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Tackling CD147 exosome-based cell-cell signaling by electrochemical biosensing for early colorectal cancer detection

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ABSTRACT

The great opportunities represented by exosomes in liquid biopsy diagnostics and the relevance of CD147 protein as diagnostic and prognostic cancer biomarker led us to develop the first bio-electroanalytical platform for the determination of exosomal CD147 (exoCD147) by exploiting micro-sized magnetic beads coated with specific anti-CD147 antibodies. The captured exosomal target protein was sandwiched by specific biotin functionalized detector antibodies followed by attaching streptavidin-HRP conjugate to perform the amperometric reading using screen-printed carbon electrodes (SPCEs) as electrode transducers in the presence of hydroquinone (HQ) and H_2O_2. The analytical and operational characteristics achieved by implementing this simple methodology allowed the sensitive (LOD 29 pg mL^{-1}) and selective determination of CD147 and the analysis of exoCD147 in different but inter-related real clinical scenarios including lysed and entire exosomes previously isolated from CRC cell lines with different metastatic potential. The obtained results, in agreement with those provided by ELISA and WB, proved the reliability of the developed immunosensor and its potential to isolate or identify specific subpopulations of exosomes based on the differential expression of characteristic surface biomarkers.

1. Introduction

Time after being considered as carriers of cellular debris (Johnstone, 1992), the smallest extracellular vesicles (EVs), known as exosomes, are recognized as pivotal parts of many cellular processes (Gurung et al., 2021), and particularly relevant in cancer research (Umwali et al., 2021). These naturally secreted nanometer-sized EVs are observed in a wide spectrum of biofluids (Zhao et al., 2021). Exosomes carry substantial bioactive cargoes able to transfer protein, lipidic, and genetic material and reveal cells' metabolic condition (Xiao et al., 2020). Besides the vast biological information niched into and onto exosomes' surfaces, the relevance of their analysis relies on the differential changes that the contained biomolecules can suffer during disease progression (Rajagopal and Harikumar, 2018), extremely useful to decipher the course of the disease and the response to a specific applied treatment.

Main bio-components of exosomes include CD9, CD63, CD81, and CD82 tetraspanins (Théry et al., 2002), a broad array of enzymes, heat-shock proteins and proteins involved in signal transduction (Pegtel and Gould, 2019), and all types of RNA and DNA (Wei et al., 2017). Attending to the huge biological information that can be extracted from these vesicles at different omics levels, exosomes play a meaningful role as unequivocal physiologically and pathologically 'bio-meters' to be accepted as potential tools for clinical application. Exosome surface profiling at the protein level provides crucial information, discriminating disease-related subtypes, and predicting and determining metastasis progression, and future organ invasions (Tian et al., 2018). Among exosomal biological molecules contained both at their inner and surface location, membrane proteins are some of the most predominantly expressed (Malla et al., 2018), especially those belonging to the tetraspanin superfamily (Kumari et al., 2015). The cluster of

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differentiation 147 (CD147), also known as extracellular matrix metalloproteinase inducer (EMMPRIN), has been described as one of the highly expressed EVs-contained molecular substances (Chang et al., 2021), playing a key role in tumour invasion and progression of different cancers (Himbert et al., 2020), including colorectal cancer (CRC) (Xu et al., 2014). Colon cancer stem cells differentiation is partially controlled by CD147, acting as a potential surrogate biomarker whose high expression is directly associated with poor survival in cancers (Fan et al., 2017). Additionally, the combination of serum CD147 and matrix metalloproteinase 9 (MMP-9) has been successfully employed for evaluating chemotherapy outcomes in non-small-cell lung cancer (NSCLC) patients (Qiao et al., 2020). Given that exosomes are considered as faithful biological vehicles to specifically decipher altered health status (Hanjani et al., 2022), and that CD147 is a promising cancer-related biomarker, the detection of exosomal CD147 is worth to be explored.

