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Chemo-spectroscopic sensor for carboxyl terminus overexpressed in carcinoma cell membrane

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Abstract

Certain carboxyl groups of the plasma membrane are involved in tumorgenesis processes. A gold core-hydroxyapatite shell (AuHA) nanocomposite is introduced as chemo-spectroscopic sensor to monitor these carboxyl groups of the cell membrane. Hydroxyapatite (HA) plays the role both of a chemical detector and of a biocompatible Raman marker. The principle of detection is based on chemical interaction between the hydroxyl groups of the HA and the carboxyl terminus of the proteins. The AuHA exhibits a surface enhanced Raman scattering (SERS) signal at 954 cm^{-1} which can be used for its localization. The bio-sensing capacity of AuHA towards human skin epidermoid carcinoma (A431) and Chinese hamster ovary (CHO) cell lines is investigated using Raman microspectroscopic imaging. The localization of AuHA on cells is correlated with scanning electron microscopy, transmission electron microscopy and structured illumination fluorescence microscopy. This qualitative approach is a step towards a quantitative study of the proteins terminus.

From the Clinical Editor: This method would enable further studies on the molecular profiling of the plasma membrane, in an attempt to provide accurate cell identification. Using a gold core-hydroxyapatite shell (AuHA) nanocomposite, the authors in this paper showed the feasibility of detecting and differentiating cell surface molecules by surface enhanced Raman scattering.

Key words: Gold-hydroxyapatite; C-terminus; SERS sensor

The molecular profiling of the plasma membrane is a prerequisite for accurate cell identification. An efficient strategy to this aim has to consider that the protein network on the membrane of every cell is unique. Several receptors or binding sites can be identified on this network. Frequently, the receptors link the definite chemical partner through a carboxyl-terminus (C-terminus). The binding site of this terminus is involved in glucose transport and glycogen synthesis.[1-3] Its overexpression enhances the glucose transport activity, glycogen and insulin synthesis[4] and tumorgenesis processes.[3] Numerous enzymatic pathways are also initiated at the carboxyl end of the plasma proteins.[5] Furthermore serotonin,[6] aquaporins[7] and the proteins of the signal transduction[8] anchor through the C-terminus. On the other hand, the virus entrance into the cell is potentially signalized by the C-terminus at the membrane´s outer site.[9]

In this dynamic context of molecular biology[10] an exact, rapid and sensitive apparatus to detect and quantify the molecules is still a key challenge. The detection method based on Raman spectroscopy permits to differentiate molecules due to their distinctive spectral signature.[11] The detection sensitivity of

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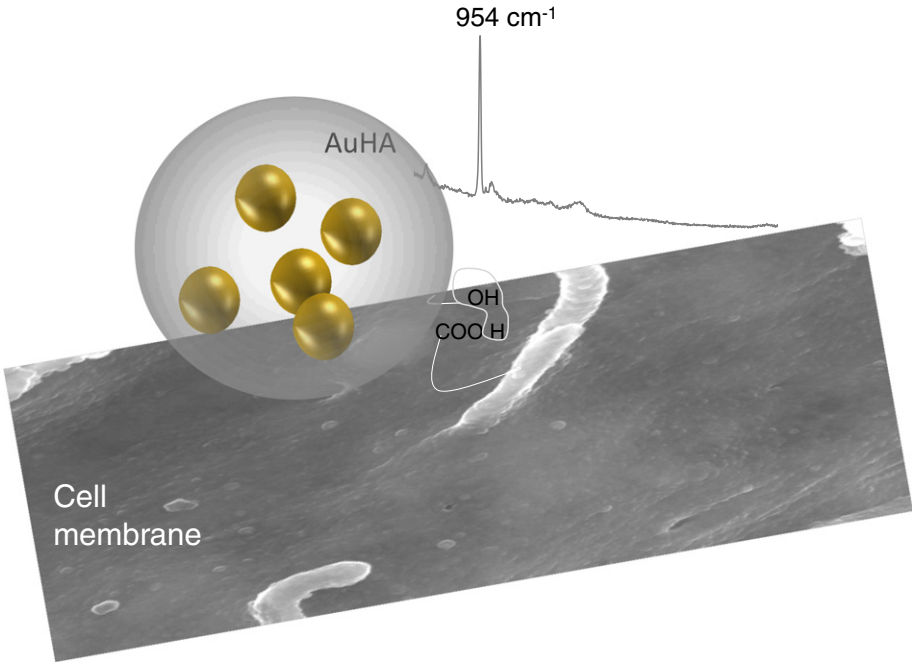

