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High-spatial resolved detection of focal adhesion of single living cells by dipole plasmonic nanoantennas

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1. Introduction and Objectives

Localized Surface Plasmon Resonance (LSPR) shows a high sensitivity spectral response able to detect small changes at local refractive index. Here, we propose a label-free methodology for the detection of cell/substrate interaction using gold plasmonic nanoantennas. Due to the recent technological advances on the nanofabrication process, it has become possible to fabricate with high repeatability a large number of these metallic nanostructures. The design consists of large area arrays (40 x 40 units) of gold features located on a glass substrate with antenna's arm length from 40 to 120 nm and gap size of 20nm.

Cell adhesion can be promoted by biofunctionalization of the metallic surfaces by using different ligands recognized by integrins. Expression of integrins on living cells is associated with the formation of focal adhesion (FA) contacts to the Extracellular Matrix (ECM) [1]. In this study a modified cRGD peptide is used for the functionalization of gold (Au) nanostructures and promotion attachment of melanoma cell by means of linking to integrin αvβ3.

Although previous studies have approached the use of single nanoparticles in living cells, no studies have been done to investigate the ability of single dipole nanoantenna to sense their focal adhesion contact. This enables the development of a topological map with information of a single cell's FA contacts. Furthermore, it has been visualized the ability of this method to provide information of composition and protein structure at these focal contacts through the excitation of nonlinear optical effect such as Raman Enhanced Scattering Spectroscopy (SERS).

2. Experiments and Results

The nanoantennas were bioconjugated with a peptide thiolated cyclic RGD for trapping murine melanoma cell B16F10-GFP; the glass substrate was passivated using a PEG-silane in order to reduce the non-specific cell attachment. The cell was cross-linked in order to avoid possible variations due to the cell membrane remodeling during the data acquisition. The Rayleigh scattering spectrum of the section of where the cells were bound was acquired and analyzed individually to determine the shift produced by the cell attachment. A map of the differences on each antennas spectrum is obtained by subtracting the initial resonance (λ_{res}) and the value after the cell attachment. The higher values of the shift are observed around the edges with a maximum value of 13 nm for a single nanoantenna.

The highly localized sensing depth allows assuring the shift detected is only due to the focal adhesion contact (10-15 nm) contact and it is not affected by close contact (30-100 nm) or variation of internal structure of the cell substrate.

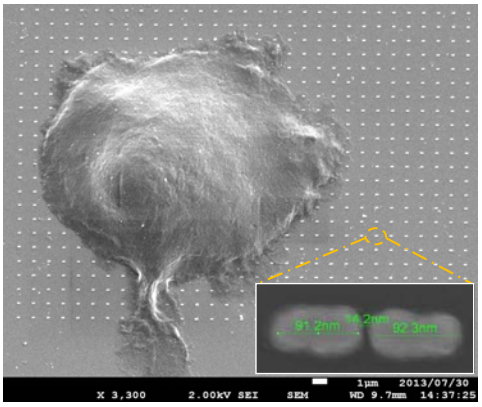

Fig1. SEM image of cell attached to an array of dipole nanoantennas bioconjugated with cRGD. Inset shows the SEM image of a single nanoantenna with its corresponding dimensions.

Important events such as cell remodeling, migration or differentiation are mediated by the localized interactions of transmembrane proteins with ECM's components. The current methods allow determining the distribution of the cell focal adhesion at the nanoscale. These focal points can be further studied by exciting Raman scattering with a laser source and analyzing the variation of the Raman peaks associated, providing biochemical information of the attachment/detachment during different cell events.

3. Summary

The capability of sensing the focal adhesion contact of single living cells using a large scale array of bioconjugated nanoantennas was demonstrated. A mapping of these contacts was obtained showing a preferred distribution of these contacts at the cell contour.

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References

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