Prevailing methodologies for exosome and exosomal-derived entities analysis include immunoblotting and enzyme-linked immunosorbent assay (ELISA), although not advised in most clinical settings (Yoshioka et al., 2014). Biosensors as fast, reliable, and precise analytical tools that have been extensively employed in the medical and biological field, blazed the trail over the past few years in exosomes' detection and quantification (Xu et al., 2020). Optical, electrochemical, and electrical-based modes are considered the leader biosensing mechanisms for exosome interrogation (Chia et al., 2017). Tian et al., developed a quantitative multiparameter analysis of single EVs using high-sensitivity flow cytometry (HSFCM) that was applied to the analysis of exosomal CD147 isolated from HCT15 and HCT116 CRC cell lines upon fluorescent staining with phycoerythrin (PE)-conjugated antibody, reaching a limit of detection (LOD) of 1.2×10^5 exosomes μL^{-1} (Tian et al., 2018). The so-called ExoCounter integrated an optical disc coated with specific antibodies against the target exoprotein and used affinity magnetic nanobeads as labelling-detection units for detecting CD147 in different cancer cells- and human blood-derived exosomes (Kabe et al., 2018). Regarding electrochemical transduction-based devices, voltammetry, amperometry, potentiometry and impedance have been employed as detection modes for exosome interrogation in biofluids such as serum (Boriachek et al., 2017), plasma (Jeong et al., 2016), blood (Kilic et al., 2018), fetal bovine serum (Dong et al., 2018), and urine (Park et al., 2017), by detecting CD63, α-CD9, CD81, and CD3 as main exosomal biomarkers. The use of nanomaterials in the preparation of electrochemical biosensors such as quantum dots (Boriachek et al., 2017) and Ag- and Cu-nanoparticles (Zhou et al., 2016), nucleic acid-based amplification strategies (Dong et al., 2018), and DNA-based nanostructures (Wang et al., 2017), as well as their integration in advanced platforms for sample manipulation, such as microfluidic chips (Kashefi-Kheyrabadi et al., 2021) and miniaturized multiplexed systems (Jeong et al., 2016), provide great advantages for exosome direction, enrichment, manipulation, and detection. The use of aptamers as bio-recognizers in electrochemical sensing devices being more frequent than that of specific immunoglobulins, antibody-based strategies for intact exosome and exosomal-derived cargoes is a research field to be explored. Despite the amount of electrochemical biosensing strategies that have been successfully developed until now for the analysis of these attractive vesicles, surprisingly, none of them has been employed for the interrogation of exoCD147. Accordingly, we have developed a simple electrochemical strategy using antibodies as bio-recognizers for the quantification and, in a trailblazer manner, for the direct detection of the yet poorly explored but critically relevant CD147 marker in lysed and entire exosomes previously isolated from different colon cancer cells.

2. Materials and methods

Apparatus and electrodes, Reagents and solutions, Colorectal cancer cell lines, and all the procedures used (Exosome isolation and purification, Protein extraction, Western-blot analysis, Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM), Assembly of sandwich-based immunocomplexes onto magnetic microbeads and Amperometric readout) are described in detail in the Supporting Information (SI).

3. Results and discussion

The strategy reported here (Scheme 1) relies on sandwich-type immunocomplexes sequentially prepared onto the surface of carboxylate-functionalized magnetic beads (HO_2C-MBs) as suitable carriers at the microscale level. Once specific anti-CD147 antibodies were covalently attached onto the HO_2C-MBs via carbodiimide/succinimide chemistry, target CD147 was efficiently captured from the corresponding sample and subsequently sandwiched and labelled with biotin-functionalized detector anti-CD147 antibodies and streptavidin-HRP conjugate (Strep-HRP), respectively. Signal transduction was carried out by amperometry in the presence of hydroquinone (HQ, redox mediator) and H_2O_2 (enzymatic substrate), giving rise to a variation in the cathodic current that, according to the immunosensing strategy, was directly proportional to the concentration of CD147 in the sample.

3.1. Optimization of experimental variables

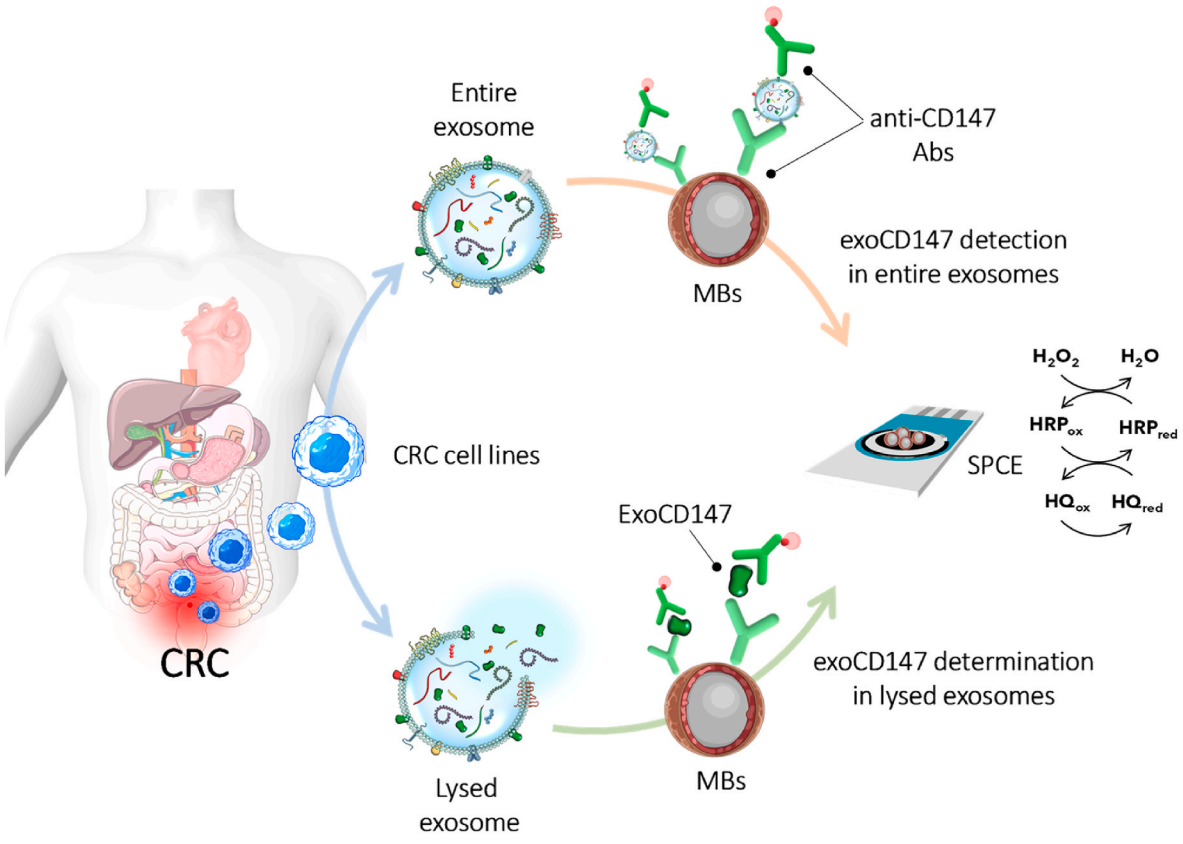
The working variables affecting the performance of the proposed immunosensing strategy were optimized by selecting the largest signal-to-blank ratio (S/B) reached in the presence (S) and in the absence (B) of 1000 pg mL^{-1} CD147 standard. The checked variables, tested ranges, and selected variable values, are summarized in Table S1, shown in Fig. S1 and critically discussed in the SI.