Figure 1. The principle of chemical link between AuHA and C-terminus of the cell membrane. The water molecule releases from a hydroxyl group (–OH) of the AuHA and an acidic group (–COOH) of the membrane. On the membrane the AuHA exhibits an enhanced Raman signal at 954 cm^{-1}, which indicates the presence of this interaction.

this technique is substantially improved by combining the molecular specificity of Raman spectroscopy with plasmonic field enhancement of nanostructured materials.[12,13] This approach is termed surface enhanced Raman scattering. In the present work this phenomenon is exploited by architecting at nanoscale a chemo-spectroscopic transducer. This transducer is synthesized by covering the gold core with hydroxyapatite, and applied to recognize chemical groups of the cell membrane (Figure 1). The AuHA exhibits an enhanced Raman signal at 954 cm^{-1} which can be used for its localization. In other studies, the SERS active nanoparticles utilize organic markers as reporters such as mercaptobenzoic acid derivatives,[14-16] methylene blue, cresyl violet or rhodamine derivatives.[17-19] However, most of the organic Raman markers are bioincompatible, water insoluble or fluorescent for a large range of excitation wavelengths. These shortcomings open the way to a variety of inorganic markers. Among them the biocompatibile[20-23] and proteinophile[24,25] hydroxyapatite could find a privileged place due to further advantages: 1) sharp bands of Raman spectrum with the bands (583, 954, and 1044 cm^{-1}) which can be easily distinguished from the characteristic Raman bands of cellular components; 2) feasible for a large range of excitation wavelengths; 3) no fluorescence; 4) easy linkage to the metallic core; 5) synthesis without organic precursors which normally can overlay the marker signals. Due to these advantages, the current paper introduces the inorganic marker hydroxyapatite which can act simultaneously as a reporter molecule and protective shell. Here, the bio-sensing capacity of AuHA sensor towards cell lines (A431 and CHO) of dissimilar proteins pattern is investigated using Raman spectroscopy or SERS. Upon excitation at 785 nm, the intimate architecture integrated hydroxyapatite molecules report the sensor distribution on the plasma membrane. We complement these data with images from scanning electron microscopy, transmission electron microscopy and fluorescence microscopy.

Methods

Colloidal gold preparation

Colloidal gold preparation followed an adapted classical routine.[26] $HAuCl_4$ was reduced by the hydrogen which is slowly released from $NaBH_4$ in aqueous solution. For synthesis two solutions, 40 mL standard solution containing 1 mL of 1% $HAuCl_4$, and the 1 mL reducing mixture of 0.01% $NaBH_4$, were added together under continuous stirring, in an ice bath. After the synthesis was completed, as evident from the red color (Figure 2, *A*), it was immediately used for the reaction with hydroxyapatite.

Synthesis of hydroxyapatite

HA was synthesized at room temperature in an atmosphere of inert gas following the reaction:

$$10Ca(NO_3)_2 + 6(NH_4)_3PO_4 + 2H_2O \rightarrow Ca_{10}(PO_4)_6(HO)_2 + 18NH_4NO_3 + 2HNO_3 \quad (R1)$$

17 mL of 1% $Ca(NO_3)_2$ was slowly added to 1 mL of 10% freshly prepared $(NH_4)_3PO_4$ into a glass beaker under stirring at 900 rpm until a white precipitate appeared. The pH value was adjusted to 10 by adding 2-3 μL of 10% $(NH_4)_3PO_4$ solution. The dispersion was stored at room temperature and prepared for recording the reference X-ray diffraction patterns (XRD) and