Maha Al-Jumaa et al., Madridge J Vaccines. 2017
http://dx.doi.org/10.18689/2638-1966.a1.006

madridge CONFERENCES

2nd International Conference and Expo on
Vaccines and Vaccination

November 20-21, 2017 Dubai, UAE

Sub-Domain Frap of Cell Surface Molecules to Monitor Cell Surface Topography in Living Cells

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It is becoming increasingly clear that many cells have a reservoir of excess plasma membrane in the form of cell surface wrinkles and micro-ridges. The released of these surface structures allow cells to change shape rapidly by permitting an apparent increase in cell surface area eg during phagocytosis or cell spreading. Although indirect methods point to this conclusion, it is only possible to visualise the cell surface topography under scanning electron microscopy and therefore cannot be used to follow surface topography changes to be monitored in living cells during changes in cell morphology. In this abstract, we describe a novel methodological approach which allows this using the measurement of fluorescence recovery after photo bleaching in sub domain at defined 2 dimension distances from the bleach front. Assuming that the diffusion of the fluorescent molecules is constant to rate of recovery at defined points reflects the diffusion distance and hence indicates the surface topography between the bleach front and the measurement domain.

Human neutrophils were isolated from blood and there plasma membrane labelled with DiI. The dye can be excited using a 543nm laser line and effectively photobleached using the 488nm line transiently elevated. A photo-bleaching zone across the region of the cell of interest was chosen and the image of the cell recording during bleaching and recovery. The bleach/recovery cycle could be repeated.

This approach was showed to reflect the cell surface topography by experimentally increasing the wrinkledness of the cell surface using hyperosmotic media. The measured apparent diffusion length increased in the increased wrinkled state and was returned to close to its pre-shrunk value by restoration of isotonicity. The methodology showed that whereas the body of neutrophils has significant ridge-like topographical features, the phagosome and the extending pseudopodia are smooth. This method also showed that elevation of cytosolic Ca^{2+} (by uncaging IP3) influenced the cell surface topography.

This methodology opens a novel way of monitoring an important cell characteristic which has not be possible to study previously.