3.2. Analytical characteristics

Under the optimized conditions, Fig. 1a displays the relationship between the amperometric responses obtained with the developed strategy and the CD147 concentration. A linear behaviour ($r^2 = 0.998$) was observed over the 0.096–5.0 ng mL^{-1} range, fitting to the following equation: i, $\mu A = (0.50 \pm 0.02)$ [CD147], ng $mL^{-1} + (0.10 \pm 0.03)$. Real amperometric readings are depicted in Fig. 1b.

The LOD and limit of quantification (LQ), 29 and 96 pg mL^{-1} CD147, respectively, were calculated according to the $3 \times s_b/m$ and $10 \times s_b/m$ criteria with s_b as the standard deviation of 10 measurements performed in the absence of CD147, and m as the slope of the calibration graph (inset in Fig. 1a). These values are significantly lower than the CD147 cut-off values established in serum to discriminate hepatocellular and pancreatic cancers from healthy individuals (>1.0 ng mL^{-1} (Wu et al., 2014)) and adequate to perform discrimination in serum samples of CRC patients ($(1.0 \pm 0.4) \times 10^2$ pg mL^{-1}) from controls ($(0.6 \pm 0.2) \times 10^2$ pg mL^{-1}) (Xiong et al., 2008). Nevertheless, since exosome biological cargos are enriched, exoCD147 content is expected to be considerably larger than that found for free serum CD147.

Importantly, the immunoplatform allowed a short analysis time (45 min after the covalent coupling of the specific capture antibodies and the CAb-MB blocking step), and a good reproducibility of the measurements for 1.0 ng mL^{-1} CD147 with a relative standard deviation (RSD) value of 6.1%, calculated using eight different immunoplatforms prepared in the same way. Additionally, the stability of CAb-MBs immunoconjugates stored in 50 μL of filtered PBS at 4 °C was checked. A control graph (Fig. S2 in the SI) was constructed by comparing the S/B ratios obtained with the immunoplatforms prepared using the stored CAb-MBs in the absence and in the presence of 1000 pg mL^{-1} CD147 for each control day. The average value of these ratios measured with three different immunoplatforms the first day of the study was set as the central value and ±3 s of this value as the upper and lower control limits. As can be seen, the stored CAb-MB immunoconjugates provided S/B ratios within control limits for 7 days, which make it possible to prepare the immunoconjugates in advance and store them until required for preparing the immunoplatforms.



Scheme 1. Schematic display of the implementation of the proposed amperometric sandwich-based immunosensing strategy for the determination of CD147 in entire (upper route) or lysed (down route) CRC isolated exosomes.

