin solution (10 µg/mL) diluted in 0.4% (w/v) **SophoMer F10** in PBS is applied to the surface of slide and incubated at 5°C in a humid environment for 45 minutes. The slide is then rinsed and dried with N_2 gas.

- The slide is further modified with a solution of biotinylated anti-human CD9 antibody (50 µg/mL) diluted in 0.4% (w/v) **SophoMer F10** in PBS, incubated at 5°C in a humid environment for 1.5 hours, followed by rinsing and drying with N_2 gas.
- To further prevent nonspecific interaction, the glass is treated with 0.4% (w/v) **SophoMer F10** in PBS and incubated at 5°C for 45 minutes in a humid environment, then rinsed and dried with N_2 gas.
- The sample of DiOC6 stained exosomes isolated from MCF-7 (HTB-22, ATCC) cell line, is diluted in 0.4% (w/v) **SophoMer F10** in PBS. The sample is introduced to the glass slide and incubated at room temperature for 1 hour in a humid environment.
- The glass slide is dipped to the large volume of PBS (2 liters) to wash away not bound exosomes from the surface of the slide. The slide with exosomes specifically bound to the surface of the slide can be observed under fluorescent microscope.

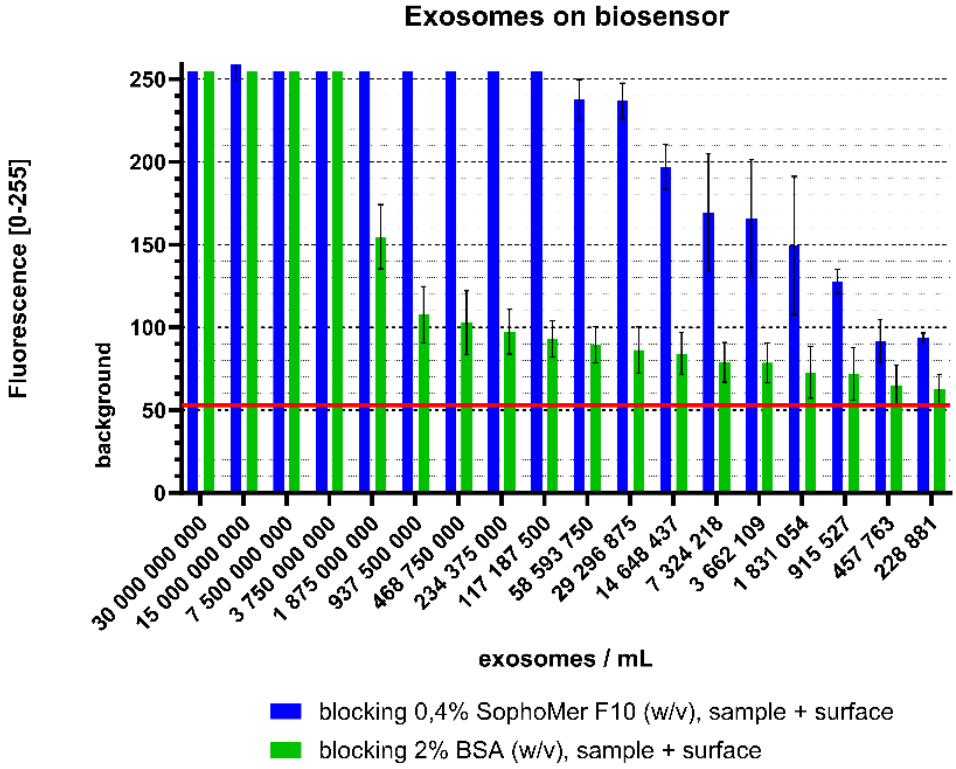

Fig. 1: Sophomer F10 was found to enhance exosome binding to the biochip surface in comparison to the BSA. Exosomes with added **Sophomer F10** was able to bind to the chips surface, crafted with the use of **Sophomer F10**, with much greater efficacy even at lower concentrations of exosomes in sample in comparison to the case where BSA was used**.**

