Drug response assessment of vascular endothelial cells by a SERS method

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Objective

Vascular endothelial cells in the innermost layer of blood vessels control vascular permeability. Lifestyle-related diseases and tumors cause a decline in this function, and in atherosclerotic lesions, the breakdown of inter-endothelial cell adhesion leads to increased permeability and inflammatory cell infiltration. In the development of disease models and therapeutic agents, it is important to have a simple system to evaluate the permeability of endothelial cells. We have proposed a combination of a Raman indicator with Raman activity and the surface enhanced Raman scattering (SERS) method based on the signal enhancement effect of a special substrate. Since the signal from the indicator is enhanced by more than several orders of magnitude only when it comes into contact with the surface of the SERS culture substrate, the generation of the SERS signal clearly reflects increased permeability of the cell layer. In this experiment, vascular endothelial cells (EA. Hy926) were seeded and cultured on a SERS culture substrate (henceforth referred to as SEA substrate).

Method

Collagen coating (Cellmatrix type IV, incubated at 37°C, CO_2 5 %, 2h) was applied to the SERS substrate which was prepared by our laboratory. After that, vascular endothelial cells (E.A. Hy 926) were seeded and cultured. The cells on the substrate were observed under an inverted microscope (100~400x) to determine if cell to cell adhesion was uniform and dense. Selected SEA substrates were then replaced with 10% PBS solution to remove the culture medium. Then various Raman indicators (Rhodamine 6G, 1,2-di(4-pyridyl)-ethylene: BPE, dimethyl sulfoxide, dichloro ruthenium, crystal violet) were added to the substrate. Finally, a portable Raman spectrometer (Hamamatsu Photonics C13560) was used to detect the SERS signal in order to determine if any of these indicators was exuding.

Results

(1) When these Raman indicators were added onto the substrate without cell seeding as a control experiment, they all showed corresponding SERS spectra, indicating that the medium did not suppress the signal from the substrate.

(2) It is essential to culture the cell membrane with low permeability. As a result of examining conditions such as cell concentration and culture time, it became possible to culture a dense layer of cells. In addition, when the indicator was added onto the SEA substrate, the SERS signal was not detected, which suggests that we succeeded in culturing model cells with low permeability. One minute after adding histamine, the Raman indicator was added. Subsequently the SERS signal was detected for all the indicators except BPE. When histamine was added, cell adhesion diminished regardless of the type of reporter indicator.

References:

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