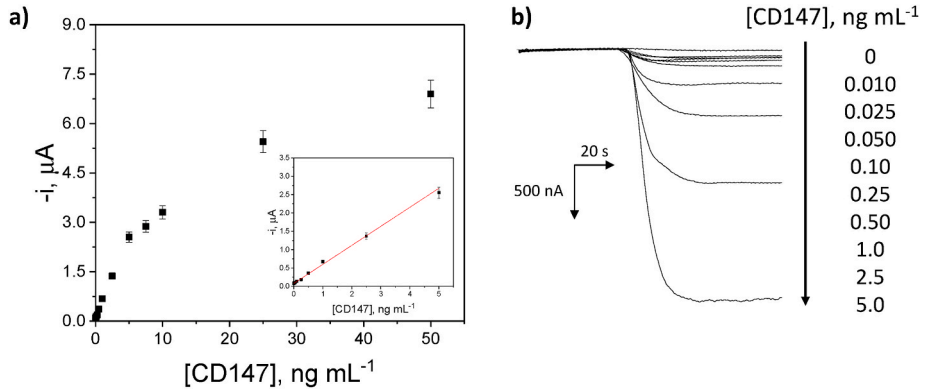


Fig. 1. Dependence of the amperometric responses with the concentration of CD147 standards prepared in buffered solution (a) and amperometric traces (b) obtained with the developed immunoplatform under optimized working conditions.

The obtained results were compared with those claimed for the only electrochemical biosensor for CD147 reported so far (Zheng et al., 2013). This biosensor used a sandwich configuration constructed onto the surface of 4-aminothiophenol and AuNPs-modified gold electrodes. Compared to the work of Zheng et al. who used AuNPs both for binding specific anti-CD147 antibodies and for amplifying the cyclic voltammetry signal, the MBs-based strategy provides a lower LOD (29 *vs.* 52 pg mL^{-1}), a wider linear range (0.096–5.0 *vs.* 0.1–1.0 ng mL^{-1}), and a significantly shorter preparation time (45 *vs.* 180 min), i.e. it provides important advantages in terms of sensitivity, preparation time and real-world applicability. All these are essential features for biosensing analytical tools used for the interrogation of biomarkers related to highly prevalent deadly diseases, such as CD147. Furthermore, as will be discussed below, the developed immunoplatform was applied to the determination of CD147 in whole exosomes and lysates from different CRC cell lines, two very different but inter-related sample scenarios that have never been explored before for interrogating the biomarker CD147. Indeed, the immunosensor by Zheng et al. was applied to its determination in cancer cells, not exosomes. On the other hand, when compared with commercially available ELISA kits for CD147 detection, some of them provide lower LODs (1.7 pg mL^{-1} https://www.abcam.com/mouse-emmprin-elisa-kit-cd147-ab205575.html, 3.45 pg mL^{-1} https://corelab.creative-biogene.com/cd147-elisa-kit-c-el-1726t-item-6747.htm, 12 pg mL^{-1} https://www.thermofisher.com/elisa/product/Human-CD147-EMMPRIN-ELISA-Kit/EH78RB). However, ELISA methods show well-known important limitations from a point-of-care (POC) perspective, including high cost and time of testing and low compatibility with miniaturized and/or portable manufacture.

3.3. Selectivity

The selectivity of the immunoplatform was assessed by contrasting the S/B ratios obtained for CD147 standards prepared in the absence and in the presence of some potential interfering substances that can coexist with the target protein in some types of samples. The amperometric responses obtained for 0.0 and 1000 pg mL^{-1} CD147 solutions supplemented with Hb, IgG, HSA, TNFα, CDH17, E-cad or HIF-1α at the concentrations indicated on the x-axis are compared in Fig. 2. As it is apparent, only the presence of HSA notably affected the S/B ratio, which is attributed to the coexistence of human anti-animal antibodies (HAAAs) in non-overly purified HSA (Muñoz-San Martín et al., 2022). However, this interference, which would only be a problem in the analysis of serum samples, was minimized upon sample dilution.

Moreover, according to the ELISA kit (DuoSet®, DY972, R&D Systems Europe) specifications, the immunoreagents used to construct the proposed immunoplatform do not show cross-reactivity against other non-target proteins, including CYPA, L1CAM/Fc Chimera, MMP-1, MMP-2, MMP-3, MMP-9, uPA and VEGF.

3.4. Detection and quantification of CD147 target protein in lysed and entire exosomes

The developed sandwich-based immunoplatform was applied to the detection and quantification of CD147 target protein in both lysed and entire exosomes previously isolated from different CRC cells with different metastatic potential.

The sample dilution factor and the suitable buffer for re-suspending the exosomes isolated from SW480 CRC cells (as a model) through ultracentrifugation (Section "Exosome isolation and purification" in the SI) were evaluated. Thus, bare MBs and CAb-MBs were incubated with SW480 exosomes suspensions prepared using different dilutions and media and subsequently labelled (Section "Assembly of sandwich-based immunocomplexes onto magnetic microbeads" in the SI). The specific (CAb) and non-specific (No CAb) amperometric signals obtained with the developed immunoplatform were compared (Fig. S3 in the SI). Regarding the exosome dilution factor (Fig. S3a), the CAb/No CAb ratio increased with increasing exosome concentration (or decreasing dilution) with a larger CAb/No CAb ratio observed for a 100-fold dilution. Lower dilutions resulted in large No CAb signals, attributed to high non-specific adsorption of exosomes onto the surface of unmodified MBs.