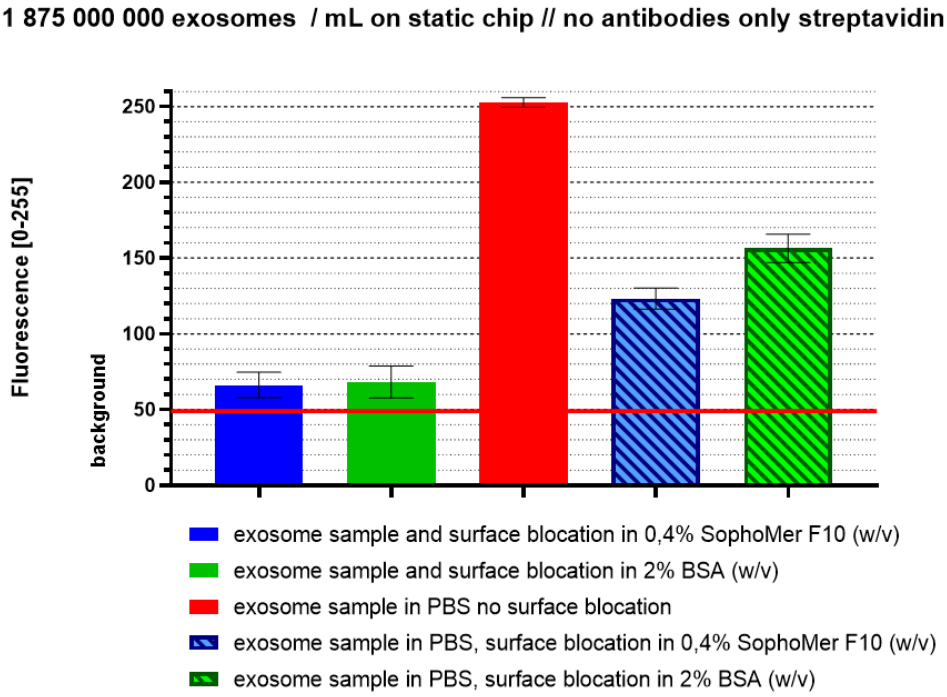

Fig. 2: Sophomer F10 lowers number of nonspecific interactions, compared to BSA. Exosomes samples has lower number of nonspecific interactions with chip surface crafted with the **Sophomer F10** when compared to surfaces crafted using BSA. Addition of **Sophomer F10** directly to the sample further lower the number of nonspecific interactions.

Conclusions

The **SophoMer F10** blocking agent demonstrates superior performance over traditional use of BSA, particularly in terms of exosome immobilization efficiency and nonspecific binding reduction. When BSA is used, the percentage of exosomes successfully immobilized on the chip surface declines rapidly, even in samples with high exosome concentrations. This effect may be due to steric shielding or insufficient surface compatibility, which limits BSA's effectiveness in maintaining stable exosome binding. In contrast, **SophoMer F10** supports a consistently high capture efficiency, even in samples with low exosome counts, by providing a surface environment that enhances specific interactions with minimal steric interference. Additionally, F10 significantly reduces nonspecific interactions, resulting in cleaner detection signals and improved overall assay sensitivity. F10, as a polymer, exhibits greater flexibility and adaptability to the biosensor's surface contours, creating a tighter, more consistent passivation layer. In contrast, BSA's rigid protein structure may leave gaps or inconsistencies in coverage, increasing the likelihood of nonspecific interactions. The use of F10 thus ensures more reliable exosome capture, even at low analyte concentrations, making it an ideal choice for high-precision biosensor applications in the field of exosomes immobilization.

For more information about SophoMer F10, please contact:

Sophomer
Rádiová 1285/7
Prague 10, Czech Republic
Phone: +420 725 435 791
Email: info@sophomer.com
Website: https://www.sophomer.com/

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Experiments were performed at the Centre for Nanomaterials and Biotechnology, Faculty of Science, Jan Evangelista Purkyně University in Ústí nad Labem, 400 96, Ústí nad Labem, Czech Republic., under the supervision of Jiří Smejkal, Ph.D.