Lipid bilayer fluidity and degree of order regulates small EVs adsorption on model cell membrane

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Small extracellular vesicles (sEVs, 30-200 nm in diameter) are known to play a pivotal role in the communication between distant cells by delivering biological information throughout the body. To date, many studies have focused on the role of sEVs characteristics such as cell origin, surface composition, and molecular cargo on the resulting uptake by the recipient cell. Yet, a full understanding of the sEV fusion process with recipient cells and in particular the role of cell membrane physical properties on the uptake are still lacking. To tackle this issue, here we isolated sEVs from a cellular model oftriple-negative breast cancer and explored their fusion pathway to a range of synthetic planar lipid bilayers featuring a variable percentage of cholesterol, and designed to mimic the formation of 'raft'-like nanodomains in plasma cell membranes. Using time-resolved Atomic Force Microscopy we were able to track the sEVs interaction with the different model membranes, showing the process to be strongly dependent on the local membrane fluidity. The strongest interaction and fusion is observed over the less fluid regions, with sEVs even able to disrupt ordered domains at sufficiently high cholesterol concentration. Our findings suggest the biophysical characteristics of recipient cell membranes to be crucial for sEVs uptake regulation. Our versatile platform can be extended to feature receptor binders within the sEVs membrane to screen, for instance, drugs designed to block the binding/cell entry of fusogenic proteins of enveloped viruses, as for the spike protein of SARS-CoV-2.