Furthermore, we tested how much different buffered media affected the specific/non-specific amperometric signals for 100-fold diluted SW480 derived exosomes. As shown in Fig. S3b, large non-specific adsorption onto bare MBs was observed when exosomes were diluted in PFBB and 1% BSA. On the contrary, the use of BB and SP buffers produced a substantial decrease in the non-specific adsorption of diluted exosomes onto the surface of unmodified MBs. The better specific-to-non-specific (CAb/No CAb) ratio obtained when using SP, led us to use this buffer medium for preparation of entire exosomes and exosomes lysates.

In addition, to demonstrate the reliability of the approach, 100-fold diluted isolated SW480 exosomes non-incubated and incubated with bare MBs and CAb-MBs were characterized using Dynamic Light Scattering (DLS), TEM and Nanosight (Fig. 3, and S4 in the SI).

The DLS results (Fig. 3a) showed only one peak corresponding to the size of the distribution for each of the four samples. The peak size was $(3.1 \pm 0.7) \times 10^2$ nm for SW480 derived exosomes whereas for bare MBs and the mixture of bare MBs and SW480 exosomes was $(1.6 \pm 0.1) \times 10^3$ nm, thus indicating that not effective capture of exosomes by bare MBs occurred. However, when CAb-MBs were incubated with 100-fold diluted target exosomes, the peak size was significantly shifted to larger values, $(2.7 \pm 0.5) \times 10^3$ nm, which proved the specific and efficient capture of exoCD147 by CAb-MBs immunoconjugates.

In addition, purified exosomes isolated by ultracentrifugation (Fig. 3b) or by using anti-CD147-coated MBs (Fig. 3c) were analysed by TEM to visualize the efficiency of exosome tethering. In both cases isolated exosomes showed a narrow size distribution (approximately 100–150 nm), that agreed with previously reported results (Yang et al., 2021). Strong similarities related to morphology were observed and distributed as single nanometric structures onto the surface of CAb-MBs, thus proving successful binding of exoCD147 by CAb-MBs conjugates. The low concentration observed for exosomes attached onto the CAb-MBs can be attributed to the 100-fold dilution of the exosomes extract where they are incubated. However, crude isolated exosomes obtained by ultracentrifugation were used to obtain the micrographs shown in Fig. 3b.

Nanosight and TEM micrographs obtained for SW620, KM12C, KM12SM, and KM12L4a CRC cells crude exosomes isolated by ultracentrifugation are shown in Fig. S4 in the SI. Differences observed in exosome size between Nanosight and DLS techniques were associated to sample polydispersity.

Quantification of CD147 in exosomes lysates was carried out by using 0.1 μg of the corresponding exosome lysate. Importantly, the obtained amperometric signals for all CRC derived exo-lysates fitted within the linear range of the calibration plot constructed for CD147 standards. In addition, no apparent matrix effect was observed when 0.1 μg of lysate was employed since the calibration slope value in buffered solution ((0.50 ± 0.02) nA mL pg^{-1}) was statistically similar to the slope value of the calibration constructed from 0.1 μg of SW480 exosome extract spiked with increasing concentrations of CD147 from 500 to 2500 pg mL^{-1} ((0.49 ± 0.16) nA mL pg^{-1}; $t_{exp} = 0.0319$ *vs.* $t_{tab} = 2.447$).

