EV-protein corona and EV surface engineering, a first study

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One strategy to improve extracellular vesicle (EV) targeting properties for drug delivery includes EV surface engineering by adding tissue-specific protein ligands. On the other hand, it has recently been acknowledged that proteins might physically adsorb on the EV surface, forming a protein corona (PC). While PC's role and composition have started to be investigated for EVs in biofluids, its formation during EV engineering is still underrated. In our contribution, we will present the first results of our findings on this aspect. Two sets of red blood cell (RBC)-EVs were used. Both sets were exposed to a DBCO-modified monoclonal antibody (Cetuximab, CTX) to obtain surface cloaked EVs with physisorbed and chemisorbed proteins with and without the clickable azido group for covalent linkage, respectively. The two sets' molecular recognition and uptake abilities were evaluated by surface plasmon resonance spectroscopy and cellular assays. The EV sets showed a corona made of a comparable amount of CTX, with the corona prepared by the covalent route mainly composed (of 75%) of covalently bound CTX. Surprisingly, the EV set functionalized by CTX physisorption showed the same affinity for EGFR (the CTX target ligand) as the EV set functionalized by covalently bound CTX. On the other hand, only the covalently functionalized EVs showed improved cellular uptake compared to the native EVs. In conclusion, the RBC-EVs can be functionalized by CTX physisorbed onto the EV surface for molecular recognition, but covalent bonding is required for improving EV cellular uptake. The EV-PC has a role and should be considered in EV surface engineering.