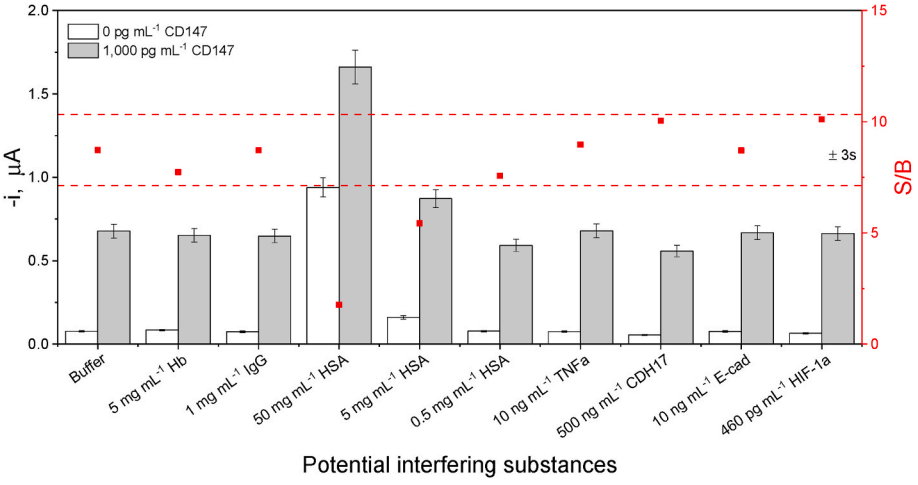
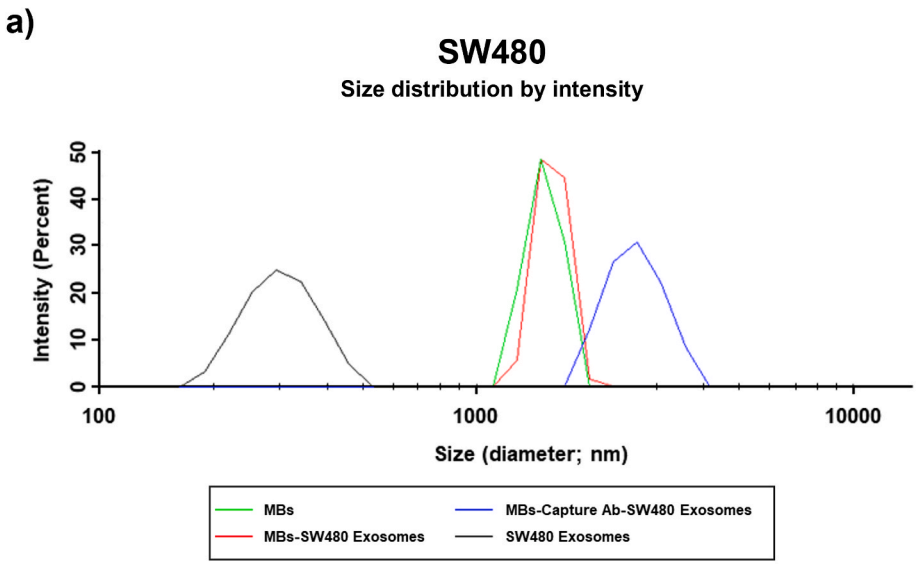


Fig. 2. Selectivity of the developed immunoplatform. S/B ratio values (in red squares) obtained with the immunoplatforms for 0 (B, white bars) and 1000 pg mL^{-1} CD147 standards (S, grey bars) prepared in the absence (buffer) and in the presence of potential interfering substances at the indicated concentration level. Upper and lower control limits (red dashed lines) were set as ± 3 s of the average value in the absence of any potential interference (n = 3). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



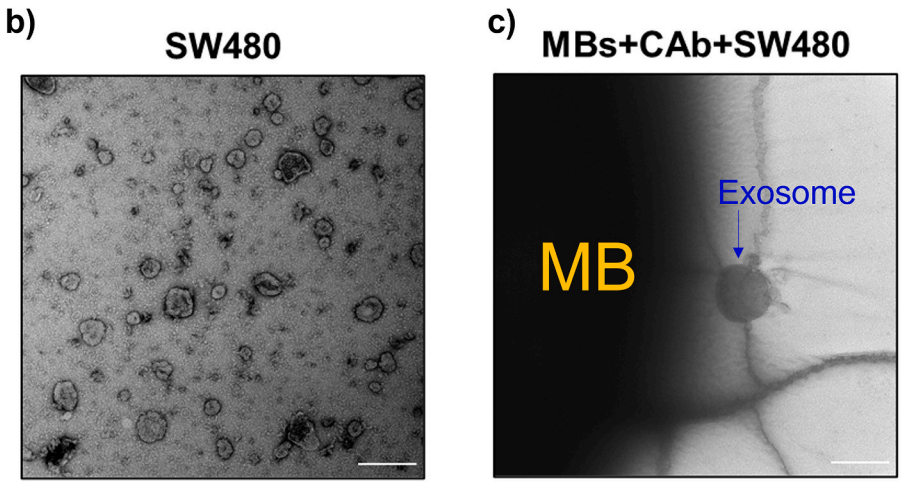


Fig. 3. Characterization of isolated SW480 exosomes by DLS (a) and TEM (b, c). DLS obtained for 100-fold diluted SW480 exosomes (black curve), unmodified MBs (green curve), and for exosomes incubated with bare MBs (red curve) and anti-CD147-MBs immunoconjugates (blue curve). TEM micrographs of SW480 exosomes isolated by ultracentrifugation (b) and by the anti-CD147-MBs immunoconjugates (after 100-fold dilution of the exosome suspension) (c). Scale bar 200 nm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

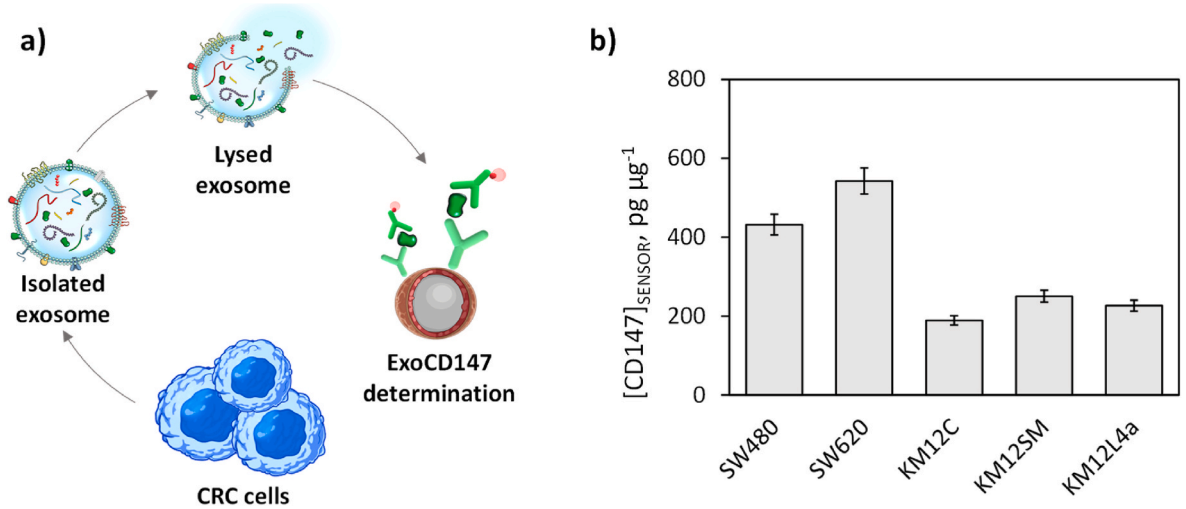


Fig. 4. Illustration of the general methodology for the exoCD147 determination in lysed exosomes isolated from CRC cells with different metastatic potential (a) and CD147 concentration (in pg μg^{-1}) determined with the developed amperometric immunoplatform in 0.1 µg of the corresponding exo-lysate from the different CRC cell lines (b).

Accordingly, the determination of exoCD147 in previously isolated and lysed exosomes from KM12C, KM12SM, KM12L4a, SW480 and SW620 CRC cells (procedure schematized in Fig. 4a) was carried out by direct interpolation of the measured amperometric signals into the calibration plot constructed with CD147 standards. The concentration levels of exoCD147 found in each analysed exosome lysate are given in Fig. 4b. The CD147 content in SW480 and SW620 exosome lysates agree with results previously reported (Xu et al., 2014) for the analysis of this exosomal target protein in different CRC cells, where the highest CD147 expression was observed in exosome lysates from SW480 and SW620 CRC cells, thus confirming the correlation existing between CD147 expression and lymph node metastasis, as well as its role as diagnosis and prognosis CRC biomarker.

Moreover, the results obtained with the amperometric immunoplatform in different exosomal lysates were compared with those provided by ELISA using the same immunoreagents. As shown in Fig. S5 and Table S2 (SI), a good correlation was reached with both methodologies, thus confirming that the developed immunoplatform can be exploited for the determination of exosomal CD147 with a reliability and accuracy similar to that of the commonly accepted ELISA method but in a simpler, faster and affordable manner, compatible with its use at the point of care.

On the other hand, CD147 was also expressed in exosome lysates from KM12C, KM12SM, and KM12L4a CRC isogenic cells. As it is apparent, a lower CD147 concentration was found for the non-metastatic KM12C CRC cells, confirming once again the direct involvement of CD147 in tumour progression, invasion, and metastasis. These results also agreed with those obtained by Western-blot (WB, Fig. S6 in the SI) which reflected the same differential expression pattern of CD147 in the exosome extracts of the five CRC cell lines although they are only semi-quantitative and required 1.0 μg of the exo-lysates.

The immunoplatform was also used for the direct detection of exosomal CD147 in entire exosomes isolated from the same CRC cells. As schematized in Fig. 5a, isolated exosomes were conveniently diluted and incubated with bare and CAb-MBs for the subsequent detection and enzymatic labelling with bDAb and Strep-HRP conjugate, respectively. The amperometric readouts obtained with the immunoplatform for the exoCD147 detection in entire SW620 and SW480 isolated exosomes are displayed in Fig. 5b. These results confirmed that the target protein can be successfully captured, detected, and enzymatically labelled directly onto the surface of entire exosomes. However, no significant differences between the use of bare and CAb-MBs were observed for exosomes isolated from KM12L4a, KM12SM, and KM12C CRC cells (Fig. S7 in the SI), which agrees with the relatively lower CD147 content found in exo-lysates from these CRC cells, compared with the isogenic SW480 and SW620 CRC cells.

These preliminary results are particularly interesting because they demonstrate the potential of the immunoplatform to isolate and identify subpopulations of exosomes by interrogating characteristic exosomal markers, such as CD147, in a method simpler and faster than those implied with other conventional methodologies. This may contribute to consolidate the potential of exosomes, whose analysis requires their prior enrichment and fractionation, as attractive sources of multiple and multi-omic target biomarkers for clinical diagnostics (Oskvold et al., 2015).

4. Conclusions

In this work, a simple amperometric sandwich-based immunoplatform suitable for the sensitive determination of CD147 in lysed and entire exosomes isolated from CRC cell lines with different metastatic capacity is reported for the first time. The proposed strategy relies on the covalent attachment of specific CD147 capture antibodies onto the surface of commercially available carboxylate magnetic microbeads and the use of biotinylated antibodies and streptavidin-HRP conjugates as detectors and enzymatic tracers, respectively, to perform the amperometric transduction at SPCEs. The excellent analytical characteristics reached allowed its applicability to the accurate analysis of CD147 in two interrelated sample scenarios of high complexity: lysed and entire exosomes isolated from several CRC cell lines. The obtained results agree with those provided by the long-adopted ELISA and WB methodologies, as well as with those reported by other authors using significantly more complex and labour-intensive methodologies.

These results demonstrate the duality of application offered by the bioplatform both to determine exosomal biomarkers and to identify and isolate subpopulations of exosomes by taking advantage of the dysregulation of characteristic biomarkers on their surface. Future efforts should focus on testing these bioplatforms on a larger number of samples and demonstrating their versatility for the simulated determination of exosomal biomarkers of the same or different omics level also trying to further minimize the nonspecific adsorption of these vesicles on unmodified MBs to achieve higher discriminations and capture efficiencies

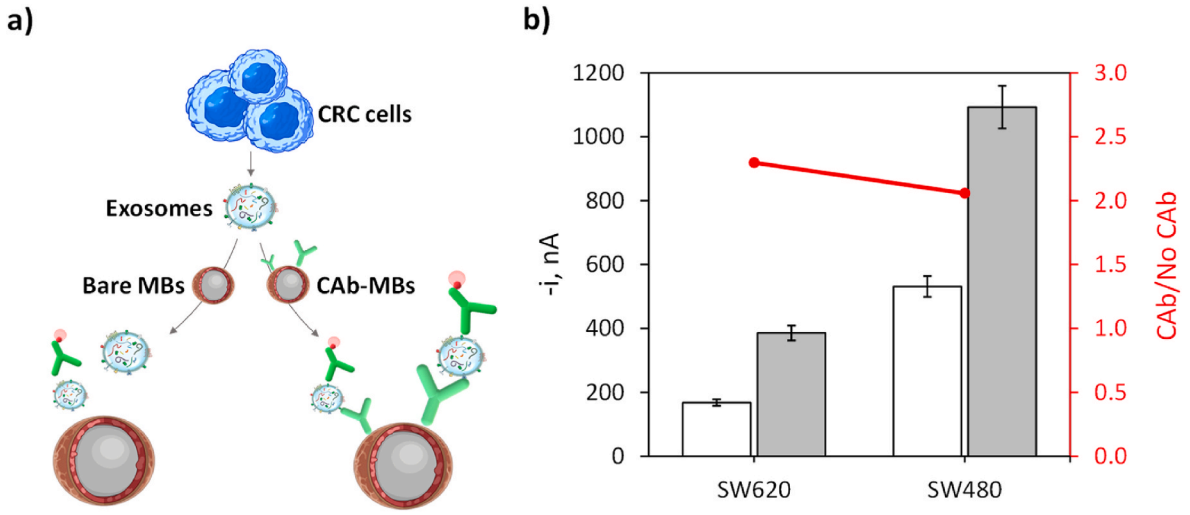


Fig. 5. Illustration of the general methodology for the detection of exoCD147 in entire exosomes isolated from CRC cells (a) and amperometric responses obtained with the developed immunoplatform after incubation and subsequent enzymatic labelling of the corresponding 100-fold diluted isolated exosomes with bare MBs (white bars) and CAb-MBs immunoconjugates (grey bars). Ratio (CAb/No CAb) between the amperometric signals obtained after incubation of exosome sample onto CAb-MBs and bare MBs is represented as red line. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

for subpopulations of interest. Despite their preliminary character, the obtained results are an important contribution to highlight the potential of exosomes and liquid biopsies and electrochemical biosensors to approach a personalized precision medicine of decentralized character and accessible to all.

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CRediT authorship contribution statement

Víctor Pérez-Ginés: Methodology, Investigation, Writing – review & editing, Writing – original draft. **Rebeca M. Torrente-Rodríguez:** Methodology, Investigation, Writing – review & editing, Writing – original draft. **Ana Montero-Calle:** Investigation, Resources, Writing – review & editing, Writing – original draft. **Guillermo Solís-Fernández:** Methodology, Investigation, Writing – review & editing, Writing – original draft. **Pablo Atance-Gómez:** Methodology, Investigation. **María Pedrero:** Conceptualization, Supervision, Resources, Writing – review & editing, Writing – original draft. **José M. Pingarrón:** Supervision, Resources, Writing – review & editing, Writing – original draft. **Rodrigo Barderas:** Conceptualization, Supervision, Resources, Writing – review & editing, Writing – original draft, Funding acquisition. **Susana Campuzano:** Conceptualization, Supervision, Resources, Writing – review & editing, Writing – original draft, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biosx.2022.100192.

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